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జాతీయోద్యమం భారత స్వాతంత్ర్య వీరాంగన కనకమ్మ

డా. గోపవరం పద్మప్రియ, పి.ఆర్.ఆర్. & వి.యస్. ప్రభుత్వ డిగ్రీ కళాశాల, విడవలూరు,
శ్రీ పొట్టి శ్రీరాములు నెల్లూరు జిల్లా. సెల్: 9177355538

భారతదేశ స్వాతంత్ర పోరాటంలో రక్తం మరిగే అంశాలు, రోమాలు నిక్కబొడుచునే సంఘటనలు ఎన్నో ఉన్నాయి. ఈ ఆజాదీకా అమృత్ మహోత్సవంలో భాగంగా వారి త్యాగాల్ని తెగువల్ని గుర్తు చేసుకుని స్ఫూర్తిని పొందడం ఈనాటి తరానికి, ఇప్పటి పరిస్థితులకు ఎంతో అవసరం, స్వాతంత్ర్యం కోసం చేసిన పోరాటంలో కేవలం పురుషులే కాదు మహిళలూ శివంగులై పురుషులతో సరిసమానంగా స్వాతంత్ర పోరాటంలో తమ భాగస్వామ్యతకు స్వాతంత్ర్యం తీసుకురావడంలో వారు చూపించిన ధైర్య సాహసాలకు కొదవలేదు. సంప్రదాయాలకు కట్టుబాట్లకు ప్రాముఖ్యతను ఇచ్చే నాటి రోజుల్లో గడప దాటి మూఢ విశ్వాసాలను ఎదిరించి స్వతంత్ర పోరాటంలో పాల్గొని భారతదేశ మహిళా పౌరషాన్ని చాటిన మహిళా మణులెందరో ఉన్నారు. వారిలో నెల్లూరుకు చెందిన భారత స్వాతంత్ర వీరాంగన పొణకా కనకమ్మ ఒకరు.

పొణకా కనకమ్మ నెల్లూరు వాస్తవ్యురాలు 1892 జూన్ 10వ తేదీన మరుపూరు కొండారెడ్డి, కామమ్మ దంపతులకు మినగల్లులో జన్మించింది (కోవూరు తాలూకా, నెల్లూరు జిల్లా) ఈమె పెరిగి పెద్దయినది పోట్లపూడిలో. వీరికి 9వ ఏటనే సుబ్బరామిరెడ్డితో వివాహం జరిగింది. వీరి పుట్టిల్లు, మెట్టిల్లు రెండూ సంపన్నమైన కుటుంబాలు. రెండు కుటుంబాలకు రాజకీయాలతో పరిచయం లేకపోయినా జాతీయోద్యమంలో చరుకైన పాత్ర వహించారు. ఈ ఉద్యమ పాత్రలో పొణకా కనకమ్మది సింహభాగం. జాతీయోద్యమంలో తన పాత్రను తన స్వీయ చరిత్రలో విఫులంగా చర్చించింది. దానిని మనం చదువుతూ ఉంటే అనుభవాల దొంతరలాగా కన్పిస్తుంది. మరుగునపడివున్న వీరి స్వీయ చరిత్రను మనకు అందించిన ఘనత కాళిదాసు పురుషోత్తమం గారిది. నేను ఈ పత్రంలో కస్తూరిదేవి విద్యాలయం, సుజనరంజని, జమీన్ రైతు, రాజకీయాలు ఇలా కొన్ని సంఘటనలను మాత్రమే పొందుపరచాను.

సుజన రంజనీ సమాజం:- 1913 మార్చి1వ తేదీన పోట్లపూడిలో పొణకా పట్టాభిరామిరెడ్డి, మరుపూరు పిచ్చిరెడ్డి, నెల్లూరు రామానాయుడు, ఇంకా కొంతమంది మిత్రులతో కలసి సమాజ సేవకై సుజనీ రంజనీ సమాజం స్థాపించారు. దీనితో పాటు వివేకానంద గ్రంథాలయం కూడా స్థాపించారు. కనకమ్మ భర్త పొణకా సుబ్బరామిరెడ్డి ఔదార్యం, సహకారం వల్ల సుజన రంజనీ సమాజం ఆంధ్రప్రదేశ్ సాంస్కృతిక పునరుజ్జీవనంలో తన పాత్రను నిర్వహించింది. ఈ సమాజ సభ్యులు హరిజన, అరుంధతీ వాడల్లో కలరా, ఇన్ఫ్లూయెంజా వ్యాపించినపుడు మందలిచ్చి, గంజి కాసి పోసేవారు. పనిపాటలు చేసుకునే బాలుర కోసం విలుకానిపల్లె, పోట్లపూడిలో హాస్టళ్ళను నిర్వహించారు. గ్రామాల్లో, వీధుల్లో నలుగురు చేరే ప్రదేశాల్లో సంఘ సంస్కరణ గురించి దేశ పరిస్థితుల గురించి ఉపన్యాసాలనిచ్చేవారు.

ఈ సమాజానికి మొదట రాజకీయ ప్రసక్తి లేకపోయినా వెన్నెలగంటి రాఘవయ్య, చతుర్వేదుల కృష్ణయ్య ప్రవేశించిన తరువాత తిన్నగా ఆమె, ఆమె మిత్రబృందం, కొద్దికాలం అతివాద విప్లవ రాజకీయాల వైపు మొగ్గి పనిచేశారు. రహస్య కార్యకలాపాలకు

పల్లెపాడులో మామిడితోపును కూడా కొనాలని తీర్మానించుకున్నారు. కాని అది ఫలవంతం కాలేదు. క్రమంగా గాంధీజీ జాతీయోద్యమంలోకి ప్రవేశించిన తరువాత పూర్తిగా వీరు అహింసా మార్గం వైపు మరలారు.

గాంధీ ప్రభావానికి లోనుకాక ముందు మద్రాసులో ఓ.వి. చిదంబరం పిళ్ళై, గుంటూరులో ఉన్నవ లక్ష్మీ నారాయణ లాంటి విప్లవకారులతో చేతులు కలపి రహస్యంగా పిస్టళ్ళు, బాంబులు దిగుమతి చేసుకొని సమయం కోసం వేచి చూసిన సాహసి ఈమె.

కస్తూరి దేవి విద్యాలయం:

ఏ దేశ ఔన్నత్యానికైనా, ఏ జాతి అభ్యుదయానికైనా విద్య, విజ్ఞానం, సంస్కృతి ముఖ్యమైన మెట్లు. ప్రేమ, త్యాగం జీవిత సూత్రాలు. ఈ సిద్ధాంతాలతో భారత స్వాతంత్రోద్యమ తొలి వసంతాల్లో గాంధీజీ జీవిత భాగస్వామి కస్తూరిబా పేరిట ఉద్భవించిన ప్రాథమిక విద్యా వ్యవస్థ కస్తూరిబా విద్యాలయం. దీనివెనుక ముందు కనకమ్మ కృషి మరువలేది. ఈ విద్యాలయాన్ని 1923వ సంఎఎలో నెల్లూరు స్త్రీల కాంగ్రెసు సంఘం తరపున ప్రకాశం పంతులు చేత ప్రారంభించబడినది. ఈ పాఠశాలలో మన సంస్కృతికి అనుగుణంగా విద్య, నూలువడకడం, నేత, కుట్టుపని మొదలగు చేతిపనులు, ఆరోగ్య సూత్రాలు, సూర్య నమస్కారాలు మొ॥ విద్యా పద్ధతులను బోధించేవారు.

ఈ విద్యాలయ నిర్వహణకు చుట్టు ప్రక్కల గ్రామాల్లో తిరిగి ధాన్యము, బియ్యము వసూలు చేసే వారు, కొన్ని చోట్ల నాటక ప్రదర్శనలు ఇచ్చి ఆ ధనాన్ని దీని నిర్వహణకే ఖర్చు చేసేవారు. 1928వ సం॥ శ్రీ కస్తూరి దేవి విద్యాలయం భవన నిర్మాణార్థం విశాఖలో "శ్రీకృష్ణ తులాభారం', 'సారంగధర' నాటకాలను ప్రదర్శించాలని సంకల్పించారు. ఆ సమయానికి ఆంధ్ర విశ్వవిద్యాలయం స్నాతకోత్సవానికి నాటి వైస్ ఛాన్సలర్ సర్వేపల్లి రాధాకృష్ణన్ అక్కడికి వచ్చియున్నారు. వీరి కోరిక మేరకు ఆ రెండు నాటకాలకు సర్వేపల్లి గారే అధ్యక్షత వహించారు.

1928లో భవన నిర్మాణార్థం పొగతోట ప్రక్కన 2 1/2 ఎకరాల స్థలాన్ని రూ.5400/- కొన్నారు. మే 12, 1929వ సంఎఎలో కస్తూరి దేవి పేరు మీద శంఖుస్థాపన కూడా చేయడం జరిగింది. 1929లో గాంధీజీ పిలుపు మేరకు శాసనోల్లంఘన ఉద్యమంలో పాల్గొన్నారు. 1930, 1932 సంఎఎలలో జైలుకు వెళ్ళడంతో ఈ విద్యాలయం 1933 వరకు మూత పడింది. వీరు జైలు నుండి విడుదలయి వచ్చిన తరువాత ఇది పునఃప్రారంభించబడింది. భవన నిర్మాణార్థం కస్తూరి దేవి నగర్లో వున్న ఒక భూమిని రేబాల పట్టాభిరామిరెడ్డికి ఇచ్చి, దర్గామిట్టలో 20 ఎకరాల భూమిని తీసుకోవడం జరిగింది. తిక్కవరపు రామిరెడ్డి 54000 రూ. భూరి విరాళంతో కస్తూరిభా నిధిని ఏర్పాటు చేసి తిరిగి విద్యాలయాన్ని నిర్మించారు.

కాని తదనంతరం జరిగిన అనేక రాజకీయ కుట్రలలో విద్యాలయం స్థాపనకు, నిర్మాణానికి, విస్తరణకు మూలబీజమైన పొణకా కనకమ్మ ఆ విద్యాలయం నుండి కార్యదక్షులు, సమర్థులు, ఎవరికీవెరవని వ్యక్తిత్వం మీద బలవంతంగా బయటకు రావలసి వచ్చింది. దీనికి సాక్ష్యం ఆమె కాంస్య విగ్రహం ఇటీవల వరకు చీకటి కొట్టులోనే మూలుగుతూ వుండటం ఇన్ని వున్నా పురుషబలం, ధనబలం ముందు తలవంచి దాని భవిష్యత్తు కోసం ఆమె చుట్టూ ముళ్ల కంచె వేసినా ఏ మాత్రం ఖాతరు చేయకుండా స్థిరంగా బలంగా అడుగు ముందుకు సాగించి, విద్యాలయ ప్రాంగణం నుంచి బయటకు వచ్చినా నిరాశ పడకుండా, రెట్టించిన ఉత్సాహంతో అడుగు ముందుకు వేసి పారిశ్రామిక పాఠశాలను స్థిరంగా నిలబెట్టింది. తిరస్కృతులు, బహిష్కృతులు, అనాథలు, అభాగినులు ఇలా రకరకాల మహిళలను చేరదీసి చేతివృత్తులు, హస్తకళలను నేర్పి, వాళ్ళకు జీవనోపాధిని కల్పించింది. కస్తూరి దేవి విద్యాలయాన్ని వదిలి వచ్చిన తరువాత ఆమె జీవితంలో ఏర్పడిన శూన్యాన్ని పారిశ్రామిక పాఠశాల పూరించంది. ఒక ఓటమి మరొక సంస్థకు పునాది అయింది. అదీ కనకమ్మ అంటే. అదే ఆమె కార్యదక్షత.

జమీన్ రైతు:

భూ వ్యవహారాలలో వెంకటగిరి రాజాతో వచ్చిన వివాదాలను లోకానికి తెలియజేసే ఉద్దేశంతో ప్రజల కొరకు, సొంత వ్యవహారాల కొరకు ఒక పత్రికను స్థాపించారు. దానిపేరే "జమీన్ రైతు". దీని వ్యవస్థాపక సంపాదకుడు పొణకా పిచ్చిరెడ్డి అని తమ స్వీయచరిత్రలో రాసుకున్నారు. "ప్రపంచాన్ని చకితమొనర్చే జమిందారీ ఉద్యమం మా కుటుంబం యొక్క మహోగ్ని జ్వాలలోనే అవతరించి దశదిశలా వ్యాపించింది. దాని నుంచే జమీన్ రైతు పుట్టింది. ఇది జగమెరిగిన సత్యం" అని తన మాటలను స్వీయ చరిత్రలో ఉటంకించారు. ఇదే విషయాన్ని ఆచార్య ఎన్.జి. రంగా కూడా జమీన్ రైతు స్వర్ణోత్సవ సంచికలో రాశారు.

కానీ తరువాత ఎక్కడా వీరి పేర్లు బయటకు రాలేదు. కారణాలు తెలియలేదు. ఇపుడు కూడా మనం గూగుల్లో జమీన్ రైతు వికీపీడియాను చూసినా వ్యవస్థాపక సంపాదకుల పేర్లు మాత్రం కనిపించవు.

రాజకీయ ముచ్చట్లు:

వీరి రాజకీయ ప్రస్థానం మన జాతీయోద్యమమంత సుదీర్ఘమైనది. కాబట్టి అక్కడక్కడ ఆమె పొందుపరచిన జ్ఞాపకాలను కొన్నింటిని మాత్రమే ఇక్కడ ప్రస్తావిస్తున్నాను. ఈ సంఘం ద్వారా వీరు తిలక్ స్వరాజ్య నిధికి నిధులను సమకూర్చడం, స్వదేశీ ఉద్యమంలో భాగంగా ఖద్దరు నేయడం లాంటి కార్యక్రమాలను నిర్వహించారు. జాతీయోద్యమమే కాక సామాజికోద్యమ నిర్వహకురాలిగా 31 రాట్నాలను కొని మహమ్మదీయ స్త్రీలకు, పతితులకు ఇవ్వడంతో పాటు, 24 మణుగుల దూదిని, 3 మణుగుల ఏకులను ఇచ్చి వారిని ప్రోత్సహిస్తూ, వారు తయారు చేసిన ఖద్దరు వస్త్రాలను వీరే కొని ఉపాధితో పాటుగా స్వదేశీ ఉద్యమాన్ని విజయవంతం చేశారు.

చెన్నపట్టణంలో రాజకీయ మహాసభ:

1927వ సంఎఎలో చెన్నపట్టణంలో రాజకీయ మహాసభలు జరిగాయి. దానిలో భాగంగా స్త్రీల రాజకీయ మహాసభకూడా నిర్వహించడం జరిగింది. ఆ సభకు పొణకా కనకమ్మను అధ్యక్షత వహించవలసిందిగా కాశీనాధుని నాగేశ్వరరావు ఒత్తిడి చేసినా ఆమె ఒప్పుకోక పులుపర్తి లక్ష్మీ నరసాంబను అధ్యక్షురాలిగా చేసి సభను నిర్వహించామని పేర్కొన్నారు.

జైలు యాత్ర:

బాపూజీ ఉప్పు సత్యాగ్రహ ఉద్యమంలో భాగంగా 1930-32 సంఎఎలలో వీరి అరెస్టు కాబడ్డారు. 1 1/2 సం॥ జైలు శిక్షను అనుభవించారు. ఇక్కడ ఆమెకు జరిగిన ఒక వివక్షను గుర్తు చేసుకుంటూ తనతో పాటు బెజవాడ గోపాలరెడ్డి కూడా అరెస్టు కాబడ్డారు. కానీ అతనికి 'ఎ' క్లాస్, తనకు 'బి' క్లాస్ జైళ్ళను ఇచ్చారని పేర్కొన్నారు.

బిపిన్ చంద్రపాల్కు ఆతిథ్యం:

ఈమెకు 9వ ఏటనే వివాహం అయి అత్తారింటికి ప్రవేశించింది. తనకు 15వ ఏట 1907లో టైఫాయిడ్ జ్వరం రావడంతో నెల్లూరుకు తీసుకుని వచ్చి వైద్యం చేయించారు. ఈ వైద్యం నిమిత్తం యడ్లవారి వీధిలో తీసుకోవటం జరిగింది. అదే సమయంలో బిపిన్ చంద్రపాల్ రాజకీయ ఉపన్యాసాలు ఇస్తూ దేశమంతా సంచరిస్తున్నారు. ఇందులో భాగంగా మద్రాసు పోతూ ఉపన్యాసాల నిమిత్తం నెల్లూరుకు రావడం జరిగింది. అప్పుడు తన తమ్ముడు మరుపూరి పిచ్చిరెడ్డి, మరిది పట్టాభిరామిరెడ్డి ఇద్దరూ బిపిన్ చంద్రపాల్ని వీరింటికి ఆహ్వానించారు. వీరికి బారకాసు సెంటర్లోని ఆగా సాహెబ్ గారి మిద్దెమీద బస ఏర్పాటు చేసినట్లు పేర్కొన్నారు. రెండు మూడు రోజులు పొణకా కనకమ్మ ఇంటిలో ఈ జంట ఆతిథ్యం స్వీకరించడం జరిగింది. వారి ఆహార విశేషాలను ఒకటి ఇక్కడ ఉటంకించారు. బిపిన్ చంద్రపాల్ గారికి ఎన్ని కూరలు చేసినా ఆ కూరల్లో వేపచిగుళ్ళు వేసుకుని తినేవారు అని పేర్కొన్నారు. ఈ సమయంలో ఈ సమావేశాలకు వచ్చిన మంగిపూడి పురుషోత్తమ శర్మ కూడా వీరి ఇంటికి వచ్చినట్లు గుర్తు చేసుకున్నారు.

తిలక్ పరిచయం:

1919లో తిలక్ గారు మద్రాసు వచ్చారు. వారిని కలవడానికి రాయప్రోలు సుబ్బారావు, రాఘవయ్య, తంబటి పుట్రయ్య తమ్ముడు పిచ్చయ్యలతో కలిసి కనకమ్మ కూడా మద్రాసుకి వెళ్ళింది. ఆ జ్ఞాపకాలను స్వీయ చరిత్రలో జ్ఞాపకం చేసుకున్నారు.

మొదటి ప్రసంగం:

1921వ సంఎఎలో వెన్నెలకంటి రాఘవయ్య, కందాడ దొరస్వామి జైలుకు వెళ్ళు సందర్భంలో పిడూరు వచ్చారు. అప్పుడు కనకమ్మను నెల్లూరుకి రమ్మని కోరారు. మర్నాడు మధ్యాహ్నానికి కనకమ్మ నెల్లూరుకి చేరుకుంది. ఆ సందర్భంలో ఏర్పాటు చేసిన సమావేశంలో మాట్లాడమని కోరడంతో మొట్ట మొదట బహిరంగం ఉపన్యాసం ఇవ్వడం జరిగింది. అప్పుడు ఆమె పొందిన ఉద్వేగాన్ని, అనుభూతుల్ని నాటి జ్ఞాపకాలను తమ స్వీయచరిత్రలో ప్రస్తావించారు.

స్త్రీల ప్రతినిధి:

1921 సం॥లో అహ్మదాబాదులో కాంగ్రెస్ వార్షికోత్సవ సభలు జరిగాయి. ఆ సభలో ఈమెను అఖిల భారత కాంగ్రెస్ సంఘ సభ్యురాలిగా చేర్చుకుని ఆంధ్ర రాష్ట్రానికి స్త్రీల ప్రతినిధిగా నియమించడం జరిగింది. 2 సంవత్సరాల పాటు ఈ బాధ్యతలను చక్కగా నిర్వహించారు.

నెల్లూరు స్త్రీల కాంగ్రెస్ సంఘం:

1922 మార్చి 10వ తేదీన నెల్లూరు జిల్లా మహిళా కాంగ్రెస్ సంఘం స్థాపించబడింది. అప్పటికీ నెల్లూరు జిల్లా కాంగ్రెస్ సంఘం ఉండటంతో రెండు జిల్లా కాంగ్రెస్ సంఘాల పేర్లపై అభ్యంతరాలు వెలువడ్డాయి. దానితో జిల్లా తీసేసి నెల్లూరు స్త్రీల కాంగ్రెస్ సంఘాన్ని స్థాపించారు. ఇందులో వీరు సభ్యురాలిగా, కార్యదర్శిగా పనిచేశారు.

మహాత్మునితో పరిచయ భాగ్యం:

1919 సం॥లో గాంధీజీ చెన్నపట్టణంలో రాజగోపాలాచారి ఇంటికి వచ్చినప్పుడు కనకమ్మ తన తల్లితో పాటు గాంధీజీని దర్శించి నూలు వడకటం జరిగింది. ఆ నేత పని నైపుణ్యానికి గాంధీజీ అబ్బురపడి సబర్మతి ఆశ్రమానికి ఆహ్వానించటం జరిగింది. దండి ఉప్పు సత్యాగ్రహంలో భాగంగా 1921 సం॥లో గాంధీజీ నెల్లూరులోని పల్లిపాడుకి వచ్చినప్పుడు స్వయంగా పొణకా కనకమ్మ భర్తే గాంధీని పల్లిపాడు ఆశ్రమానికి తీసుకురావటం జరిగింది.

ఇలా చెప్పుకుంటూ పోతే ఎన్నో జ్ఞాపకాలు తన స్వీయ చరిత్రలో నదీ ప్రవాహంలా మనకు తారసపడుతూనే ఉంటాయి.

ఆధార గ్రంథాలు:

1. కనకపుష్య రాగం (పొణకా కనకమ్మ స్వీయ చరిత్ర) – సంపాదకుడు డా. కాళిదాసు పురుషోత్తం

2. వివిధ పత్రికా వ్యాసాలు.

ప్రభుత్వ మహిళా కళాశాల (స్వ.ప్ర.)

గుంటూరు, ఆంధ్రప్రదేశ్

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Azadi Ka Amrit Mahotsav

అంతర్జాతీయ అంతర్జాల సదస్సు

జాతీయోద్యమం - అనుభవాలు, జ్ఞాపకాలు, సాహిత్యం

"National Movement – Experiences, Memories, Literature"

10 & 11 ఆగస్టు, 2022

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మూసీ ప్రత్యేక సంచిక

సాహిత్య సాంస్కృతిక చారిత్రక మాస పత్రిక

నిర్వహణ

తెలుగు శాఖ

ప్రభుత్వ మహిళా కళాశాల, (స్వ.ప్ర.) గుంటూరు

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విషయసూచిక

జాతీయోద్యమం భారత స్వాతంత్ర్య వీరాంగన కనకమ్మ

డా. గోపవరం పద్మప్రియ, పి.ఆర్.ఆర్. & వి.యస్. ప్రభుత్వ డిగ్రీ కళాశాల, విడవలూరు,
శ్రీ పొట్టి శ్రీరాములు నెల్లూరు జిల్లా. సెల్: 9177355538

భారతదేశ స్వాతంత్ర పోరాటంలో రక్తం మరిగే అంశాలు, రోమాలు నిక్కబొడుచునే సంఘటనలు ఎన్నో ఉన్నాయి. ఈ ఆజాదీకా అమృత్ మహోత్సవంలో భాగంగా వారి త్యాగాల్ని తెగువల్ని గుర్తు చేసుకుని స్ఫూర్తిని పొందడం ఈనాటి తరానికి, ఇప్పటి పరిస్థితులకు ఎంతో అవసరం, స్వాతంత్ర్యం కోసం చేసిన పోరాటంలో కేవలం పురుషులే కాదు మహిళలూ శివంగులై పురుషులతో సరిసమానంగా స్వాతంత్ర పోరాటంలో తమ భాగస్వామ్యతకు స్వాతంత్ర్యం తీసుకురావడంలో వారు చూపించిన ధైర్య సాహసాలకు కొదవలేదు. సంప్రదాయాలకు కట్టుబాట్లకు ప్రాముఖ్యతను ఇచ్చే నాటి రోజుల్లో గడప దాటి మూఢ విశ్వాసాలను ఎదిరించి స్వతంత్ర పోరాటంలో పాల్గొని భారతదేశ మహిళా పౌరషాన్ని చాటిన మహిళా మణులెందరో ఉన్నారు. వారిలో నెల్లూరుకు చెందిన భారత స్వాతంత్ర వీరాంగన పొణకా కనకమ్మ ఒకరు.

పొణకా కనకమ్మ నెల్లూరు వాస్తవ్యురాలు 1892 జూన్ 10వ తేదీన మరుపూరు కొండారెడ్డి, కామమ్మ దంపతులకు మినగల్లులో జన్మించింది (కోవూరు తాలూకా, నెల్లూరు జిల్లా) ఈమె పెరిగి పెద్దయినది పోట్లపూడిలో. వీరికి 9వ ఏటనే సుబ్బరామిరెడ్డితో వివాహం జరిగింది. వీరి పుట్టిల్లు, మెట్టిల్లు రెండూ సంపన్నమైన కుటుంబాలు. రెండు కుటుంబాలకు రాజకీయాలతో పరిచయం లేకపోయినా జాతీయోద్యమంలో చరుకైన పాత్ర వహించారు. ఈ ఉద్యమ పాత్రలో పొణకా కనకమ్మది సింహభాగం. జాతీయోద్యమంలో తన పాత్రను తన స్వీయ చరిత్రలో విఫులంగా చర్చించింది. దానిని మనం చదువుతూ ఉంటే అనుభవాల దొంతరలాగా కన్పిస్తుంది. మరుగునపడివున్న వీరి స్వీయ చరిత్రను మనకు అందించిన ఘనత కాళిదాసు పురుషోత్తమం గారిది. నేను ఈ పత్రంలో కస్తూరిదేవి విద్యాలయం, సుజనరంజని, జమీన్ రైతు, రాజకీయాలు ఇలా కొన్ని సంఘటనలను మాత్రమే పొందుపరచాను.

సుజన రంజనీ సమాజం:- 1913 మార్చి1వ తేదీన పోట్లపూడిలో పొణకా పట్టాభిరామిరెడ్డి, మరుపూరు పిచ్చిరెడ్డి, నెల్లూరు రామానాయుడు, ఇంకా కొంతమంది మిత్రులతో కలసి సమాజ సేవకై సుజనీ రంజనీ సమాజం స్థాపించారు. దీనితో పాటు వివేకానంద గ్రంథాలయం కూడా స్థాపించారు. కనకమ్మ భర్త పొణకా సుబ్బరామిరెడ్డి ఔదార్యం, సహకారం వల్ల సుజన రంజనీ సమాజం ఆంధ్రప్రదేశ్ సాంస్కృతిక పునరుజ్జీవనంలో తన పాత్రను నిర్వహించింది. ఈ సమాజ సభ్యులు హరిజన, అరుంధతీ వాడల్లో కలరా, ఇన్ఫ్లూయెంజా వ్యాపించినపుడు మందలిచ్చి, గంజి కాసి పోసేవారు. పనిపాటలు చేసుకునే బాలుర కోసం విలుకానిపల్లె, పోట్లపూడిలో హాస్టళ్ళను నిర్వహించారు. గ్రామాల్లో, వీధుల్లో నలుగురు చేరే ప్రదేశాల్లో సంఘ సంస్కరణ గురించి దేశ పరిస్థితుల గురించి ఉపన్యాసాలనిచ్చేవారు.

ఈ సమాజానికి మొదట రాజకీయ ప్రసక్తి లేకపోయినా వెన్నెలగంటి రాఘవయ్య, చతుర్వేదుల కృష్ణయ్య ప్రవేశించిన తరువాత తిన్నగా ఆమె, ఆమె మిత్రబృందం, కొద్దికాలం అతివాద విప్లవ రాజకీయాల వైపు మొగ్గి పనిచేశారు. రహస్య కార్యకలాపాలకు

పల్లెపాడులో మామిడితోపును కూడా కొనాలని తీర్మానించుకున్నారు. కాని అది ఫలవంతం కాలేదు. క్రమంగా గాంధీజీ జాతీయోద్యమంలోకి ప్రవేశించిన తరువాత పూర్తిగా వీరు అహింసా మార్గం వైపు మరలారు.

గాంధీ ప్రభావానికి లోనుకాక ముందు మద్రాసులో ఓ.వి. చిదంబరం పిళ్ళై, గుంటూరులో ఉన్నవ లక్ష్మీ నారాయణ లాంటి విప్లవకారులతో చేతులు కలపి రహస్యంగా పిస్టళ్ళు, బాంబులు దిగుమతి చేసుకొని సమయం కోసం వేచి చూసిన సాహసి ఈమె.

కస్తూరి దేవి విద్యాలయం:

ఏ దేశ ఔన్నత్యానికైనా, ఏ జాతి అభ్యుదయానికైనా విద్య, విజ్ఞానం, సంస్కృతి ముఖ్యమైన మెట్లు. ప్రేమ, త్యాగం జీవిత సూత్రాలు. ఈ సిద్ధాంతాలతో భారత స్వాతంత్రోద్యమ తొలి వసంతాల్లో గాంధీజీ జీవిత భాగస్వామి కస్తూరిబా పేరిట ఉద్భవించిన ప్రాథమిక విద్యా వ్యవస్థ కస్తూరిబా విద్యాలయం. దీనివెనుక ముందు కనకమ్మ కృషి మరువలేది. ఈ విద్యాలయాన్ని 1923వ సంఎఎలో నెల్లూరు స్త్రీల కాంగ్రెసు సంఘం తరపున ప్రకాశం పంతులు చేత ప్రారంభించబడినది. ఈ పాఠశాలలో మన సంస్కృతికి అనుగుణంగా విద్య, నూలువడకడం, నేత, కుట్టుపని మొదలగు చేతిపనులు, ఆరోగ్య సూత్రాలు, సూర్య నమస్కారాలు మొ॥ విద్యా పద్ధతులను బోధించేవారు.

ఈ విద్యాలయ నిర్వహణకు చుట్టు ప్రక్కల గ్రామాల్లో తిరిగి ధాన్యము, బియ్యము వసూలు చేసే వారు, కొన్ని చోట్ల నాటక ప్రదర్శనలు ఇచ్చి ఆ ధనాన్ని దీని నిర్వహణకే ఖర్చు చేసేవారు. 1928వ సం॥ శ్రీ కస్తూరి దేవి విద్యాలయం భవన నిర్మాణార్థం విశాఖలో "శ్రీకృష్ణ తులాభారం', 'సారంగధర' నాటకాలను ప్రదర్శించాలని సంకల్పించారు. ఆ సమయానికి ఆంధ్ర విశ్వవిద్యాలయం స్నాతకోత్సవానికి నాటి వైస్ ఛాన్సలర్ సర్వేపల్లి రాధాకృష్ణన్ అక్కడికి వచ్చియున్నారు. వీరి కోరిక మేరకు ఆ రెండు నాటకాలకు సర్వేపల్లి గారే అధ్యక్షత వహించారు.

1928లో భవన నిర్మాణార్థం పొగతోట ప్రక్కన 2 1/2 ఎకరాల స్థలాన్ని రూ.5400/- కొన్నారు. మే 12, 1929వ సంఎఎలో కస్తూరి దేవి పేరు మీద శంఖుస్థాపన కూడా చేయడం జరిగింది. 1929లో గాంధీజీ పిలుపు మేరకు శాసనోల్లంఘన ఉద్యమంలో పాల్గొన్నారు. 1930, 1932 సంఎఎలలో జైలుకు వెళ్ళడంతో ఈ విద్యాలయం 1933 వరకు మూత పడింది. వీరు జైలు నుండి విడుదలయి వచ్చిన తరువాత ఇది పునఃప్రారంభించబడింది. భవన నిర్మాణార్థం కస్తూరి దేవి నగర్లో వున్న ఒక భూమిని రేబాల పట్టాభిరామిరెడ్డికి ఇచ్చి, దర్గామిట్టలో 20 ఎకరాల భూమిని తీసుకోవడం జరిగింది. తిక్కవరపు రామిరెడ్డి 54000 రూ. భూరి విరాళంతో కస్తూరిభా నిధిని ఏర్పాటు చేసి తిరిగి విద్యాలయాన్ని నిర్మించారు.

కాని తదనంతరం జరిగిన అనేక రాజకీయ కుట్రలలో విద్యాలయం స్థాపనకు, నిర్మాణానికి, విస్తరణకు మూలబీజమైన పొణకా కనకమ్మ ఆ విద్యాలయం నుండి కార్యదక్షులు, సమర్థులు, ఎవరికీవెరవని వ్యక్తిత్వం మీద బలవంతంగా బయటకు రావలసి వచ్చింది. దీనికి సాక్ష్యం ఆమె కాంస్య విగ్రహం ఇటీవల వరకు చీకటి కొట్టులోనే మూలుగుతూ వుండటం ఇన్ని వున్నా పురుషబలం, ధనబలం ముందు తలవంచి దాని భవిష్యత్తు కోసం ఆమె చుట్టూ ముళ్ల కంచె వేసినా ఏ మాత్రం ఖాతరు చేయకుండా స్థిరంగా బలంగా అడుగు ముందుకు సాగించి, విద్యాలయ ప్రాంగణం నుంచి బయటకు వచ్చినా నిరాశ పడకుండా, రెట్టించిన ఉత్సాహంతో అడుగు ముందుకు వేసి పారిశ్రామిక పాఠశాలను స్థిరంగా నిలబెట్టింది. తిరస్కృతులు, బహిష్కృతులు, అనాథలు, అభాగినులు ఇలా రకరకాల మహిళలను చేరదీసి చేతివృత్తులు, హస్తకళలను నేర్పి, వాళ్ళకు జీవనోపాధిని కల్పించింది. కస్తూరి దేవి విద్యాలయాన్ని వదిలి వచ్చిన తరువాత ఆమె జీవితంలో ఏర్పడిన శూన్యాన్ని పారిశ్రామిక పాఠశాల పూరించంది. ఒక ఓటమి మరొక సంస్థకు పునాది అయింది. అదీ కనకమ్మ అంటే. అదే ఆమె కార్యదక్షత.

జమీన్ రైతు:

భూ వ్యవహారాలలో వెంకటగిరి రాజాతో వచ్చిన వివాదాలను లోకానికి తెలియజేసే ఉద్దేశంతో ప్రజల కొరకు, సొంత వ్యవహారాల కొరకు ఒక పత్రికను స్థాపించారు. దానిపేరే "జమీన్ రైతు". దీని వ్యవస్థాపక సంపాదకుడు పొణకా పిచ్చిరెడ్డి అని తమ స్వీయచరిత్రలో రాసుకున్నారు. "ప్రపంచాన్ని చకితమొనర్చే జమిందారీ ఉద్యమం మా కుటుంబం యొక్క మహోగ్ని జ్వాలలోనే అవతరించి దశదిశలా వ్యాపించింది. దాని నుంచే జమీన్ రైతు పుట్టింది. ఇది జగమెరిగిన సత్యం" అని తన మాటలను స్వీయ చరిత్రలో ఉటంకించారు. ఇదే విషయాన్ని ఆచార్య ఎన్.జి. రంగా కూడా జమీన్ రైతు స్వర్ణోత్సవ సంచికలో రాశారు.

కానీ తరువాత ఎక్కడా వీరి పేర్లు బయటకు రాలేదు. కారణాలు తెలియలేదు. ఇపుడు కూడా మనం గూగుల్లో జమీన్ రైతు వికీపీడియాను చూసినా వ్యవస్థాపక సంపాదకుల పేర్లు మాత్రం కనిపించవు.

రాజకీయ ముచ్చట్లు:

వీరి రాజకీయ ప్రస్థానం మన జాతీయోద్యమమంత సుదీర్ఘమైనది. కాబట్టి అక్కడక్కడ ఆమె పొందుపరచిన జ్ఞాపకాలను కొన్నింటిని మాత్రమే ఇక్కడ ప్రస్తావిస్తున్నాను. ఈ సంఘం ద్వారా వీరు తిలక్ స్వరాజ్య నిధికి నిధులను సమకూర్చడం, స్వదేశీ ఉద్యమంలో భాగంగా ఖద్దరు నేయడం లాంటి కార్యక్రమాలను నిర్వహించారు. జాతీయోద్యమమే కాక సామాజికోద్యమ నిర్వహకురాలిగా 31 రాట్నాలను కొని మహమ్మదీయ స్త్రీలకు, పతితులకు ఇవ్వడంతో పాటు, 24 మణుగుల దూదిని, 3 మణుగుల ఏకులను ఇచ్చి వారిని ప్రోత్సహిస్తూ, వారు తయారు చేసిన ఖద్దరు వస్త్రాలను వీరే కొని ఉపాధితో పాటుగా స్వదేశీ ఉద్యమాన్ని విజయవంతం చేశారు.

చెన్నపట్టణంలో రాజకీయ మహాసభ:

1927వ సంఎఎలో చెన్నపట్టణంలో రాజకీయ మహాసభలు జరిగాయి. దానిలో భాగంగా స్త్రీల రాజకీయ మహాసభకూడా నిర్వహించడం జరిగింది. ఆ సభకు పొణకా కనకమ్మను అధ్యక్షత వహించవలసిందిగా కాశీనాధుని నాగేశ్వరరావు ఒత్తిడి చేసినా ఆమె ఒప్పుకోక పులుపర్తి లక్ష్మీ నరసాంబను అధ్యక్షురాలిగా చేసి సభను నిర్వహించామని పేర్కొన్నారు.

జైలు యాత్ర:

బాపూజీ ఉప్పు సత్యాగ్రహ ఉద్యమంలో భాగంగా 1930-32 సంఎఎలలో వీరి అరెస్టు కాబడ్డారు. 1 1/2 సం॥ జైలు శిక్షను అనుభవించారు. ఇక్కడ ఆమెకు జరిగిన ఒక వివక్షను గుర్తు చేసుకుంటూ తనతో పాటు బెజవాడ గోపాలరెడ్డి కూడా అరెస్టు కాబడ్డారు. కానీ అతనికి 'ఎ' క్లాస్, తనకు 'బి' క్లాస్ జైళ్ళను ఇచ్చారని పేర్కొన్నారు.

బిపిన్ చంద్రపాల్కు ఆతిథ్యం:

ఈమెకు 9వ ఏటనే వివాహం అయి అత్తారింటికి ప్రవేశించింది. తనకు 15వ ఏట 1907లో టైఫాయిడ్ జ్వరం రావడంతో నెల్లూరుకు తీసుకుని వచ్చి వైద్యం చేయించారు. ఈ వైద్యం నిమిత్తం యడ్లవారి వీధిలో తీసుకోవటం జరిగింది. అదే సమయంలో బిపిన్ చంద్రపాల్ రాజకీయ ఉపన్యాసాలు ఇస్తూ దేశమంతా సంచరిస్తున్నారు. ఇందులో భాగంగా మద్రాసు పోతూ ఉపన్యాసాల నిమిత్తం నెల్లూరుకు రావడం జరిగింది. అప్పుడు తన తమ్ముడు మరుపూరి పిచ్చిరెడ్డి, మరిది పట్టాభిరామిరెడ్డి ఇద్దరూ బిపిన్ చంద్రపాల్ని వీరింటికి ఆహ్వానించారు. వీరికి బారకాసు సెంటర్లోని ఆగా సాహెబ్ గారి మిద్దెమీద బస ఏర్పాటు చేసినట్లు పేర్కొన్నారు. రెండు మూడు రోజులు పొణకా కనకమ్మ ఇంటిలో ఈ జంట ఆతిథ్యం స్వీకరించడం జరిగింది. వారి ఆహార విశేషాలను ఒకటి ఇక్కడ ఉటంకించారు. బిపిన్ చంద్రపాల్ గారికి ఎన్ని కూరలు చేసినా ఆ కూరల్లో వేపచిగుళ్ళు వేసుకుని తినేవారు అని పేర్కొన్నారు. ఈ సమయంలో ఈ సమావేశాలకు వచ్చిన మంగిపూడి పురుషోత్తమ శర్మ కూడా వీరి ఇంటికి వచ్చినట్లు గుర్తు చేసుకున్నారు.

తిలక్ పరిచయం:

1919లో తిలక్ గారు మద్రాసు వచ్చారు. వారిని కలవడానికి రాయప్రోలు సుబ్బారావు, రాఘవయ్య, తంబటి పుట్రయ్య తమ్ముడు పిచ్చయ్యలతో కలిసి కనకమ్మ కూడా మద్రాసుకి వెళ్ళింది. ఆ జ్ఞాపకాలను స్వీయ చరిత్రలో జ్ఞాపకం చేసుకున్నారు.

మొదటి ప్రసంగం:

1921వ సంఎఎలో వెన్నెలకంటి రాఘవయ్య, కందాడ దొరస్వామి జైలుకు వెళ్ళు సందర్భంలో పిడూరు వచ్చారు. అప్పుడు కనకమ్మను నెల్లూరుకి రమ్మని కోరారు. మర్నాడు మధ్యాహ్నానికి కనకమ్మ నెల్లూరుకి చేరుకుంది. ఆ సందర్భంలో ఏర్పాటు చేసిన సమావేశంలో మాట్లాడమని కోరడంతో మొట్ట మొదట బహిరంగం ఉపన్యాసం ఇవ్వడం జరిగింది. అప్పుడు ఆమె పొందిన ఉద్వేగాన్ని, అనుభూతుల్ని నాటి జ్ఞాపకాలను తమ స్వీయచరిత్రలో ప్రస్తావించారు.

స్త్రీల ప్రతినిధి:

1921 సం॥లో అహ్మదాబాదులో కాంగ్రెస్ వార్షికోత్సవ సభలు జరిగాయి. ఆ సభలో ఈమెను అఖిల భారత కాంగ్రెస్ సంఘ సభ్యురాలిగా చేర్చుకుని ఆంధ్ర రాష్ట్రానికి స్త్రీల ప్రతినిధిగా నియమించడం జరిగింది. 2 సంవత్సరాల పాటు ఈ బాధ్యతలను చక్కగా నిర్వహించారు.

నెల్లూరు స్త్రీల కాంగ్రెస్ సంఘం:

1922 మార్చి 10వ తేదీన నెల్లూరు జిల్లా మహిళా కాంగ్రెస్ సంఘం స్థాపించబడింది. అప్పటికీ నెల్లూరు జిల్లా కాంగ్రెస్ సంఘం ఉండటంతో రెండు జిల్లా కాంగ్రెస్ సంఘాల పేర్లపై అభ్యంతరాలు వెలువడ్డాయి. దానితో జిల్లా తీసేసి నెల్లూరు స్త్రీల కాంగ్రెస్ సంఘాన్ని స్థాపించారు. ఇందులో వీరు సభ్యురాలిగా, కార్యదర్శిగా పనిచేశారు.

మహాత్మునితో పరిచయ భాగ్యం:

1919 సం॥లో గాంధీజీ చెన్నపట్టణంలో రాజగోపాలాచారి ఇంటికి వచ్చినప్పుడు కనకమ్మ తన తల్లితో పాటు గాంధీజీని దర్శించి నూలు వడకటం జరిగింది. ఆ నేత పని నైపుణ్యానికి గాంధీజీ అబ్బురపడి సబర్మతి ఆశ్రమానికి ఆహ్వానించటం జరిగింది. దండి ఉప్పు సత్యాగ్రహంలో భాగంగా 1921 సం॥లో గాంధీజీ నెల్లూరులోని పల్లిపాడుకి వచ్చినప్పుడు స్వయంగా పొణకా కనకమ్మ భర్తే గాంధీని పల్లిపాడు ఆశ్రమానికి తీసుకురావటం జరిగింది.

ఇలా చెప్పుకుంటూ పోతే ఎన్నో జ్ఞాపకాలు తన స్వీయ చరిత్రలో నదీ ప్రవాహంలా మనకు తారసపడుతూనే ఉంటాయి.

ఆధార గ్రంథాలు:

1. కనకపుష్య రాగం (పొణకా కనకమ్మ స్వీయ చరిత్ర) – సంపాదకుడు డా. కాళిదాసు పురుషోత్తం

2. వివిధ పత్రికా వ్యాసాలు.

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Spectroscopic and luminescent properties of Ce^{3+} -doped TeO_2-WO_3-GeO_2 glasses

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ABSTRACT

Different concentrations of Ce^{3+}-doped TeO_2-WO_3-GeO_2-Ce_2O_3 (TWGCe) glasses were prepared by melt-quenching method and characterized. Different spectroscopic and luminescence properties were evaluated using room temperature absorption and emission spectra. The absence of sharp X-ray diffraction peaks and the presence of broad band from 18° to 35° shows the amorphous phase of studied glasses. The Fourier transform infrared absorption spectra show the presence of various vibrational bonds. The optical properties of TWGCe glasses were studied by exciting the glass samples at 323 nm ultraviolet wavelength. The quenching in luminescence is noticed at × = 0.5 mol% along with a red-shift at higher Ce^{3+} concentrations. The decay time was found decrease with increase of concentration of Ce^{3+} ions. These glasses emit intense blue luminescence with CIE coordinates (0.161, 0.018) when excited at 323 nm radiation. These glasses are favourable as blue light sources in the design of phosphor-based white LEDs.

1. Introduction

The multi-component glasses are of most significant compared to single crystals. They are of interest due to their low fabrication cost, flexibility in manufacturing different sizes and shapes. The glassy materials have been used to produce various optical components with excellent transparency. They exhibit intense emission in near ultraviolet and visible regions, possesses relatively high density and more rare earth (RE) ion solubility [1–4]. They have tremendous applications as host materials for variety of RE ions doped laser sources, fiber amplifiers in communication, dispersion-control devices, sensors in industry, scintillators in radio-physics, etc…[5,6]. The trivalent cerium (Ce^{3+}) ions are the most commonly used REs for intense luminescence in the spectral range of 300–600 nm. These are most significant in designing oxide-based scintillators owing to their high fluorescence yield strength and short lifetime of the order of 10^{-8} – 10^{-9} s. The Ce^{3+} -doped scintillators find wide range of applications in the fields of bio-imaging, defense, communication and high-energy physics [7,8]. The emission of strong blue light from Ce^{3+} activated multi-component glass or glass-ceramics have been used to generate white light in phosphor based white light emitting diodes (w-LEDs) [9,10].

The tellurite-based glasses are of noteworthy due to their excellent non-linear optical properties with low phonon energy, relatively good thermal stability with chemical durability, low manufacturing temperature. These glasses are more advantages in enhancing the fluorescence efficiency by reducing multi-phonon relaxations rates [11,12]. The presence of TeO_4, TeO_{3+1}/TeO_3, WO_6, WO_4 and GeO_6 structural units which help in strengthening glass network, glass forming ability and hence thermal stability. The structural, physical and thermal properties [13] and the shielding properties [14] of TeO_2-WO_3-GeO_2 glasses have been studied. Certain RE^{3+} ions activated TeO_2-WO_3-GeO_2 glasses were fabricated and characterized for various visible and fiber laser sources [15–17]. This work reports on the structural and photoluminescence properties of TeO_2-WO_3-GeO_2-CeO_2 (TWGCe) glasses. The optimization of Ce^{3+} concentration and photometric properties were also discussed.

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2. Materials, method of preparation and instrumentation

The TWGCe*x* glasses of composition (85-*x*) TeO_2-5 WO_3-10 GeO_2-*x* Ce_2O_3 (*x* = 0, 0.1, 0.5 and 1.0 mol%) were prepared by melt-quenching method using TeO_2 (≥99.5 %), WO_3(≥99 %), GeO_2(99.999 %) and Ce_2O_3(99.9 %) as precursor materials. The stoichiometric amounts of precursor materials of 20 g batch each were thoroughly mixed in the presence of acetone and melted at 875 °C for 40 min using alumina crucible. The melt was air-quenched by pouring onto a preheated brass mould and annealed at 300 °C for 10 h and then cooled to room temperature producing bubble-free and transparent glasses (see Fig. 1**a**). All these glass samples were polished for optical quality with 0.18 cm thick and then used for other characterizations. The prepared glass samples were referred as TWGCe*x* glasses, where × stands for the concentration of Ce^{3+} ions in mol%.

The Archimedes principle was applied to obtain density measurements using double-distilled water as an immersion-fluid. The Abbes' refractometer illuminated with sodium vapour lamp (λ = 5893 Å) was used to find the refractive indices. The room temperature optical absorption spectra were recorded on Agilent Cary 60 UV–Visible spectrometer. The transmittance mode infrared absorption spectra were recorded with PerkinElmer FT-IR spectrometer following KBr technique. The scanning electron microscopy-energy dispersive X-ray spectrum (SEM-EDS) was recorded on VEGA3 TESCAN provided with BrukerEasy EDS equipment. The excitation and emission spectra were carried out with Jobin Yvon Fluorolog-3 spectrofluorimeter and the lifetime measurements were done using FLS 1000 photoluminescence spectrometer with light source in pulsed mode. All the optical measurements were performed at room temperature.

3. Results and discussion

3.1. Basic characteristic parameters

The phase of TWGCe*x* glasses has been examined by powder X-ray diffraction (XRD) studies. The XRD profiles shown in Fig. 1**b** reveal a broad-bump range from 18° to 35° and it has been assigned to the combined effect of multiple-scatterings at non-uniformly distributed atoms leading to exhibit amorphous phase of studied glasses. Some of the significant basic parameters of TWGCe*x* glasses are summarized in Table 1. The values of refractive index (n) and density (d) increase with increase of Ce^{3+} concentration and their variation are illustrated in Fig. 2. The decrease in molar volume (V_m) with Ce^{3+} concentration leads compactness of elements. The reflection loss is found to be ~ 18 % for all the samples indicating negligible loss of incident light intensity or the studied glasses allow most of the incident light to absorb or transmit through them. The ratio of molar refraction (R_m) to molar volume (V_m) has been used to predict metallic or non-metallic behaviour of glass samples. (R_m/V_m) > 1 for metallic nature of solids while (R_m/V_m) < 1 for non-metallic nature of solids [18]. In the present investigation, the value of (R_m/V_m) is found to be ~ 0.63 for all TWGCe*x* glasses indicating their non-metallic nature. The increased concentration of Ce^{3+} ions (C) cause a decrease in inter-ionic distance (r_i) resulting to energy transfer among the excited Ce^{3+} ions at higher concentrations.

In order to evaluate the optical band gap energy of prepared TWGCe*x* glass samples, the room temperature optical absorption spectra were measured and presented in Fig. 1**c**. The optical band gap energy is one of

Table 1
Comparison of basic parameters of TWGCe*x* glasses.

Parameter	TWGCe0	TWGCe0.1	TWGCe0.5	TWGCe1.0
Optical path length, *l* ± 0.01 cm	0.18	0.18	0.18	0.18
Density, d ± 0.001 g/cm^3	4.454	5.067	5.086	5.102
Refractive index, n ± 0.001	2.448	2.453	2.458	2.462
Reflection losses, R_L ± 0.02 %	~18	~18	~18	~18
Molar refraction, R_m ± 0.01 cm^3	22.12	19.48	19.45	19.42
Molar volume, V_m ± 0.01 cm^3	35.41	31.13	31.02	30.94
(R_m / V_m) ± 0.02	~0.63	~0.63	~0.63	~0.63
Concentration, C ± 0.02 × 10^{20} ions/cm^3	–	1.10	5.53	11.03
Inter-ionic distance, r_i ± 0.01 Å	–	20.84	12.18	9.68
Indirect band gap energy, E_g ± 0.02 eV	2.56	2.40	2.24	2.16
Direct band gap energy, E_g ± 0.02 eV	2.84	2.74	2.54	2.42

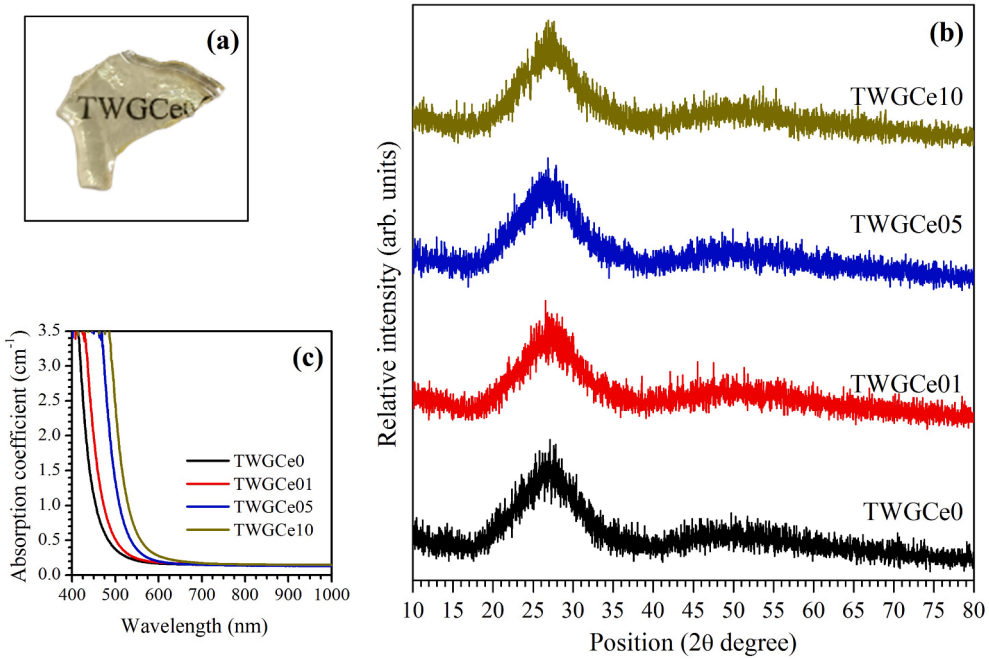

Fig. 1. Image of TWGCe05 glass (a), XRD profiles(b) and absorption spectra (c) of TWGCe*x* glasses.

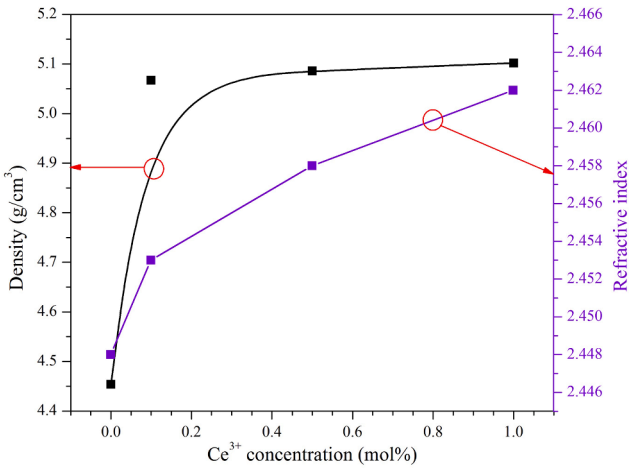

Fig. 2. Variation of density and refractive index of TWGCe*x* glasses as a function of Ce^{3+} concentration.

the significant properties to analyze the optical transitions of active ions doped in a glassy matrix and it can be determined using UV–VISabsorption spectra (see Fig. 1**c**). The absorption coefficient (α = Absorbance /optical path length) near the absorption edge and the photon energy (hν)of incident radiation are related as shown below [19].

$$\alpha\,.\,(h\nu) \;=\; B\,(h\nu - E_{opt})^{q} \qquad (1)$$

where B is a constant and it is independent of photon energy. The integer q represents the type of transition (direct/indirect) and it has 1/2, 1/3, 2 and 3 for direct allowed, direct forbidden, indirect allowed and indirect forbidden transitions, respectively. This condition is applicable under negligible reflectance. In case of glassy material, the reflectance can be minimized by polishing them to optically high quality [20–24]. The value of indirect and direct band gap energies has been obtained by extrapolating the linear region of Tauc's plots to meet the hν axis as shown in Fig. 3. In the present investigation, both indirect and direct band gap energies found decrease with increase of Ce^{3+} concentration (see Table 1). The addition of low concentrations of dopant ions decreases the energy band gap and it could be due to small separation distance between dopant ions [25].

3.2. FTIR and SEM analysis

The FTIR spectra help us to know various structural vibrations present in the glass host. The FTIR spectra of TWGCe0 and TWGCe05 glasses shown in Fig. 4 are similar and they are identical to other tellurite glass [26]. The IR bands at ~ 3437, ~2361 and ~ 1713 cm^{-1} belongs to the symmetric stretching vibrations of HO^- group [5,27], the IR band noticed at ~ 871 cm^{-1} is related to asymmetric stretching vibrations of Ge-O-Ge and W-O-W units [26]. The bands at ~ 721 and 642 cm^{-1} have been ascribed to Te-O stretching vibrations in TeO_4 and TeO_3 units respectively [28]. The presence of OH^- content influence the luminescence properties of RE active ions in a glassy matrix [29] and it can be evaluated using the equation,$\alpha_{OH} = \ln(T_0/T_D)/l$, where T_o is the maximum transmittance, T_D is transmittance corresponding to 3000 $cm^{-1}$by assuming the reflectance as zero and *l* is the optical path length.

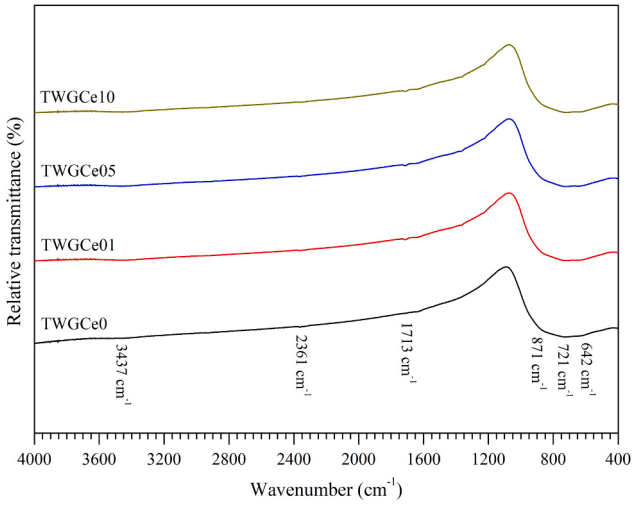

Fig. 4. FTIR spectra of TWGCe*x* glasses.

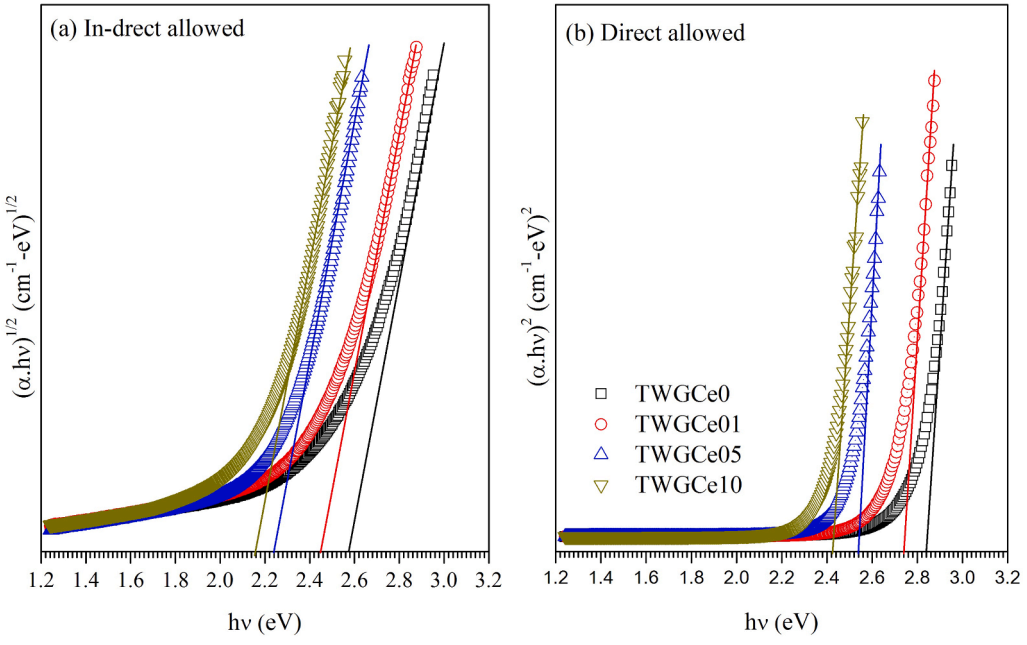

Fig. 3. Tauc's plots for indirect (a) and direct (b) allowed transition in TWGCe*x* glasses.

Lower the OH^- content lesser will be the non-radiative losses. The value of α_{OH} is found to be 0.51 and 0.48 ± 0.02 cm^{-1} for TWGCe0 and TWGCe05 glasses, respectively. The addition of Ce_2O_3 content reduces the α_{OH} value resulting to enhance the luminescence properties.

The SEM micrograph with EDS spectrum of TWGCe05 glass is shown in Fig. 5. As can see the SEM micrograph, one can notice that the prepared glass samples have good amorphous phase and they are free from air-bubbles, clusters and defects. The EDS spectrum shows the presence of all elements distributed uniformly indicating the proposed chemical composition. The atomic weight percentages of different elements present in the TWGCe05 glass are 61.77 at%, 4.52 at%, 31.53 at%, 1.69 at% and 0.49 at% for oxygen (O), germanium (Ge), tellurium (Te), tungsten (W) and cerium (Ce) and the corresponding mole percentages are 23.98 mol%, 23.60 mol%, 30.81 mol%, 16.87 mol% and 4.74 mol%.

3.3. Luminescence properties

The excitation and emission spectra of TWGCe*x* glasses are shown in Fig. 6. The excitation spectra monitoring the emission at 408 nm consists of a broad band extended from 243 nm to 360 nm and has been assigned to Ce^{3+}: $4f \rightarrow 5d$ transition [see Fig. 6**(a)**]. This broad band appears having three sub-peaks with maximum at ~ 255, ~274 and ~ 323 nm. The emission spectra recorded at 323 nm excitation consists of a broad and intense band due to Ce^{3+}: $5d \rightarrow 4f$ (408 nm) transitions. The intensity of excitation band increases with increase of Ce^{3+} concentration, while the intensity of emission bands increases with increase of Ce^{3+} concentration reach to maximum at × = 0.5 mol% and then decrease for further rise of concentration showing luminescencequenching phenomenon beyond × = 0.5 mol% due to energy transfer (ET) among the excited Ce^{3+} ions at higher concentrations. The emission profiles of TWGCe glasses are similar to those reported for gadolinium oxyfluoroborate [5] and P_2O_5-Li_2CO_3-$GdBr_3$-Al_2O_3 [30] glasses.

As can see the normalized emission spectra shown as inset of Fig. 6 **(b)**, one can notice a red-shift in emitted luminescence with increase of Ce^{3+} concentration. The peak maximum of Ce^{3+}: $5d \rightarrow 4f$ transition shifts from 403 nm for × = 0.1 mol% to 408 nm for × = 0.5 mol% and finally to 410 nm for × = 1.0 mol%. The observed red-shift has been ascribed to the site distribution of Ce^{3+} ions in the ligand fields [31]. It is well known that when the energy of incident light photons is equal or greater than band gap energy of the material then the photons are absorbed by the material and excites the active ions to higher energy levels. When excited at 323 nm wavelength, the ground state Ce^{3+}: $4f$ ions get excited to Ce^{3+}: $5d$ emission state resulting to exhibit intense luminescence through Ce^{3+}: $5d \rightarrow 4f$ transition. The emission mechanism of Ce^{3+} ions in TWGCe*x* glasses at 323 nm excitation is illustrated in Fig. 7**(a)**.

The Commission International de I'Eclairage (CIE-1931) chromaticity coordinates evaluated from the emission spectral profiles have been used to know the colour purity (CP) and correlated colour temperature (CCT) of emitted luminescence. For TWGCe*x* glasses excited at 323 nm, the CIE chromaticity coordinates are obtained as (x = 0.161, y = 0.018).The CP and CCT values have been determined using the equations shown below.

$$CP = \frac{\sqrt{(x - x_e)^2 + (y - y_e^2)}}{\sqrt{(x_d - x_e)^2 + (y_d - y_e^2)}} \times 100 \quad (2)$$

$$CCT = -449\,z^3 + 3525\,z^2 - 6823.3\,z + 5520.33 \quad (3)$$

where (x_e, y_e) represents the coordinates of epicenter point and (x_d, y_d) represents the coordinates for dominant-wavelength-point. Generally, the typical coordinates for equal-energy point are (0.333, 0.333). The constant z can be obtained using the formula, $z = (x - x_e)/(y - y_e)$. The evaluated CIE coordinates are located in the intense blue region of CIE diagram shown in Fig. 7**(b)**. The colour purity is estimated to be 98 % and the corresponding CCT rating is 1802 K. It is well known that the light sources with CCT < 3000 K emit warm light useful for living room illumination. It is known that the generation of white light from phosphor-based LEDs requires blue light excitation. The observed results show that the studied glasses emits intense and warm blue light suitable for decorative lighting sources as well as replacement for blue light source in phosphor-based w-LEDs.

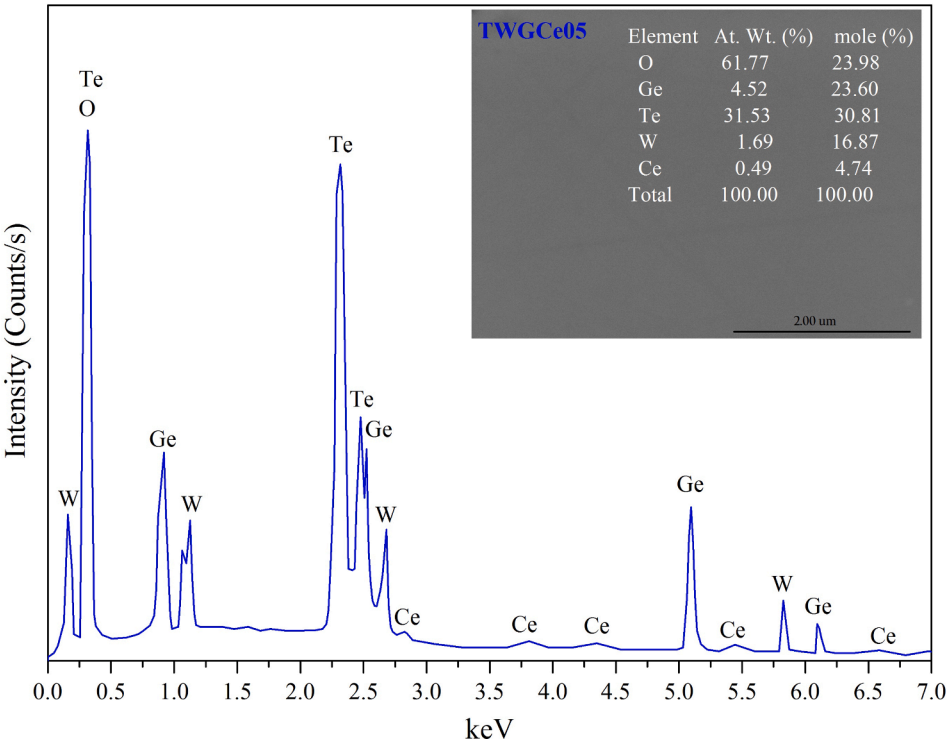

Fig. 5. EDS profile of TWGCe05 glass. Insets show the SEM micrograph and elemental analysis.

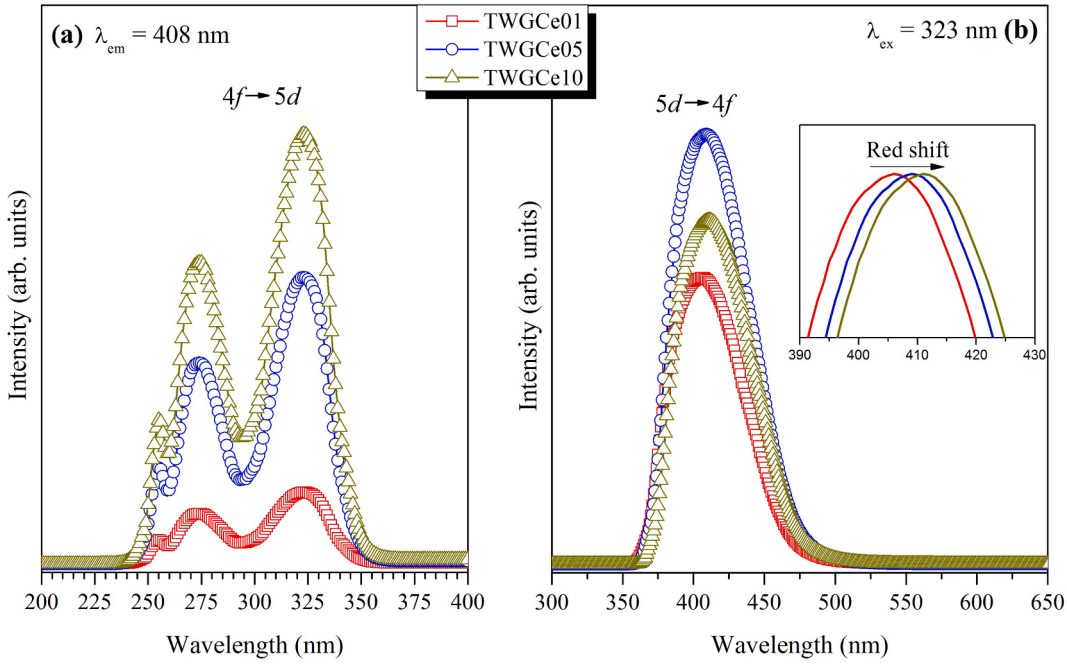

Fig. 6. Excitation (a) and emission (b) spectra of TWGCe*x* glasses. Inset shows a red-shift in emission spectra at 323 nm excitation.

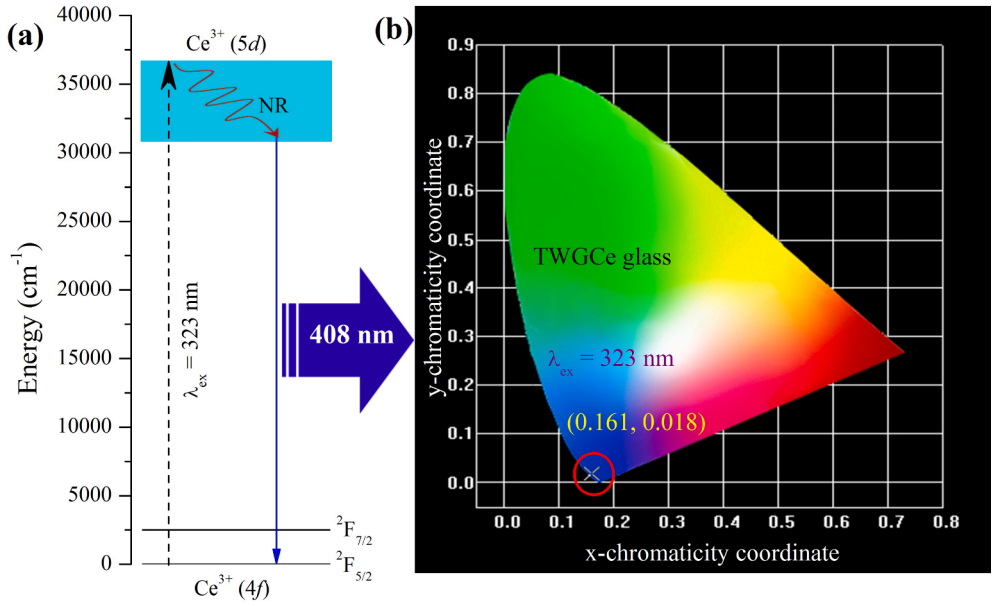

Fig. 7. Emission mechanism of Ce^{3+} ions at 323 nm excitations (a) and the chromaticity diagram of TWGCe*x* glasses.

The process of ET among the excited active ions occurs either due to exchange interaction or multi-polar interaction process depending upon the critical transfer distance but not on inter-ionic distance. According to Dexter's theory of sensitized luminescence in solids [32], the exchange interaction type of ET is possible for $R_c \leq 5$ Å and beyond this limit ($R_c >$ 5 Å) the multi-polar interaction come into play for the transfer of energy among the excited ions. The inter-ionic distance (r_i) is the average distance among the sites of Ce^{3+} ions in TWGCe glass host matrix, while the critical transfer distance (R_c) represents the mean distance among the Ce^{3+} ions for which threshold transfer of energy occurs at suitable excitation. The value of R_c responsible for ET mechanism has been estimated using the following formula [33].

$$R_c = \left(\frac{M}{N_A . d . C}\right)^{1/3} \quad (4)$$

where M is the molar mass, N_A is the Avagadro's number, d is the density and C is the concentration of active ions (in g/mol). The value of R_c is found to be 5.56, 3.82 and 3.03 Å for × = 0.1, 0.5 and 1.0 mol%, respectively. These values suggest that the exchange interaction mechanism play a key role in ET for TWGCe*x* glasses.

3.4. Luminescence decay

The luminescence decay curves of Ce^{3+} in TWGCe*x* glasses monitoring the emission and excitation wavelengths at 408 nm and 323 nm

respectively are illustrated in Fig. 8. These decay curves are well fitted to a double exponential function, $I(t) = A_1 . e^{-t/\tau_1} + A_2 . e^{-t/\tau_2}$, where I(t) represent the emission intensity as a function of time t, A_1 & A_2 are constants and τ_1 & τ_2 are the short and long decay times, respectively. The average decay time $\langle\tau\rangle$ has been estimated using well known equation, $\langle\tau\rangle = \frac{A_1 . \tau_1^2 + A_2 . \tau_2^2}{A_1 . \tau_1 + A_2 . \tau_2}$. In the present case, the values of $\langle\tau\rangle$ are determined as 23.92, 22.15 and 19.32 ns for TWGCe0.1, TWGCe0.5 and TWGCe1.0 glasses, respectively. The gradual decrease in decay time with the addition of Ce^{3+} ions in TWG glass host is an indication of energy transfer among the excited Ce^{3+} ions at higher concentrations.

4. Conclusions

The TWGCe*x* glasses have been prepared with perfect amorphous phase by conventional melt-quench method are free from bubbles and impurities. The ratio of molar refraction to molar volume is found lesser than unity [$(R_m/V_m) > 1$] showing non-metallic nature of TWGCe*x* glasses. The OH^- content of the order 0.51 – 0.48 cm^{-1} is favourable to improve the luminescence properties. The SEM micrograph confirms the absence of impurities and bubbles and the EDS results show the presence of all the elements distributed uniformly. These glasses exhibit deep-blue luminescence through Ce^{3+}: $5d \rightarrow 4f$ transition when excited at 323 nm wavelength. The observed concentration quenching has been ascribed to the exchange interaction among the excited Ce^{3+} ions at higher concentrations. For all TWGCe*x* glasses, the lifetime is found to be 1.02 ns. The colour perception and CCT rating values are found to be 99 % and 1802 K, respectively are more favourable for decorative lighting sources. The intense and fast luminescence signal is more suitable to be used as a scintillator material. The observed results suggest that the TWGCe05 glass is more favourable to design decorative blue light sources for living rooms and a suitable blue light source to design w-LEDs.

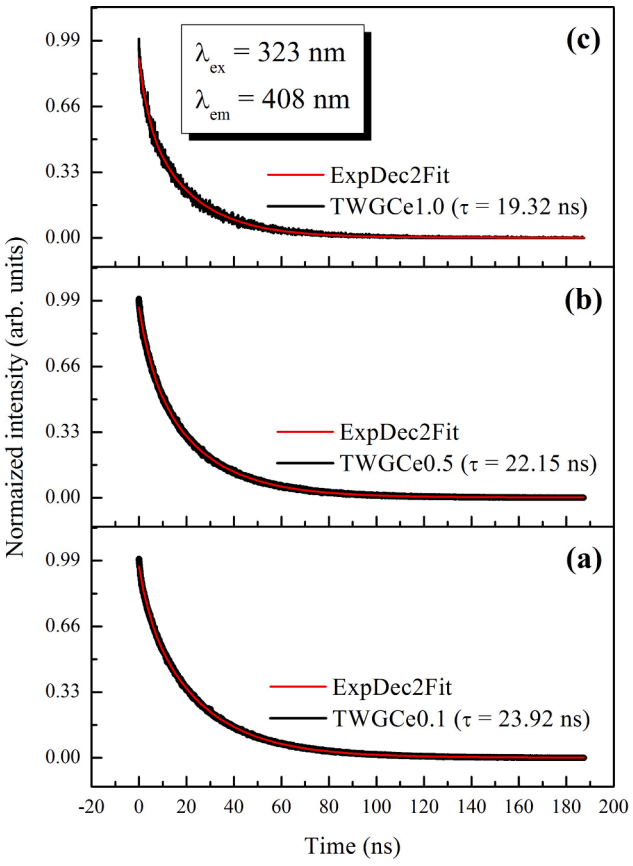

Fig. 8. Luminescence decay profiles of TWGCe*x* glasses.

CRediT authorship contribution statement

G. Pullaiah: Methodology, Writing – original draft. **K. Venkata Rao:** Software, Investigation, Writing – review & editing, Supervision. **B.C. Jamalaiah:** Conceptualization, Software, Investigation, Writing – review & editing. **N. Madhu:** Software, Validation, Writing – review & editing. **Venkatramaiah Nutalapati:** Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on reasonable request.

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Structural and optical analysis of $YAl_3(BO_3)_4$: Pr^{3+} phosphors for lighting applications

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ABSTRACT

A series of Pr^{3+} activated $YAl_3(BO_3)_4$ phosphors were fabricated by solid-state reaction technique and they were characterized via phase, morphology and photoluminescence studies. Powder X-ray diffraction peaks were suitably matched to JCPDS No. 72–1978. The presence of various vibrational bonds was identified using Fourier transform infrared spectroscopy. High resolution scanning electron microscopic investigations show that the phosphor particles were distributed uniformly and presented a rod-like shape. The photoluminescence results show that the studied phosphors were easily excited by visible wavelength to produce intense-yellow to pale-yellow tunable emission. The decrease in luminescence intensity and lifetime was assigned to dipole-dipole interaction energy-transfer among excited Pr^{3+} ions. Observed results show that the 0.05 mol% of Pr^{3+} activated phosphor produce intense-yellow luminescence at 480 nm excitation.

1. Introduction

Currently, there is a need for new, novel, low power consumption, low pollution and high efficiency materials in the fields of science & engineering, agriculture, medicine, defense, industry and communication [1–3]. Compared to other lighting sources, the light-emitting-diode (LED) based devices are more favorable owing to their high brightness, long lifetime, less energy consumption, low heat production [4]. The phosphor converted LEDs have been the most suitable light sources in various fields owing to their reliability, long-life, low energy consumption, high fluorescence efficiency and environmental friendly behavior as they are free from toxic elements like Cd, S and Cl [5]. The superior qualities of pc-LEDs initiate many researchers to design and develop new red or yellow emitting materials for white-LEDs. Recently, the oxide-based phosphors have been the subject for many applications such as white light generation for screens in PDPs and FEDs, for green emission in horticulture due to their great resistance to moisture, excellent thermal and chemical stability compared to other phosphors like silicates, nitrides and so on[6–9].

Trivalent rare-earth (RE) ions doped $YAl_3(BO_3)_4$ phosphors have been the potential candidates for spectacular optoelectronic applications owing to their extensive iso-morphous substitutions, UV transparency, thermal constancy, chemical strength and non-

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linear optical properties [10,11]. In $YAl_3(BO_3)_4$ structure, the O^{2-} ions forms trigonal prisms with Y^{3+} ions at the center and they are separated from each other by $(BO_3)^{3-}$ groups [12]. Thus, the structure of $YAl_3(BO_3)_4$ lattice facilitates to activate relatively huge concentrations of RE^{3+} active ions without considerable agglomeration as they do not require any charge compensation. It is well known that the Pr^{3+} ions show complex emission spectral profiles compared to other RE ions. Since the emission transitions of Pr^{3+} ions are sensitive to the host environment around it, they exhibit blue (~480 nm), green (~540 nm), yellow (~580 nm), red (~600–650 nm), near UV (~800–1500 nm) luminescence through $^1D_2$, $^3P_1$,$^3P_0$ and $^1G_4$ excited energy states to their lower lying energy states [13,14]. The presence of prominent emission levels responsible for outstanding emission transitions in near-UV, visible and near-IR regions facilitate the trivalent praseodymium (Pr^{3+}) ions to used in the design of various optoelectronic devices. Based on the diverse applications, the Dy^{3+}, Tm^{3+}, Dy^{3+}/Tm^{3+}, Dy^{3+}/Ce^{3+}, Sm^{3+}/Tb^{3+}, Eu^{3+}/Tb^{3+} and Ho^{3+}/Tm^{3+} ions activated $YAl_3(BO_3)_4$ phosphors have been prepared and analyzed for various photonic applications [15–21]. The characteristic features of $YAl_3(BO_3)_4$ phosphor matrix initiated to design and investigate the optical properties of Pr^{3+} ions to meet the current technological applications.

The $Ca_9Y(PO_4)_7$:Ln^{3+} (Ln = Ce, Gd, Pr) phosphors for high luminescence efficiency and thermal-stability [22], the O^{2-}-Pr^{2+} charge transfer and luminescence properties of Pr^{3+} ions in YF_3–Y_2O_3 system [23], the YOF:Pr nanoparticles for temperature sensing applications [24] and the Y_2MoO_6: $x$$Pr^{3+}$ phosphors for white LED applications [25] were reported. Further, the influence of Pr^{3+} concentration on the luminescence of $BaY_{1.94}Al_4SiO_{12}$:0.06Ce^{3+} phosphor prepared by solid state method has been reported for white LEDs [26]. The present research work explores the fabrication, structural, and optical characterization, optimization of Pr^{3+} concentration for efficient luminescence, color purity of emitted luminescence and the decay analysis of Pr^{3+}:$^1D_2$ emission state. The energy transfer (ET) phenomenon among excited Pr^{3+} ions also discussed.

2. Experimental procedure

2.1. Materials, method of preparation

High purity and analytical grade Y_2O_3 (99.99 % purity), Al_2O_3 (99.9 % purity), H_3BO_3 (≥99.5 % purity), and Pr_2O_3 (99.9 % purity) were used directly for the synthesis of YABPrx phosphors of composition $Y_{(1-x)}Al_3(BO_3)_4$: $x$$Pr^{3+}$, where x = 0,0.01, 0.02, 0.05,0.1,0.5,1.0 and 2.0 mol% via solid-state reaction technique. A batch composition of about 5.0 g homogeneous-powders (in stoichiometric-amounts) with an additional-amount of 3.0 wt% of H_3BO_3 (to avoid its evaporation at higher temperatures) were prepared and heat treated at 200 °C and 600 °C temperatures for 3 h following intermediate grinding. These grinded powders were sintered at 1200 °C for 3 h and cooled to room temperature to produce YABPrx phosphors.

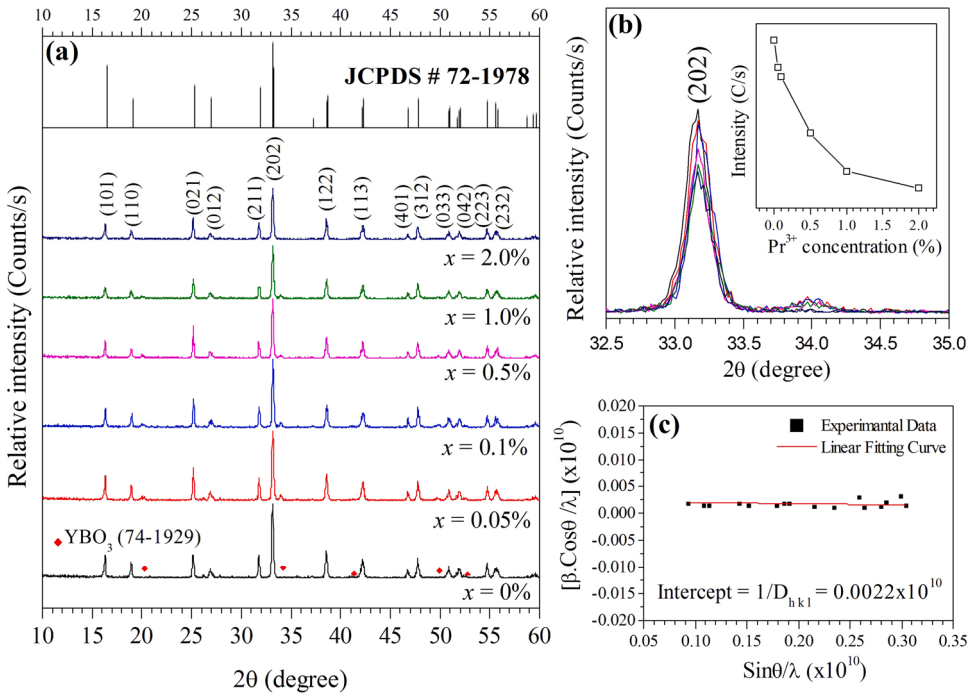

Fig. 1. PXRD patterns of YABPrx phosphors (a), magnified image of PXRD peak corresponding (202) Miller plane along with intensity vs. Pr^{3+} concentration (b) and the H-W plot for YABPr1.0 phosphor.

2.2. Recording of various characteristics

Powder X-ray diffraction (PXRD) measurements were carried out with X′ Pert Pro Materials Research Diffractometer provided with Cu-Kα radiation of wavelength 1.5406 Å. The Fourier transform infrared spectral analysis was carried out by KBr technique with a PerkinElmer FT-IR spectrometer. The morphology of studied samples was examined with FEI-Quanta-FEG-200 F high-resolution scanning electron microscope (HR-SEM). The photoluminescence excitation, emission and decay analysis were carried out with FLS-1000 photoluminescence spectrometer containing xenon flash lamp in pulsed mode as a light source.

3. Results and discussion

3.1. Structure and morphology

The PXRD profiles of YABPr*x* ($0 \leq x \leq 2.0$ mol%) phosphors are illustrated in Fig. 1(a). These patterns are suitably fitted to JCPDS card No. 72–1978 belonging to $CaMg_3(CO_3)_4$ mineral huntite structure [27]. A two-dimensional crystal structure of $YAl_3(BO_3)_4$ lattice is shown in Fig. 2. As can see the PXRD profiles, one can notice a negligible YBO_3 phase and it has fever effect on the optical properties of RE ions and it has been ascribed to loss of boron at higher temperatures and slow reaction of Al_2O_3 with Y_2O_3 and H_3BO_3 [28]. A monotonous decrease in intensity of PXRD patterns with the addition of concentration of Pr^{3+} ions is observed. The magnified image of a PXRD peak corresponding to (202) Miller plane in the 2θ ranges from 32.5 to 35°and the variation of its intensity versus Pr^{3+} ions concentration is described as inset of Fig. 1(b). The close ionic radii of Y^{3+} (0.090 nm) and Pr^{3+} (0.1013 nm) may lead suitable replacement of Pr^{3+} ions in place of Y^{3+} sites. The mean size of crystallite has been determined as ~46 nm using to Debye-Scherrer method [29] and it is very close to the Hall-Williamson's (H-W) method (~45 nm) [30]. The H-W plot (β.cosθ/λ vs. sinθ/λ) for YABPr1.0 phosphor is presented in Fig. 1(c). The small deviation in ionic radii of Y^{3+} and Pr^{3+} may cause negligible lattice distortions resulting micro strains of the order 0.0074 which is found smaller than that of $SrEuMgAl_{10}O_{17}$ (~0.00751 nm) phosphor [31].

The FT-IR spectral profiles of YABPr*x* ($x = 0$ and 0.5 mol%) phosphors shown in Fig. 3 exhibit fourteen IR absorption bands showing the presence of various vibrations responsible for optical properties of RE active ions. A broad-band with a peak utmost at ~3030 cm^{-1} and a group of bands at ~2354, ~2322 and ~2247 cm^{-1} belongs to O–H stretching vibration and moisture in the sample, while the band at ~1355 cm^{-1} is related to phonon frequency of YAB phosphor [32,33]. The IR bands observed at ~1414, ~1252 and ~500 cm^{-1} belongs to B-O asymmetric stretching and asymmetric bending vibrations in orthoborate group, O-B-O bending vibrations, respectively, while the band at ~707 cm^{-1} belongs to both B-O-B bending and Al-O bending vibrations [34,35]. The IR bands positioned at ~1064, 918 and 869 cm^{-1}have been related to the YBO_3 phase [28,36] and the IR band at ~776 cm^{-1} has been ascribed to Al-O stretching vibrations [37]. The IR bands noticed at ~613, 542 and 461 cm^{-1} have attributed to Y-O stretching vibrations [38].

The shape, size, orientation and distribution of particles have been known by studying the morphology. The HR-SEM micrograph of YABPr0 phosphor shown in Fig. 4 presented a rod-like shape with uniformly distributed particles. From this image one can notice that all the particles are oriented distinctly in a particular direction. This type of morphology helps in improving the optical properties of RE active ions [39]. The mean size of particles is calculated as ~105 nm with length varies from 500 to 1000 nm supporting the nano-crystalline phase of YABPr*x* phosphors.

3.2. Photoluminescence analysis

In order to gain access to higher energy states and hence to select appropriate excitation wavelength producing efficient emission, the photoluminescence excitation (PLE) spectrum of YABPr*x* phosphors have been recorded controlling the emission at about 598 nm corresponding to $Pr^{3+}: {}^1D_2 \rightarrow {}^3H_4$ transition. These spectra reveal three excitation bands at around 448, 470 and 480 nm

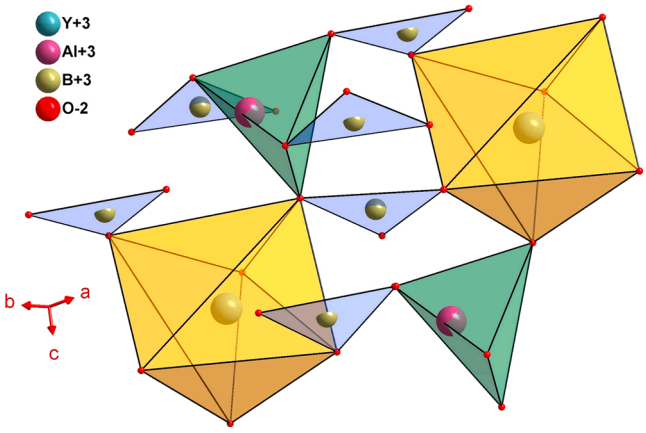

Fig. 2. Representation of two-dimensional YAB lattice.

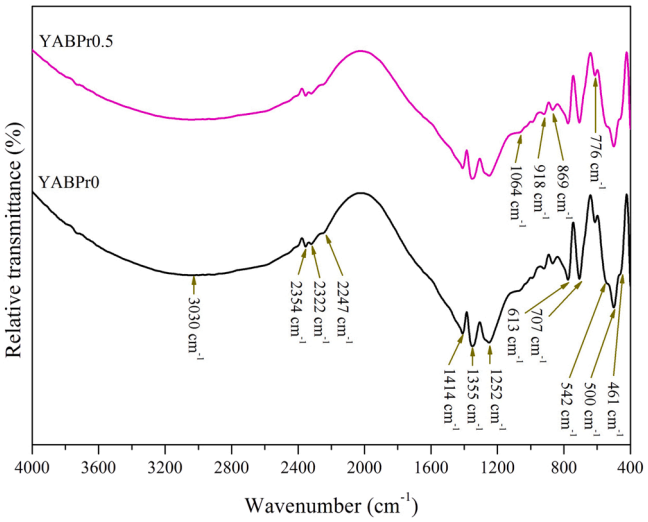

Fig. 3. FT-IR spectra of YABPr*x* ($x = 0$ and 0.5 mol%) phosphors.

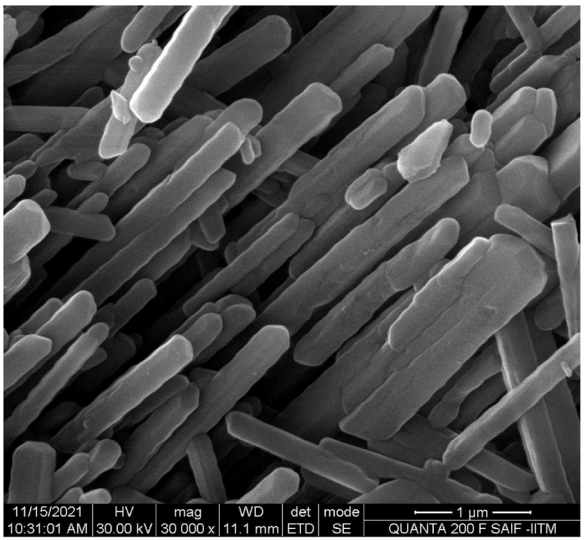

Fig. 4. HR-SEM micrograph of YABPr0 phosphor.

corresponding to $Pr^{3+}: {}^3H_4 \rightarrow {}^3P_{2,1,0}$transitions, respectively. The observed spectral profiles are identical to those reported for Li_2SrSiO_4: Ce^{3+}, Pr^{3+} Phosphors [40]. The excitation spectra of YABPr*x* phosphors with the assignment of PLE bands noticed are depicted in Fig. 5. The intensity of $Pr^{3+}: {}^3H_4 \rightarrow {}^3P_J$ ($J = 0,1,2$) transitions increased with enhancement of concentration of Pr^{3+} ions. The inset of Fig. 5 shows the variation of strength of $Pr^{3+}: {}^3H_4 \rightarrow {}^3P_J$ transitions as a function of Pr^{3+} concentration. Since the PLE band with peak maximum at ~480 nm ($Pr^{3+}: {}^3H_4 \rightarrow {}^3P_0$) is broad and intense, this wavelength can be used to excite YABPr*x* phosphors more efficiently compared to other two wavelengths.

Upon 480 nm excitation, the emission spectra of YABPr*x* phosphors in the spectral region from 500 to 725 nm illustrated in Fig. 6 show different transitions originated from ${}^3P_0$ and ${}^1D_2$ emission states. The first group of emission bands (523, 528, 534, 538 and 542 nm) with peak maximum at 528 nm has been attributed to ${}^3P_0 \rightarrow {}^3H_4$transition, the second group of bands (554, 558 and 563 nm) with maximum at 563 nm has been attributed to ${}^3P_0 \rightarrow {}^3H_5$ transition, the third group of bands (594, 598, 603 and 611 nm) with maximum at 598 nm has been assigned to ${}^1D_2 \rightarrow {}^3H_4$ transition, the fourth group of bands (625, 630 and 633 nm) with maximum at 625 nm has been allocated to ${}^3P_0 \rightarrow {}^3H_6$ transition, the fifth group of bands (637, 647 and 656 nm) with maximum at 637 nm has been allotted to ${}^3P_0 \rightarrow {}^3F_2$ transition, the sixth group of bands (663, 670, 676 and 684 nm) with maximum at 670 nm has been ascribed to ${}^1D_2 \rightarrow {}^3H_5$ transition, and the seventh group of bands (696 and 706 nm) with maximum at 696 nm has been assigned to ${}^3P_0 \rightarrow {}^3F_3$ transition [41].

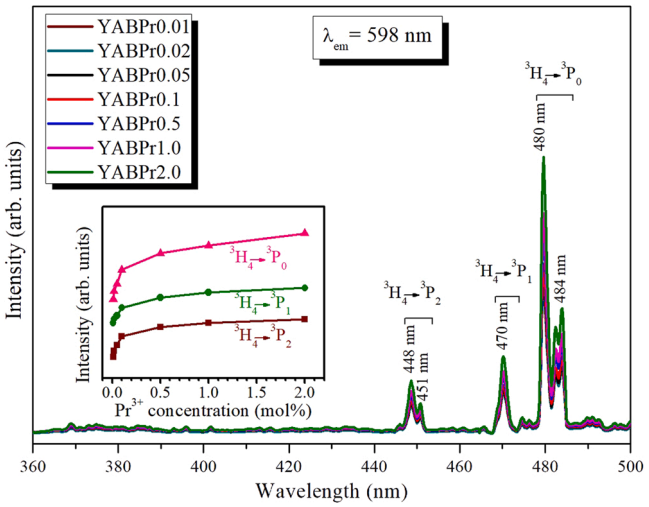

Fig. 5. PLE spectra of YABPr*x* phosphors (λ_{em} = 598 nm). Inset shows the variation of intensity of Pr^{3+} : $^3H_4 \rightarrow {}^3P_{2,1,0}$transitions as a function of Pr^{3+} concentration.

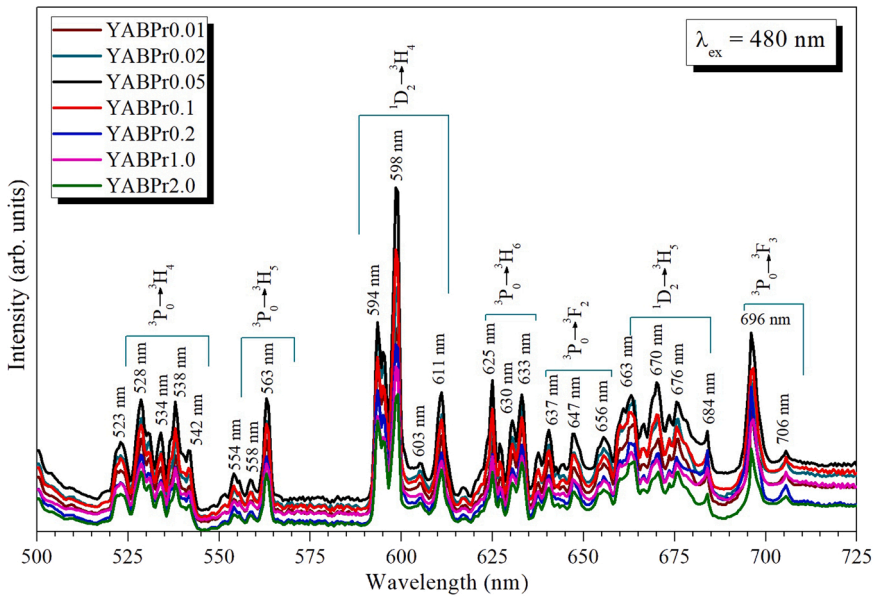

Fig. 6. Emission spectra of YABPr*x* phosphors at 480 nm excitation.

From the emission spectra, it is found that the strength of observed transitions increases reaches to maximum at $x = 0.05$ mol% and then decrease monotonically with the enhancement of Pr^{3+} concentration signifying efficient energy transfer (ET) among the exited Pr^{3+} ions at 480 nm excitation. It is due to the reason that at lowest concentrations the absence of energy transfer among the Pr^{3+} active ions initiates feeble interactions resulting to enhance the intensity of emitted luminescence. This process continues up to a limiting concentration called the optimum concentration ($x = 0.05$ mol%). As the concentration increases beyond the optimum, the energy transfer among the Pr^{3+} ions increase slowly due to increased interactions among Pr^{3+} ions enhancing non-radiative transitions resulting a decrease in luminescence intensity with increase of Pr^{3+} concentration. This process of variation in intensity with the concentration of active ions is known as luminescence quenching. To understand the luminescence quenching process, the magnified emission spectra in the spectral region from 590 nm to 604 nm and the variation of intensity of Pr^{3+} : $^1D_2 \rightarrow {}^3H_4$ transition versus Pr^{3+} concentration are described in Fig. 7(a) and Fig. 7(b), respectively.

Upon 480 nm excitation, the incident light photons interact with the ground state Pr^{3+} ions and excite them to Pr^{3+}: $^3P_0$ emission state resulting to produce radiative transitions through $^3P_0 \rightarrow {}^3H_{4,5,6}$ and $^3P_0 \rightarrow {}^3F_{2,3}$transitions along with non-radiative (NR) transitions through $^3P_0 \rightarrow {}^1D_2$transition. The population of $^1D_2$ emission state increases enormously due to its long lifetime compared to

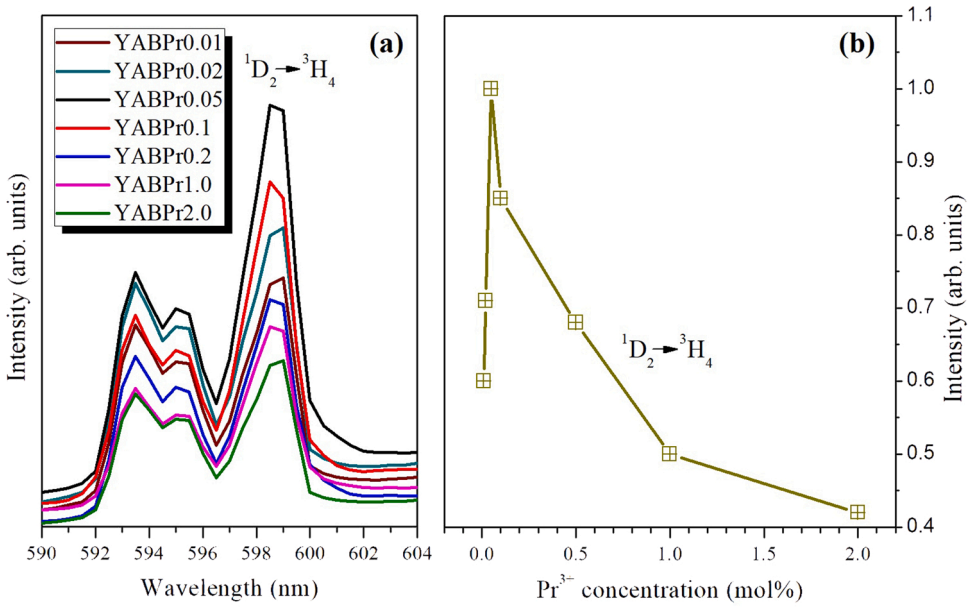

Fig. 7. Magnified image of $^1D_2 \rightarrow {}^3H_4$ transition of YABPr*x* phosphors at $\lambda_{ex} = 480$ nm (a) and its intensity variation as a function of Pr^{3+} concentration (b).

$^3P_0$ state. It is well known that the intensity of emitted luminescence due to $\Psi J \rightarrow \Psi^l J^l$ transition varies proportionally to the population density of higher energy state (ΨJ). The higher population of $^1D_2$ energy state results a maximum intensity for 598 nm ($^1D_2 \rightarrow {}^3H_4$) luminescence. The emission mechanism of Pr^{3+} ions in YABPr*x* phosphors at 480 nm excitation has been described in a partial energy level diagram shown in Fig. 8.

3.3. Color-purity and color-temperature

The analysis of *colour purity*(CP) and *correlated colour temperature* (CCT) of emitted-light has been done by evaluating *Commision International de l'Eclairage* ($CIE - 1931$)color coordinates(x, y). The Pr^{3+} ions exhibit either red or yellow or green luminescence depending upon the host environment in which they are located. The YVO_4: Pr^{3+} [42] and $CaLaB_7O_{13}$: Pr^{3+} [43] emit red luminescence, the $LaMgAl_{11}O_{19}$: Pr^{3+} [44] emit yellow luminescence and the Pr^{3+}: NGW [8] emits green luminescence. The evaluated CIE coordinates show a tunable luminescence from intense-yellow to pale yellow as illustrated in CIE diagram shown in Fig. 9. The YABPr0.05 phosphor with coordinates (x = 0.492, y = 0.485) exhibits an intense yellow luminescence similar to that of $LaMgAl_{11}O_{19}$: Pr^{3+}(0.4497, 0.5306) [44] and Ba_2GdNbO_6: Sm^{3+} (0.496, 485) [45]. According to McCamy [46], the appearance of color of emitted luminescence has been expressed by CCT and the color sensitivity by CP. The values of CIE coordinates, CCT rating and CP for YABPr*x* phosphors are abridged in Table 1. The *CCT*rating of YABPr*x* phosphors is in the vicinity of warm-white light indicating safer for environmental and human activities.

3.4. Luminescence-decay dynamics

Generally, the lifetime (τ) of an emission state will remain constant in the presence of radiative transitions and it decreases due to non-radiative (NR) transitions which cause ET among excited active ions or active and sensitizer ions. The decay curves of Pr^{3+}: $^1D_2$ emission state of YABPr*x* phosphors at $\lambda_{em} = 598$ nm and $\lambda_{ex} = 480$ nm are shown in Fig. 10(a). All-these decay-profiles are fitted to one-exponential function, $I = I_0.e^{-t/\tau}$, where I_0 represents the intensity at $t = 0$ *s*. Since the deviation in lifetimes of YABPr0.01 (74.8 $\pm$ 0.2 μs) and YABPr0.02 (74.7 $\pm$ 0.2 μs) phosphors are negligible and found almost the same as that of YABPr0.05, the decay profiles of YABPr0.01 and YABPr0.02 phosphors are not depicted in Fig. 10(a). The lifetime values are estimated to be 74.6, 68.7, 47.1, 40.4 and 38.6($\pm$ 0.2 μs) for different values of *x*, respectively and they shrink with enhance of Pr^{3+} ions concentration. The decrease in strength of luminescence intensity and the value of τ is mainly due to ET amongst the excited Pr^{3+} ions at higher concentrations. Fig. 10 (b) demonstrates the variation of lifetime as a function of Pr^{3+} ions concentration.

The working performance of an optical material has been estimated by evaluating its quantum efficiency, $\eta = \int I(t).\, dt/\tau$, where I (t) is the integrated emission intensity and τ is the lifetime [47]. For optimized concentration (YABPr0.05), the value of quantum efficiency is estimated to be 38 %. Blasse [48] reported that either exchange or multi-polar interaction mechanisms such as dipole-dipole (d-d), dipole-quadrupole (d-q) and quadrupole-quadrupole (q-q) play a key role for ET among the activators and also activator and sensitizer ions. The exchange interaction mechanism type of ET is possible up to a critical transfer distance (R_c) of up to

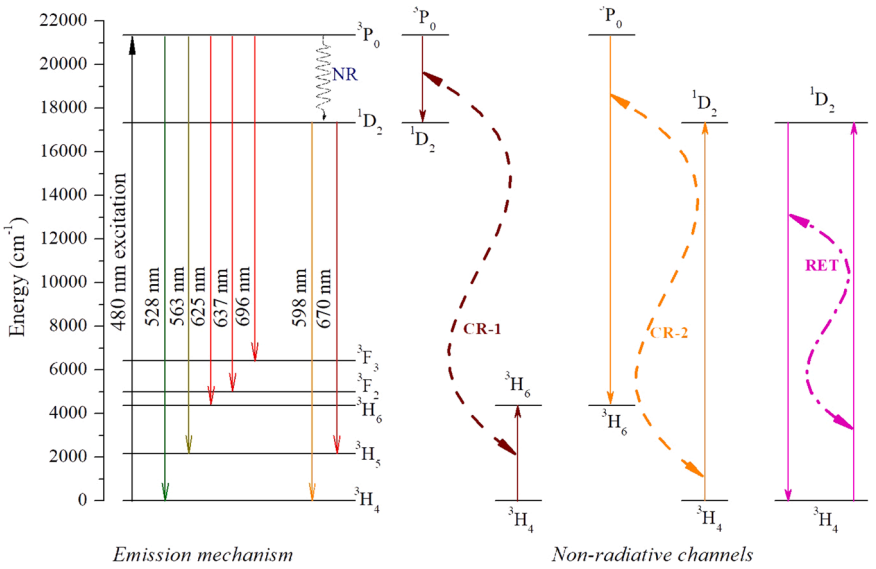

Fig. 8. Partial energy level diagram showing the emission mechanism of YABPr*x* phosphors at 480 nm excitation and the possible non-radiative channels responsible for energy transfer among the excited Pr^{3+} ions.

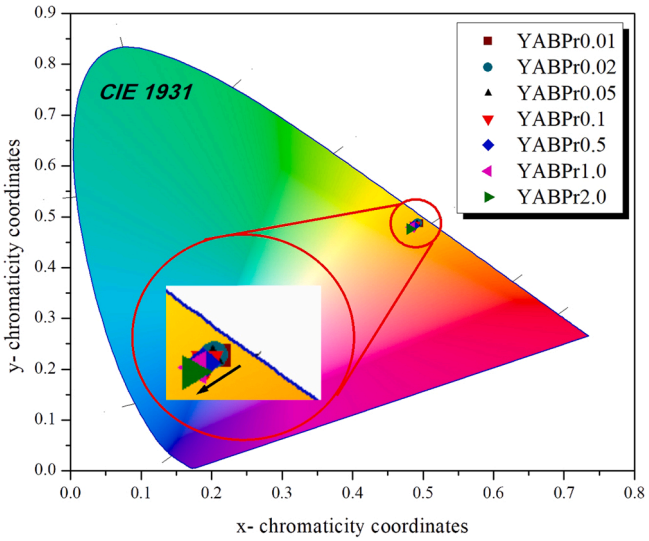

Fig. 9. CIE chromaticity diagram of YABPr*x* phosphors at 480 nm excitation.

Table 1
Comparison of CIE coordinates, CCT rating and CP values of YABPr*x* phosphors.

Doping, *x* (mol%)	CIE coordinates (x, y)	CCT rating (K)	CP (%)
$x = 0.01$	(0.495, 0.486)	2783	~93
$x = 0.02$	(0.493, 0.484)	2794	~93
$x = 0.05$	(0.492, 0.485)	2811	~93
$x = 0.1$	(0.489, 0.483)	2893	~93
$x = 0.5$	(0.487, 0.481)	2844	~92
$x = 1.0$	(0.484, 0.478)	2861	~91
$x = 2.0$	(0.482, 0.475)	2866	~90

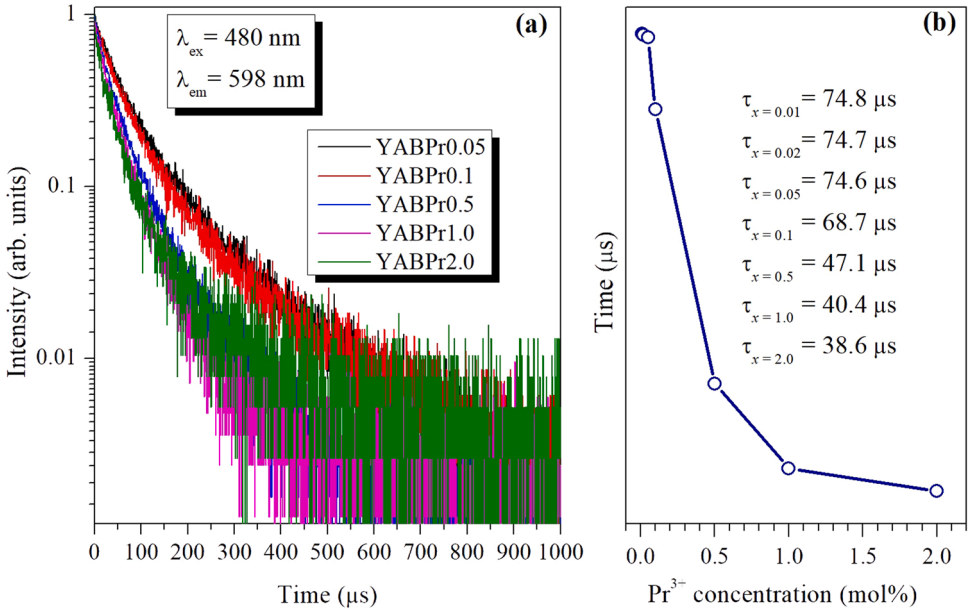

Fig. 10. Decay curves of Pr^{3+}: $^1D_2$ emission state in YABPr*x* phosphors (a) and the variation of lifetime as a function of Pr^{3+} concentration (b).

5 Å only [49]. In case of YABPr*x* phosphors, the number of available crystallographic sites per unit cell for the active ions is 3, the volume of unit cell is 541.94 Å and the critical concentration is 0.05 mol% and the corresponding value of $R_c$is ~19.0 Å leading to multi-polar interaction mechanism. According to Dexter's theory of sensitized-luminescence in solids [49], the slope of logarithmic plot of emission intensity per activator versus activator concentration [Log(I/C) vs. Log(C)] is equal to Q/3 which helps us to identify the type of interaction mechanism accountable for ET. The ET parameter, Q = 3, 6, 8, 10 for exchange, d-d, d-q, q-q interactions, respectively. From Log(I/C) vs. Log(C) plot presented in Fig. 11, the value of Q is found to be nearly 6 indicating $d-d$ interaction ET among the excited Pr^{3+} ions at 480 nm excitation. This transfer of energy is possible through non-radiative channels like cross-relaxation (CR) and resonant energy transfer (RET) channels. The probable CR and RET channels responsible for ET are shown below and they are illustrated in Fig. 8.

$$CR-1: \left[{}^3P_0,\ {}^3H_4\right] \rightarrow \left[{}^1D_2,\ {}^3H_6\right]$$

$$CR-2: \left[{}^3P_0,\ {}^3H_4\right] \rightarrow \left[{}^3H_6,\ {}^1D_2\right]$$

$$RET: \left[{}^1D_2,\ {}^3H_4\right] \rightarrow \left[{}^3H_4,\ {}^1D_2\right]$$

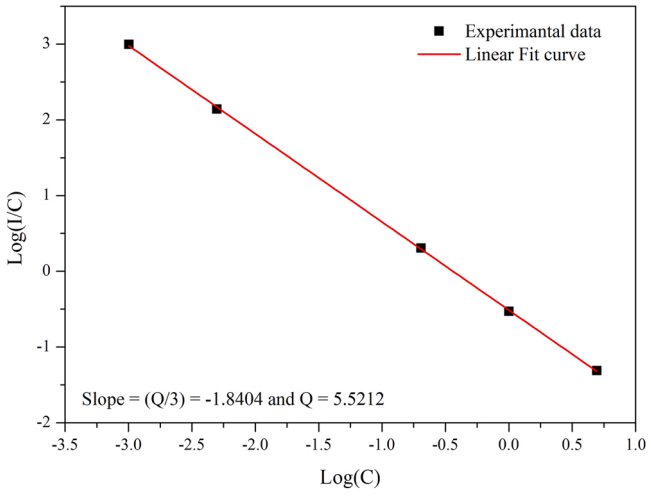

Fig. 11. Logarithmic plot of emission intensity per activator versus activator concentration in YABPr*x* phosphors.

4. Conclusions

The optical analysis of different concentrations of Pr^{3+} ions activated YABPrx ($0 \leq x \leq 2.0$ mol%) phosphors fabricated by solid-state reaction technique have been investigated. The PXRD analysis state that the studied phosphors are crystallized with an average size of 45–46 nm and suitably matched to JCPDS No. 72–1978. The HR-SEM micrograph presented a rod-like shape with uniform distribution of particles. The PLE spectra obtained by controlling the emission at 598 nm show $^3H_4 \rightarrow {}^3P_{2,1,0}$ transitions positioned at 448, 470 and 480 nm, respectively. Upon 480 nm excitation, the emission spectra exhibit different transitions originated from $^3P_0$ and $^1D_2$ emission states to their lower lying energy states leading to produce tunable luminescence from intense yellow to pale yellow. The decay curves of Pr^{3+}: $^1D_2$ emission state are suitably fitted to one exponential function. The addition of Pr^{3+} concentration results a decrease in luminescence intensity and the lifetime of $^1D_2$ emission state due to ET through $d-d$ interaction among the excited Pr^{3+} ions. Based on the observed results it can be concluded that the YABPr0.05 phosphor generates intense and pure yellow luminescence at 480 nm excitation and it is useful for solid state lighting.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on reasonable request.

Acknowledgments

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ORIGINAL RESEARCH ARTICLE

Photoluminescence Properties of $SrAl_2O_4$: Pr^{3+} Phosphors for Red Light Sources

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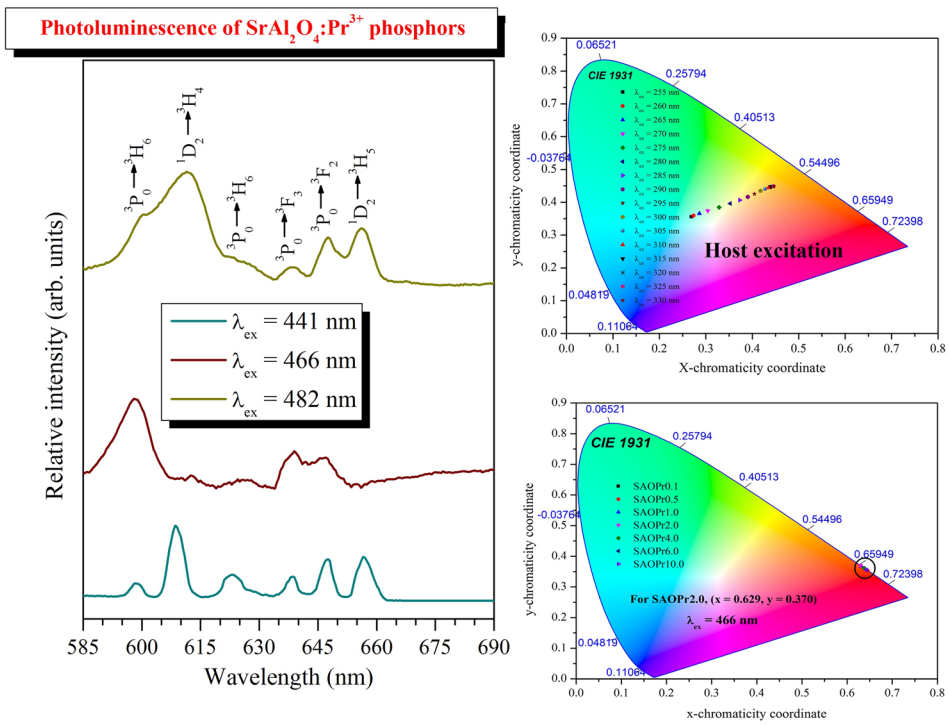
Abstract

A series of trivalent praseodymium (Pr^{3+}) ion-activated $SrAl_2O_4$ phosphors were prepared by solid-state reaction and characterized. The morphology of prepared phosphors was examined through SEM and TEM studies. The adjacent spacing of lattice fringes was estimated to be ~0.28 nm for the (310) crystal plane of the $SrAl_2O_4$ structure. The luminescence properties were studied by exciting the prepared phosphors within the host as well as Pr^{3+} ions. For efficient luminescence, the concentration of Pr^{3+} ions was optimized to be 2.0 mol.%. The colour perception of emitted luminescence was examined by evaluating the chromaticity coordinates. The observed quenching in luminescence and the gradual decrease in lifetime with increase of Pr^{3+} concentration was assigned to the multi-polar interaction mechanism among the excited Pr^{3+} ions at higher concentrations. The studied phosphors show more proficiency for solid-state red light sources.

Graphical Abstract



Keywords $SrAl_2O_4$ phosphor · praseodymium ions · photoluminescence · solid-state lighting device · red light sources

Extended author information available on the last page of the article

Introduction

Among the various energy saving materials, the trivalent rare earth (RE) ion-activated phosphors are more useful due to their excellent absorption from vacuum ultraviolet to near infrared (near-IR), good thermal and chemical stability, sharp emission lines, good colour rendering index and eco-friendly properties.[1–4] They are more favourable in designing various photonic devices owing to their low energy consumption, compact size, long lifetime and good quantum efficiency.[5,6] They effectively enhance the working efficiency of different kinds of photonic devices such as solid-state lasers, flat panel displays, optical sensors, surface coatings, energy-storage devices and light emitting devices.[7–10] The selection of $SrAl_2O_4$ host, its importance and applications in solid state lighting (SSL) technology was described in detail in our previous work.[11,12] Different RE ion-activated $SrAl_2O_4$ phosphors have been synthesized and characterized for various technological applications for the benefit of mankind and the environment. $SrAl_2O_4$:Ce^{3+}-Li^{3+} co-doped phosphors were investigated to enhance white light emission.[13] The luminescence enhancement of $SrAl_2O_4$: Eu^{3+} phosphors in the presence of B^+, Li^+, Na^+, and K^+ was reported for white LED applications.[14,15] $SrAl_2O_4$:Eu^{3+} phosphors[16] prepared by combustion were investigated for the applications of mechanoluminescence dosimetry. Long-lasting and the afterglow photoluminescence properties of $SrAl_2O_4$:Eu^{3+}, Dy^{3+} phosphors were studied for the fabrication of various photonic devices.[17,18] Sm^{3+}, Tb^{3+} and Tb^{3+}/Bi^{3+} -doped $SrAl_2O_4$ phosphors[19–21] were developed and characterized for various lighting applications.

Among the RE ions, the Pr^{3+} ion is a user-friendly luminescent center due to its closely packed energy level structure and prominent emission transitions in ultraviolet (UV), visible and near-IR spectral regions. The emission spectra of Pr^{3+} ions are more sensitive to the host environment and hence significantly differ in different hosts resulting to produce red, yellow, green and blue emissions as well as NIR emission. The emission transitions from $^1D_2$, $^3P_1$ and $^3P_0$ energy states to their lower lying energy states are responsible for red, yellow and green luminescence, the transitions from $^1S_0$ energy state results a blue luminescence while the transitions corresponding to 4f-5d energy state are responsible for UV emission.[22–25] These features have attracted the researchers to investigate Pr^{3+} activated different phosphor materials as colour centers. Different concentrations of Pr^{3+} ions activated $PbGdB_7O_{13}$ phosphors[22] were prepared and characterized for potential red luminescence. Under blue light irradiation, green to yellow tunability with an increase of Pr^{3+} concentration in BaY_2ZnO_5:Pr^{3+} phosphors has been reported.[23] $NaGd(WO_4)_2$:Pr^{3+} phosphors[24] synthesized by sol-gel method were investigated for intense green luminescence. Guan et al.[25] studied the concentration-dependent luminescence and energy transfer among the Pr^{3+} ions in $BaGd_2(MoO_4)_4$ crystals.

The broad yellow-orange emitting $SrAl_2O_4$:Pr^{3+} phosphors[26] containing 2.0 to 4.0 mol.% of Pr^{3+} ions mixed with 1.0 wt.% of carbon powder were sintered at 1200°C and investigated through powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), photoluminescence excitation (PLE), emission and time-resoled decay studies for SSL applications. Broad emission through Pr^{3+}:($^3P_0$, $^1D_2$) → ^3H_J transition has been assigned to the crystal field effect of $SrAl_2O_4$. In the present work, the $SrAl_2O_4$:Pr^{3+} phosphors have been prepared varying the concentration of Pr^{3+} ions from 0.1 to 10.0 mol.% without mixing the carbon powder and sintered at a relatively low temperature on the order of 1050°C for 3 h.

The current research work focused a light on the synthesis and characterization of $SrAl_2O_4$:Pr^{3+} phosphors through PXRD, SEM, transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, FT-Raman spectroscopy, excitation, emission and luminescence decay studies. The concentration dependent luminescence was investigated by exciting the samples within the host matrix as well as dopant (Pr^{3+}) ions. The optimization of concentration of Pr^{3+} ions for efficient luminescence was reported. The possible reasons for quenching in luminescence and decay time were also reported. The colour perception studies illustrate that the $SrAl_2O_4$:Pr^{3+} phosphor samples show an efficient red luminescence upon different excitations. The obtained results show that the studied phosphors exhibit quite interesting luminescence properties under different excitations compared to earlier work.[26–28]

Experimental

Materials Used and Preparation Technique

Different concentrations of Pr^{3+} ion-activated $Sr_{(1-x)}Al_2O_4$:x Pr^{3+} (x = 0 mol.%, 0.1 mol.%, 0.5 mol.%, 1.0 mol.%, 2.0 mol.%, 4.0 mol.%, 6.0 mol.% and 10.0 mol.%) phosphors were prepared by solid-state reaction using high-grade $SrCO_3$ (99%), Al_2O_3 (99.9%) and Pr_2O_3 (99.9%). The batch compositions of about 2.0 g of homogeneous powders were prepared with stoichiometric ratios. Additionally, 2.0 wt.% of H_3BO_3 (99.5%) was added as a flux material for easy decomposition of $SrCO_3$. These powder samples were heat-treated at 200°C for 3 h and sintered at 1050°C for 3 h following intermediate grinding producing milky-white $Sr_{(1-x)}Al_2O_4$:x Pr^{3+} phosphors. The obtained phosphor

materials were labeled as SAOPr*x*, where *x* stands for the concentration of Pr^{3+} ions (in mol.%).

Characterizations

The crystalline phase of SAOPr*x* ($0 \le x \le 10.0$ mol.%) phosphors was studied with an X' Pert Pro Materials Research Diffractometer ($\lambda_{CuK\alpha}$ = 1.5406 Å). A KBr pellet was employed to study the Fourier transform infrared (FTIR) absorption spectrum with a Perkin Elmer Spectrum One FTIR spectrometer. A Bruker RFS27 FT-Raman spectrometer using a Nd-YAG laser (λ = 1064 nm) was used to record Raman spectra. Morphological study was carried out with a Hitachi SU-70 scanning electron microscope (SEM) and an FEI-TECNAI G2 F20 high-resolution transmission electron microscope (HR-TEM). The photoluminescence excitation and emission studies were carried out witha Jobin Yvon Fluorolog-3 spectrofluorimeter with a xenon lamp of 450 W in continuous mode, while the decay dynamics were carried out on the same instrument with a xenon lamp in pulsed mode. All the characterizations were recorded at room temperature only.

Results and Discussion

Structure, Composition and Morphology Studies

The PXRD profiles of SAOPr*x* phosphors for a 2θ range from 15 to 50° are shown in Fig. 1a. These profiles are well fitted to the monoclinic $SrAl_2O_4$ structure (JCPDS No. 34-0379). All the PXRD profiles are found to be identical except in their intensity showing the same crystalline phase. The intensity of PXRD profiles decreases with the increase in concentration of Pr^{3+} ions showing the uniform distribution of Pr^{3+} ions in SAOPr*x* lattice. The variation of intensity of the PXRD peak corresponding to the (310) crystal plane was seen at $2\theta = 35.28°$ as a function of Pr^{3+} ion concentration is shown in Fig. 1b.

The monoclinic crystalline phase of $SrAl_2O_4$ phosphor has been confirmed from the Rietveld refinement following Fullprof software[19] and the corresponding lattice parameters were noted as a = 8.4419 Å, b = 8.8217 Å and c = 5.1592 Å. The mean crystallite size (D) of SAOPr*x* phosphors is estimated to be 36 nm using Debye-Scherrer's

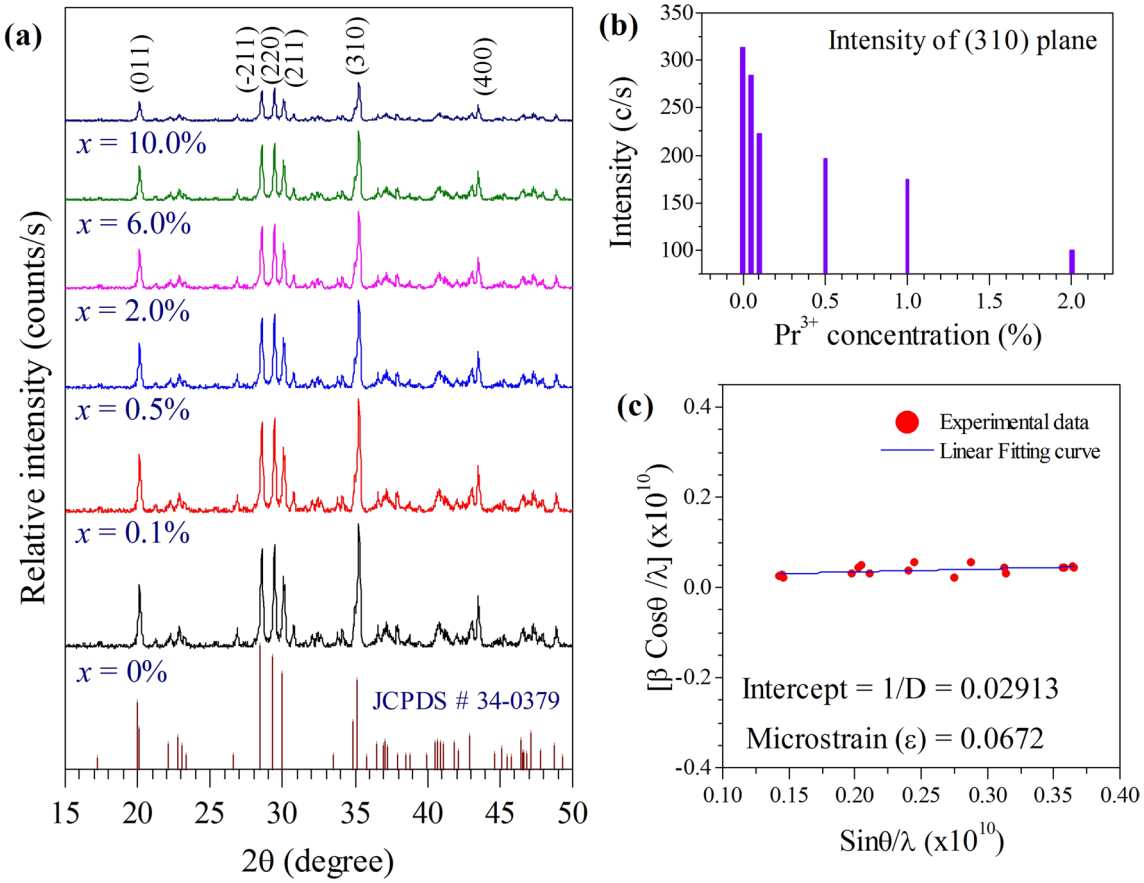


Fig. 1 PXRD profiles of SAOPrx phosphors (a), variation of intensity of (310) crystal plane as a function of Pr^{3+} concentration (b) and the Williamson–Hall curve of SAOPr1.0 phosphor (c).

formula, $D = \frac{0.89\,\lambda}{\beta\,.\,\cos\theta}$, where λ is the wavelength of x-rays (1.5406 Å), β is the full-width at half maximum (in radians) and θ is the diffraction angle. The size of crystallites has also been obtained using the Williamson–Hall (W–H) equation.[29] According to W–H, the crystallite size (D) and the angle of diffraction (θ) are related as

$$\frac{\beta\cos\theta}{\lambda} = \frac{1}{D} + \frac{\varepsilon\sin\theta}{\lambda} \quad (1)$$

The mean crystallite size and the magnitude of microstrain (ε) present in the material can be found from the $\left(\frac{\beta\cos\theta}{\lambda}\right)$ *versus* $\left(\frac{\sin\theta}{\lambda}\right)$ plot, known as the W–H curve and it is shown in Fig. 1c. The slope and reciprocal of the intercept of the W–H curve represent the microstrain and the mean crystallite size (D), respectively. The magnitude of microstrain and the mean crystallite size are found to be 0.0672 and 34 nm, respectively. The value of D obtained from the W–H curve is close to that obtained from Debye-Scherrer's formula.

The FTIR profile of SAOPr1.0 phosphor in the spectral range from 950 to 400 cm^{-1} shown in Fig. 2a reveals a number of significant vibrational modes associated with Al–O and Sr–O. The IR bands located from 892 to 838 cm^{-1} belong to the formation of $SrAl_2O_4$.[30] The IR peaks identified at 802, 772 and 712 cm^{-1} have been assigned to the AlO_6 and AlO_4 groups, respectively.[31] The IR peaks noticed at around 668 cm^{-1} and 642 cm^{-1} have been ascribed to the Sr-O vibration.[32] The peaks at around 614, 594 and 556 cm^{-1} have been attributed to the mixed stretching and bending mode of Al-O-Al.[33] The IR bands located at 532, 518 and 502 cm^{-1} have been attributed to the Al–O vibration.[32] The IR peaks observed at 446, 444 and 414 cm^{-1} have been assigned to O–Al–O symmetric vibration.[34] The FT-Raman spectrum of SAOPr1.0 phosphor shown in Fig. 2b reveals the presence of various molecular units as well as the confirmation of monoclinic phase. This spectrum consists of nearly 15 weak Raman modes possible for monoclinic $SrAl_2O_4$. These weak Raman modes have been ascribed to the overlap of certain symmetry vibrations. An intense and sharp peak at around 467 cm^{-1} has also been noticed along with weak modes confirming the monoclinic phase of SAOPr1.0 phosphor and it can be attributed to the O-Al-O bending vibrations.[35,36]

The SEM/TEM analysis provides not only the surface morphology but also the details regarding the distribution of particles and their orientation. The TEM analysis has also been used to confirm the crystallite size, i.e., whether they crystallized into nanoscale or not. The elemental analysis is also possible from both SEM and TEM

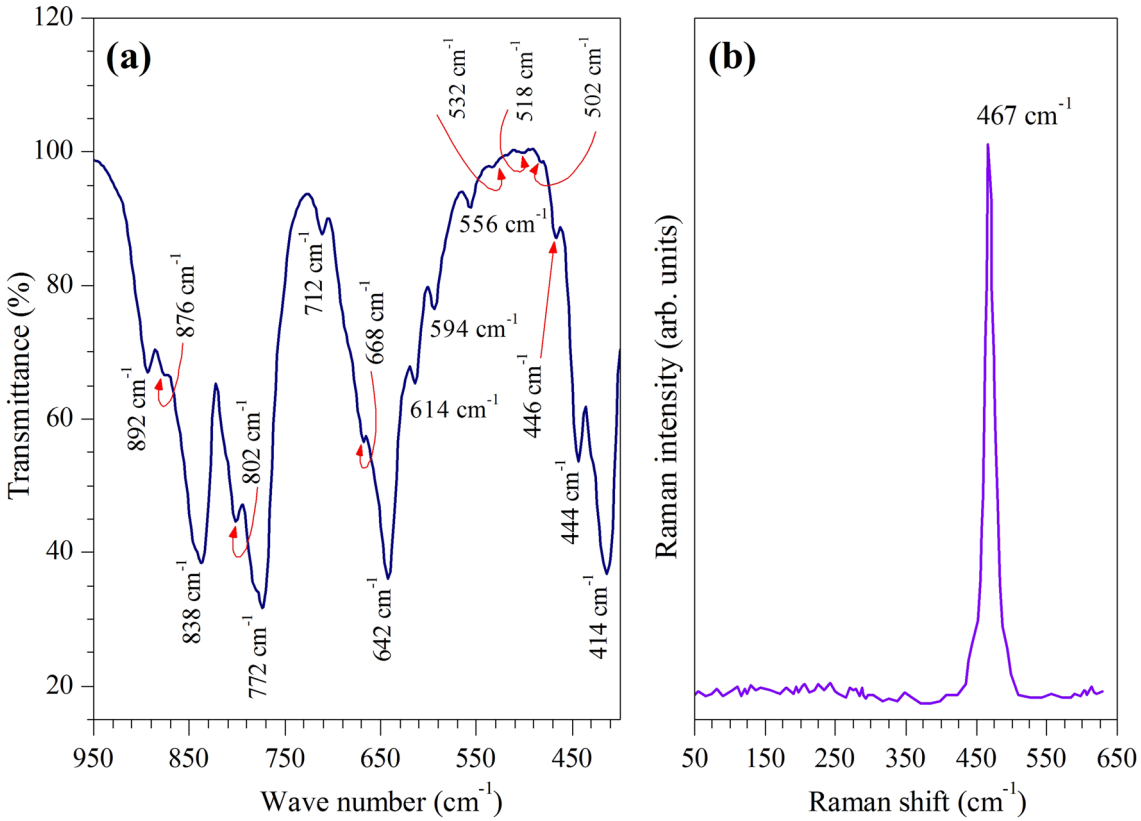


Fig. 2 FTIR (a) and FT-Raman (b) spectra of SAOPr1.0 phosphor.

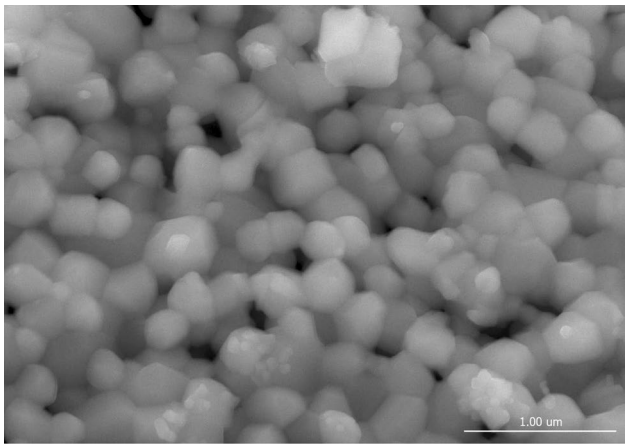


Fig. 3 SEM micrograph of SAOPr1.0 phosphor.

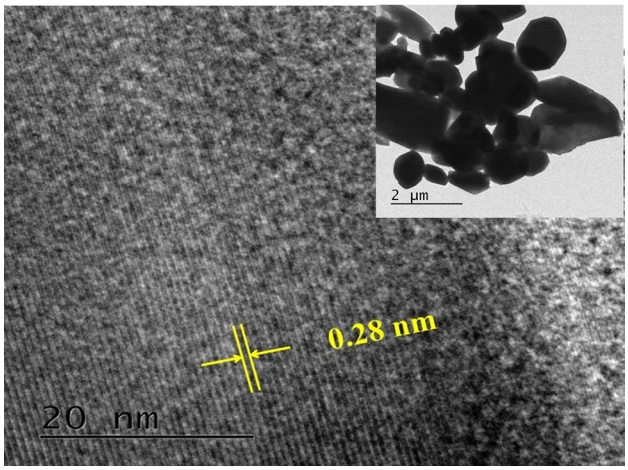


Fig. 4 HR-TEM image of SAOPr1.0 phosphor. Inset shows its TEM micrograph.

micrographs. The present research work is not focused on the elemental analysis. The SEM image of SAOPr1.0 phosphor shown in Fig. 3 represents homogeneous distribution of particles crystallized into spherical shape with different size. The TEM image of SAOPr1.0 phosphor (see inset of Fig. 4) reveals the uniform distribution of $SrAl_2O_4$ particles that are crystallized into nanoscale. The adjacent spacing of the lattice fringes corresponding to (310) crystal plane is estimated to be 0.28 nm from the HR-TEM image illustrated in Fig. 4. The interplanar spacing was also evaluated using the following equation.[37]

$$\frac{1}{d_{hkl}^2} = \frac{1}{\sin^2\beta}\cdot\left[\frac{h^2}{a^2} + \frac{k^2\sin^2\beta}{b^2} + \frac{l^2}{c^2} - \frac{2hl\cos\beta}{a.c}\right] \quad (2)$$

For the monoclinic SAOPr1.0 phosphor, the lattice parameters have been obtained as $a = 8.4419$ Å, $b = 8.8217$ Å and $c = 5.1592$ Å from the Rietveld refinement (not shown) and β is on the order of 93.415º. For the (310) crystal plane, the value of d_{hkl} is found to be 0.267 nm which is very close to that obtained from the HR-TEM analysis.

Photoluminescence Excitation

The photoluminescence (PL) analysis has been carried out through excitation, emission and lifetime studies. The excitation spectra of SAOPr2.0 phosphor has been recorded controlling the emission at 488 nm, 548 nm, 609 nm, 648 nm and 658 nm and presented in Fig. 5a. These spectra are comparable to other reports[28] and they contain a broad excitation band in the spectral region from 240 to 300 nm which can be assigned to the $SrAl_2O_4$ host excitation band.[11] These spectra reveal five excitation bands due to Pr^{3+}: $^3H_4 \rightarrow {}^3P_2$ (~441 nm), Pr^{3+}: $^3H_4 \rightarrow {}^1I_6$ (~448 nm), Pr^{3+}: $^3H_4 \rightarrow {}^3P_1$ (~466 nm), Pr^{3+}: $^3H_4 \rightarrow {}^3P_0$ (~482 nm) and Pr^{3+}: $^3H_4 \rightarrow {}^1D_2$ (~583 nm) transitions.[22,37–40] To study the effect of Pr^{3+} concentration, the excitation spectra of SAOPr*x* phosphors have been recorded monitoring the emission at 648 nm (Pr^{3+}: $^3P_0 \rightarrow {}^3F_2$) wavelength (not shown). The intensity of observed excitation bands increases with increase of Pr^{3+} concentration showing uniform distribution of Pr^{3+} ions in SAOPr lattice. The variation of intensity of $^3H_4 \rightarrow {}^3P_1$ (466 nm) transition as a function of Pr^{3+} ions concentration is shown in Fig. 5b. Since, the wavelengths corresponding to Pr^{3+}: $^3H_4 \rightarrow ({}^3P_J, {}^1D_2)$ transitions are covered by the emission of commercial InGaN LED chip (440 nm – 490 nm), the SAOPr*x* phosphors can be suitably excited by it. From the excitation spectra, it is also clear that the SAOPr phosphors can be suitably excited within the host and the dopant (Pr^{3+}) ions at 441 nm (Pr^{3+}:$^3H_4 \rightarrow {}^3P_2$), 466 nm ($Pr^{3+}$:$^3H_4 \rightarrow {}^3P_1$) and 482 nm ($Pr^{3+}$:$^3H_4 \rightarrow {}^3P_0$) wavelengths.

Host Excited Luminescence Properties

Upon different excitations within the host matrix (250 nm ≤ λ_{ex} ≤ 330 nm), the emission spectra of SAOPr2.0 phosphor in the spectral region from 460 to 740 nm are presented in Fig. 6a. These spectra reveal a broad and strong emission band corresponding to the host emission[11] with sub-peaks at about 468 nm , 488 nm, 548 nm, 609 nm, 648 nm and 658 nm corresponding to $^3P_1 \rightarrow {}^3H_4$, $^3P_0 \rightarrow {}^3H_4$, $^3P_2 \rightarrow {}^3F_2$, $^1D_2 \rightarrow {}^3H_4$, $^3P_0 \rightarrow {}^3F_2$ and $^1D_2 \rightarrow {}^3H_5$ transitions, respectively. The intensity of observed emission transitions increases with the increase of excitation wavelength reaching a maximum at λ_{ex} = 270 nm and then decreases for higher excitation wavelengths. This could be due to variation in population of Pr^{3+} ions at various emission states. The variation of intensity of $^3P_0 \rightarrow {}^3F_2$ (648 nm) transition as a function of

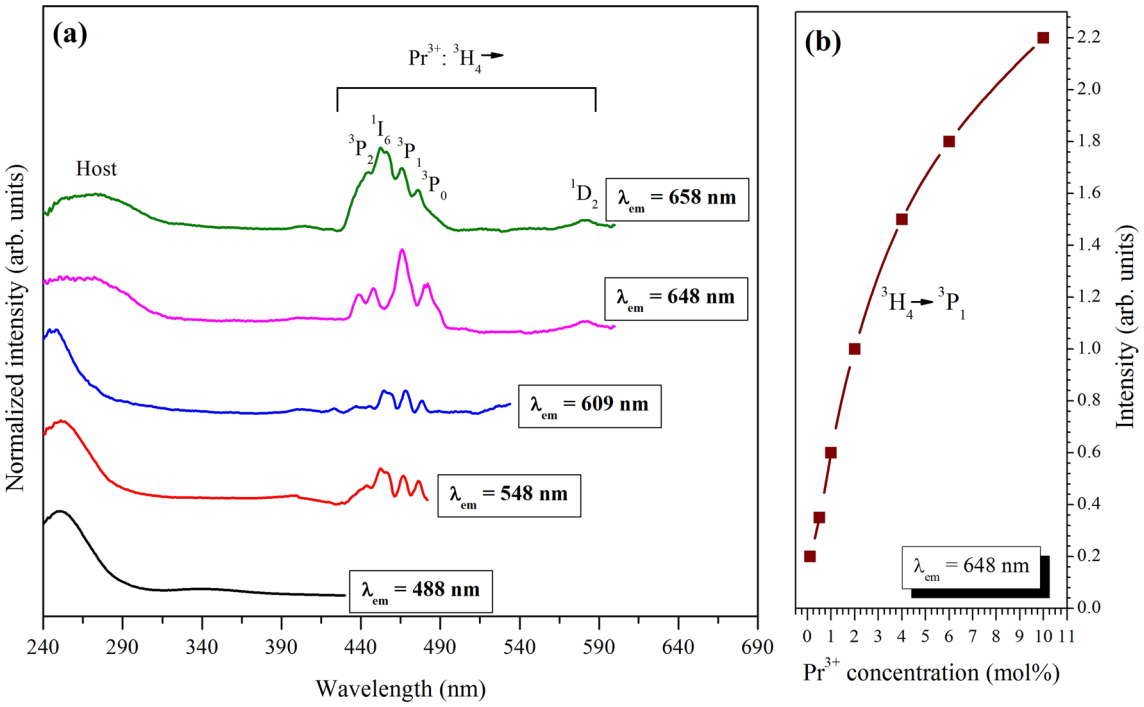


Fig. 5 Excitation spectra of SAOPr2.0 phosphor at different emission wavelengths (a) and the variation of intensity of $^3H_4 \rightarrow {}^3P_1$ (466 nm) transition as a function of Pr^{3+} concentration at $\lambda_{em} = 648$ nm.

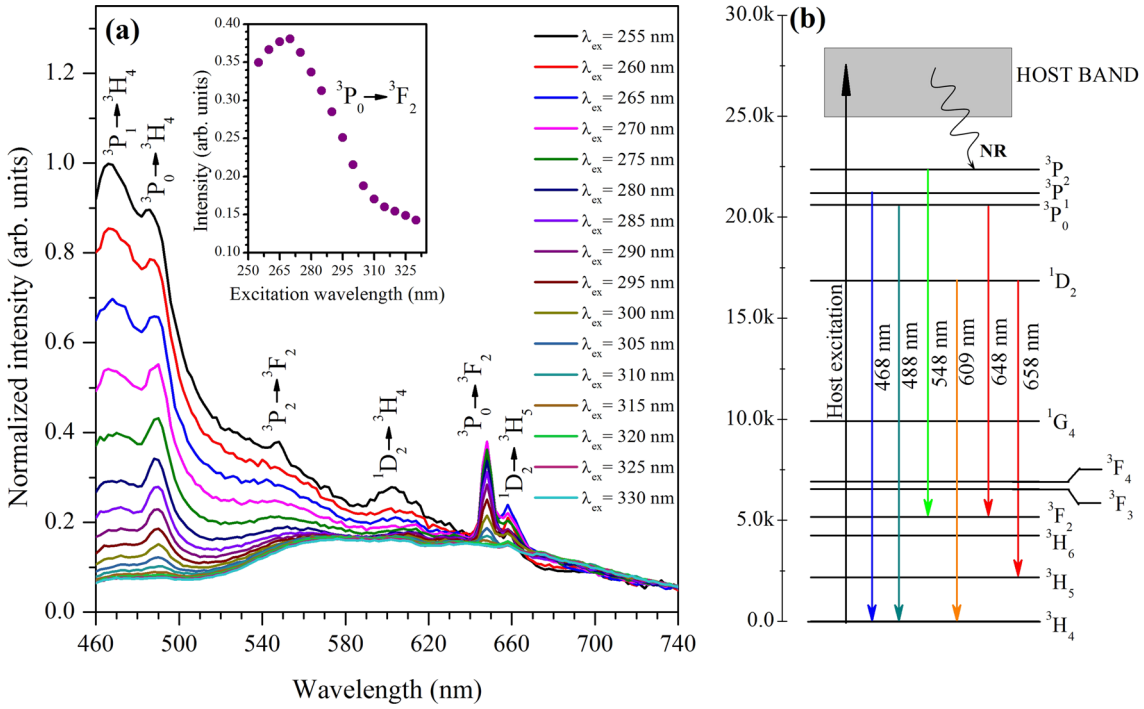


Fig. 6 Emission spectra of SAOPr2.0 phosphor under different host excitations (a) Inset shows the variation of intensity of $^3P_0 \rightarrow {}^3F_2$ (648 nm) transition as a function of Pr^{3+} concentration. The partial energy level diagram showing the emission mechanism of Pr^{3+} ions under host excitation (b).

Pr^{3+} ions concentration is described as inset of Fig. 6a. The emission mechanism of SAOPr2.0 phosphor under different host excitation wavelengths is illustrated in a partial energy level diagram shown in Fig. 6b.

The colour perception of emitted luminescence has been studied by evaluating the Commission International de I'Eclairage (CIE) 1931 chromaticity coordinates (*x*, *y*), which are summarized in Table I, and they are represented in the CIE diagram shown in Fig. 7. From this figure one can notice that the colour of emitted luminescence varies from bluish-green to yellow when the excitation wavelength changes from 255 to 330 nm. The variation in population density at different emission energy states results the tunability in emitted luminescence.

Dopant (Pr^{3+}) Ion Excited Luminescence Properties

Luminescence Properties at 466 nm Excitation

When excited at 466 nm (Pr^{3+}: $^3H_4 \rightarrow {}^3P_1$), the SAOPr*x* phosphors show emission profiles as shown in Fig. 8a. These spectra reveal six emission bands at about 598, 609, 622, 638, 648 and 656 nm corresponding to $^3P_1 \rightarrow {}^3H_6$, $^1D_2 \rightarrow {}^3H_4$, $^3P_1 \rightarrow {}^3F_2$, $^3P_1 \rightarrow {}^3F_3$, $^3P_0 \rightarrow {}^3F_2$ and $^1D_2 \rightarrow {}^3H_5$ transitions, respectively. The intensity of emitted transitions originating from Pr^{3+}: ^3P_J emission states show a luminescence quenching beyond $x = 2.0$ mol.%, while the transitions originating from the Pr^{3+}: $^1D_2$ emission state shows luminescence quenching beyond $x = 4.0$ mol.%. The variation in intensity of $^3P_1 \rightarrow {}^3H_6$ and $^1D_2 \rightarrow {}^3H_4$ transitions as a function of Pr^{3+} ion concentration is presented in Fig. 8b. From the literature, it is well known that the emission from the Pr^{3+}: $^1I_6$ energy state is not significant in many hosts.[22–28]

When excited at 466 nm, the interacting photons stimulate the ground state Pr^{3+} ions and excite to $^3P_1$ state (~21460 cm^{-1}). These excited Pr^{3+} ions transit to lower lying $^3P_0$ and $^1D_2$ emission states through a fast non-radiative (NR) process producing $^3P_0 \rightarrow {}^3F_2$ (648 nm), $^1D_2 \rightarrow {}^3H_4$ (609 nm) and $^1D_2 \rightarrow {}^3H_5$ (656 nm) transitions. However, some of the Pr^{3+} ions decay radiatively resulting $^3P_1 \rightarrow {}^3H_6$ (598 nm), $^3P_1 \rightarrow {}^3F_2$ (622 nm) and $^3P_1 \rightarrow {}^3F_3$ (638 nm) transitions. These emission spectral profiles are similar to $CaTa_2O_6$:Pr^{3+}[39] phosphors. The emission mechanism of SAOPr*x* phosphors at 466 nm excitation is described in the partial energy level diagram shown in Fig. 9a.

To study the colour perception, the CIE chromaticity coordinates have been calculated using the emission spectra shown in Fig. 8a and they are summarized in Table II. These chromaticity coordinates are close to those reported for National Television Standard Committee (NTSC) ($x = 0.67$, $y = 0.33$), Y_2O_3:Eu^{3+} commercial red emitting phosphor ($x = 0.655$, $y = 0.345$), $Ca_{2.96}Sm_{0.02}Na_{0.02}B_2O_6$ ($x = 0.608$, $y = 0.365$),[41] $NaSrPO_4$:Sm^{3+} ($x = 0.60$, $y = 0.39$),[42] SAOSm10 (x = 0.605, y = 0.362)[21] phosphors. The location of these chromaticity coordinates is presented in Fig. 10. From this figure it is clear that all the samples show an intense red luminescence when excited at 466 nm wavelength.

Table I CIE chromaticity coordinates of SAOPr2.0 phosphor at different excitation wavelengths

S. No.	Excitation wavelength (nm)	CIE chromaticity coordinates	
		x	*y*
1	255	0.269	0.357
2	260	0.274	0.360
3	265	0.87	0.366
4	270	0.304	0.375
5	275	0.329	0.386
6	280	0.353	0.397
7	285	0.373	0.407
8	290	0.391	0.417
9	295	0.405	0.426
10	300	0.418	0.435
11	305	0.428	0.442
12	310	0.436	0.446
13	315	0.441	0.448
14	320	0.444	0.447
15	325	0.446	0.448
16	330	0.447	0.449

Luminescence Properties at 482 nm Excitation

In the case of 482 nm (Pr^{3+}: $^3H_4 \rightarrow {}^3P_0$) excitation, the SAOPr*x* phosphors show a different emission profile. These emission spectra show six emission bands at about 600, 612, 623, 638, 647 and 656 nm corresponding to $^3P_1 \rightarrow {}^3H_6$, $^1D_2 \rightarrow {}^3H_4$, $^3P_0 \rightarrow {}^3H_6$, $^3P_0 \rightarrow {}^3F_3$, $^3P_0 \rightarrow {}^3F_2$ and $^1D_2 \rightarrow {}^3H_5$ transitions, respectively. For reference, the emission spectrum of the SAOPr2.0 phosphor is shown in Fig. 11a. Among the observed emission transitions, the $^1D_2 \rightarrow {}^3H_4$ (612 nm) transition is found to be more intense and broader compared to other transitions. The variation of intensity of the $^1D_2 \rightarrow {}^3H_4$ transition as a function of Pr^{3+} ion concentration is presented as the inset of Fig. 11a.

The colour perception of SAOPr*x* phosphors has been investigated by evaluating the CIE chromaticity coordinates using the emission spectra obtained by exciting the samples at 482 nm wavelength and they are also summarized in Table II. These chromaticity coordinates are close to those obtained at 466 nm excitation and they are well located in the red region of the CIE diagram shown in Fig. 12.

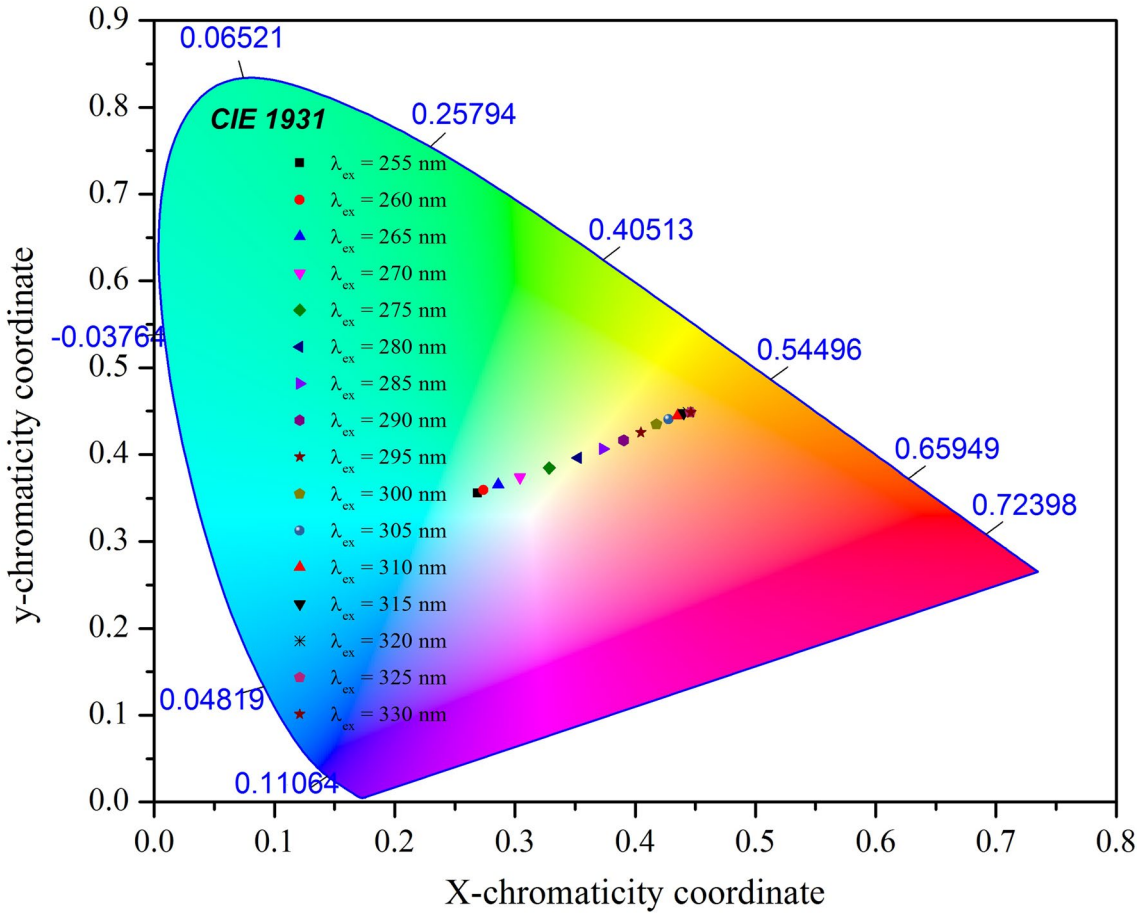


Fig. 7 CIE chromaticity diagram of SAOPr2.0 phosphor under different host excitations.

Luminescence Properties at 441-nm Excitation

Upon 441 nm (Pr^{3+}: $^3H_4 \rightarrow {}^3P_2$) excitation, the SAOPr*x* phosphors show interesting emission properties. These emission spectra reveal six transition peaks at around 598, 609, 623, 638, 647 and 657 nm corresponding to $^3P_1 \rightarrow {}^3H_6$, $^1D_2 \rightarrow {}^3H_4$, $^3P_0 \rightarrow {}^3H_6$, $^3P_0 \rightarrow {}^3F_3$, $^3P_0 \rightarrow {}^3F_2$ and $^1D_2 \rightarrow {}^3H_5$ transitions, respectively. For reference, the emission spectrum of SAOPr2.0 phosphor in the region from 580 to 680 nm is shown in Fig. 13a. Among the observed emission transitions, the $^1D_2 \rightarrow {}^3H_4$ (609 nm) transition is found to be more intense compared to other transitions. The variation of intensity of $^1D_2 \rightarrow {}^3H_4$ transition as a function of Pr^{3+} ion concentration is presented in Fig. 13b. Upon 441-nm excitation, the SAOPr*x* phosphors emit red luminescence and the corresponding chromaticity diagram is shown in Fig. 14. The CIE chromaticity coordinates of SAOPr*x* phosphor under 441-nm excitation are also close to those obtained at 466-nm excitation and they are summarized in Table II.

The comparative emission spectra of SAOPr2.0 phosphor under 441-, 466- and 482-nm excitations is shown in Fig. 15. These spectra show that the studied phosphors have different emission profiles with slight deviation in emission peak maxima. Additionally, the Pr^{3+} concentration hardly influences the emission color upon excitation at 441, 466 and 482 nm. The PXRD studies reveal that the crystalline peak positions do not vary with the increase of Pr^{3+} ions concentration indicating suitable occupancy of Pr^{3+} ions in Sr^{2+} ion sites. A slight variation in ionic radii and electronic configuration leads to a slight deviation in emission peak maxima at different excitation wavelengths.[43]

Luminescence Quenching

As seen the Figs. 8b, 11a and 13b, one can notice a quenching in luminescence at higher Pr^{3+} ion concentration. Upon 441- and 482-nm excitations, luminescence quenching has been observed beyond 2.0 mol.% of Pr^{3+} ion concentration for all the emitted energy states. When excited at 466 nm, the luminescence quenching is noticed beyond 4.0 mol.% of Pr^{3+} ion concentration for $^1D_2$ emission state and beyond 2.0 mol.% for other excited energy states. This quenching

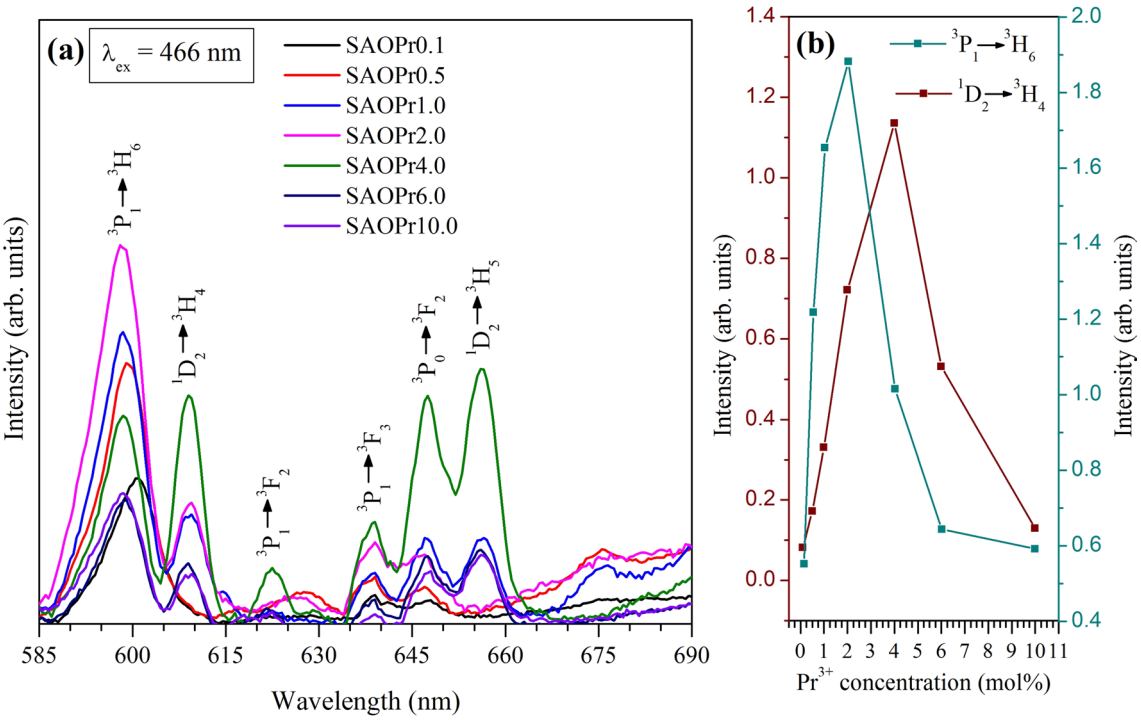


Fig. 8 Emission spectra of SAOPrx phosphors at λ_{ex} = 466 nm (a) and the variation of intensity of $^3P_1 \rightarrow {}^3H_6$ and $^1D_2 \rightarrow {}^3H_4$ transitions as a function of Pr^{3+} concentration (b).

in luminescence is mainly due to the efficient energy transfer (ET) among the excited Pr^{3+} ions at higher concentrations. It is well known that the transfer of energy among the exited RE ions at higher concentration is mainly due to the exchange and/or multi-polar interaction mechanism such as dipole-dipole (d-d), dipole-quadrupole (d-q) and quadrupole-quadrupole (q-q) mechanisms. Based on the critical transfer distance (R_c), the exchange or multi-polar interaction mechanism play a key role in ET.[44] The magnitude of R_c can be obtained using the formula shown below.

$$R_c = 2\left(\frac{3V}{4\pi x_c N}\right)^{1/3} \tag{3}$$

where V, x_c and N represents the volume of unit cell, critical concentration of active ions and number of crystallographic-sites available for the occupation of active ions. The exchange interaction mechanism has been responsible when the value of R_c is less than 5 Å only.[45] In case of SAOPr phosphors, the volume of unit cell is found to be 383.68 $Å^3$, the critical concentration of Pr^{3+} ions is around 2.0 mol.% and the number of crystallographic-sites occupied by Pr^{3+} ions is 6. The value of R_c is found to be 18.28 Å which indicates that the multi-polar interaction mechanism is responsible for the transfer of energy among the excited Pr^{3+} ions.

When the migration of energy among the excited RE active ions becomes prominent than the direct energy transfer, then the luminescence quenching is a function of the concentration of active ions.[46] The type interaction mechanism responsible for ET among the excited RE active ions has been known using the following equation considering the relationship between the emission intensity and the concentration of active ions.

$$\ln\left(\frac{I}{C}\right) = K - \left(\frac{S}{3}\right).\ln(C) \tag{4}$$

where I is the emission peak intensity, C is the concentration of RE active ions and the constant K belongs to a particular excitation of the material. The parameter S = 3 for exchange interaction, S = 6 for d-d interaction, S = 8 for d-q interaction and S = 10 for q-q interaction. The value of S has been found from the slope (S/3) of $\ln\left(\frac{I}{C}\right)$vs. $\ln(C)$ plot. Upon 466-nm excitation, the $\ln\left(\frac{I}{C}\right)$ vs. $\ln(C)$ plots are drawn for $^3P_1 \rightarrow {}^3H_6$ (598 nm) and $^1D_2 \rightarrow {}^3H_4$ (609 nm) transitions and they are illustrated in Fig. 16a and Fig. 16b, respectively. From these plots the value of the slope is found to be 1.76 ± 0.13 and 3.38 ± 0.25 for $^3P_1 \rightarrow {}^3H_6$ (598 nm) and $^1D_2 \rightarrow {}^3H_4$ (609 nm) transitions and the corresponding S values are 5.28 and 10.14, respectively. These results confirm that both d-d and q-q interaction mechanisms are responsible for ET among the excited Pr^{3+} ions when excited at 466-nm radiation.

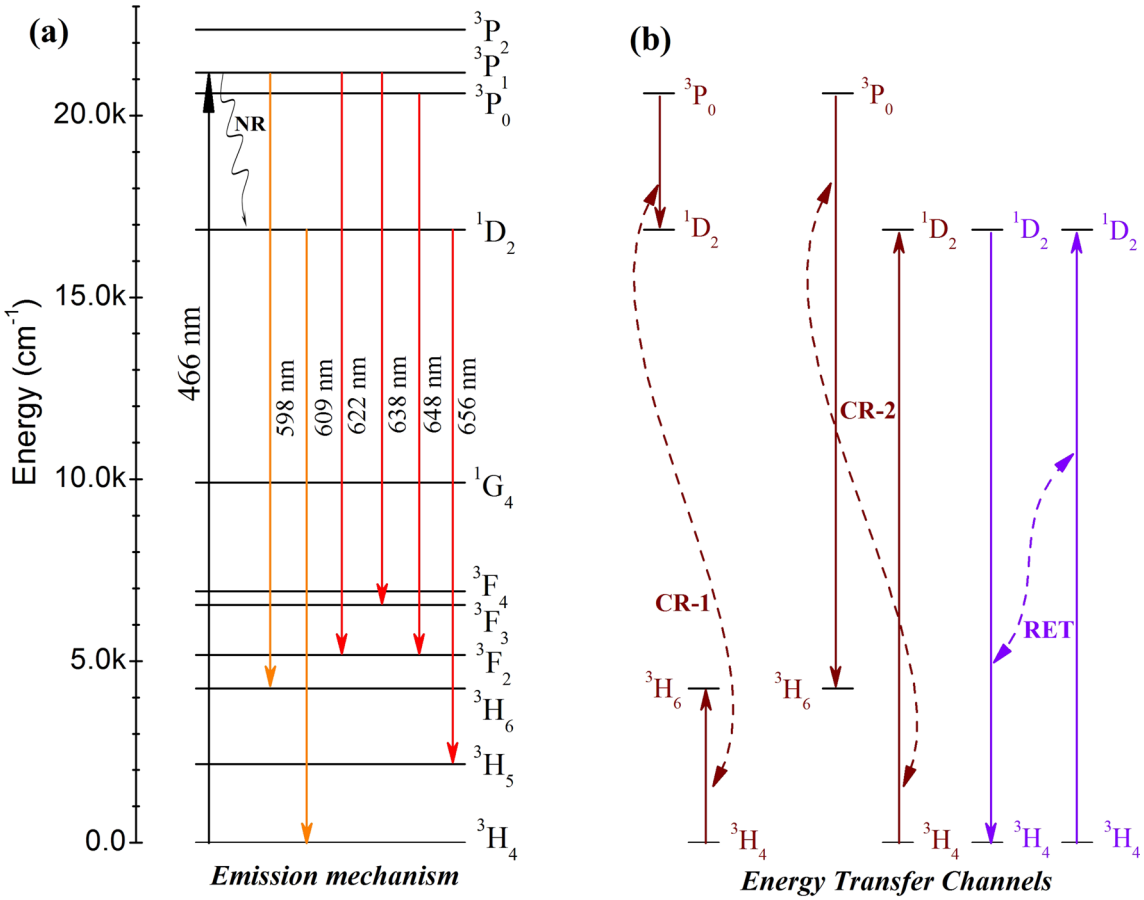


Fig. 9 Partial energy level diagram showing the emission mechanism of Pr^{3+} ions at 466 nm excitation (a) and the possible energy transfer channels (b).

Table II CIE chromaticity coordinates of SAOPrx phosphors

Doping *x* (mol%)	CIE chromaticity coordinates					
	λ_{ex} = 441 nm		λ_{ex} = 466 nm		λ_{ex} = 482 nm	
	x	*y*-	*x*	*y*-	*x*	*y*-
0.1	0.633	0.367	0.637	0.363	0.641	0.359
0.5	0.632	0.368	0.645	0.354	0.632	0.368
1.0	0.633	0.366	0.631	0.368	0.637	0.362
2.0	0.633	0.366	0.629	0.370	0.631	0.368
4.0	0.634	0.365	0.637	0.363	0.642	0.358
6.0	0.633	0.367	0.642	0.357	0.646	0.354
10.0	0.632	0.367	0.642	0.358	0.646	0.353

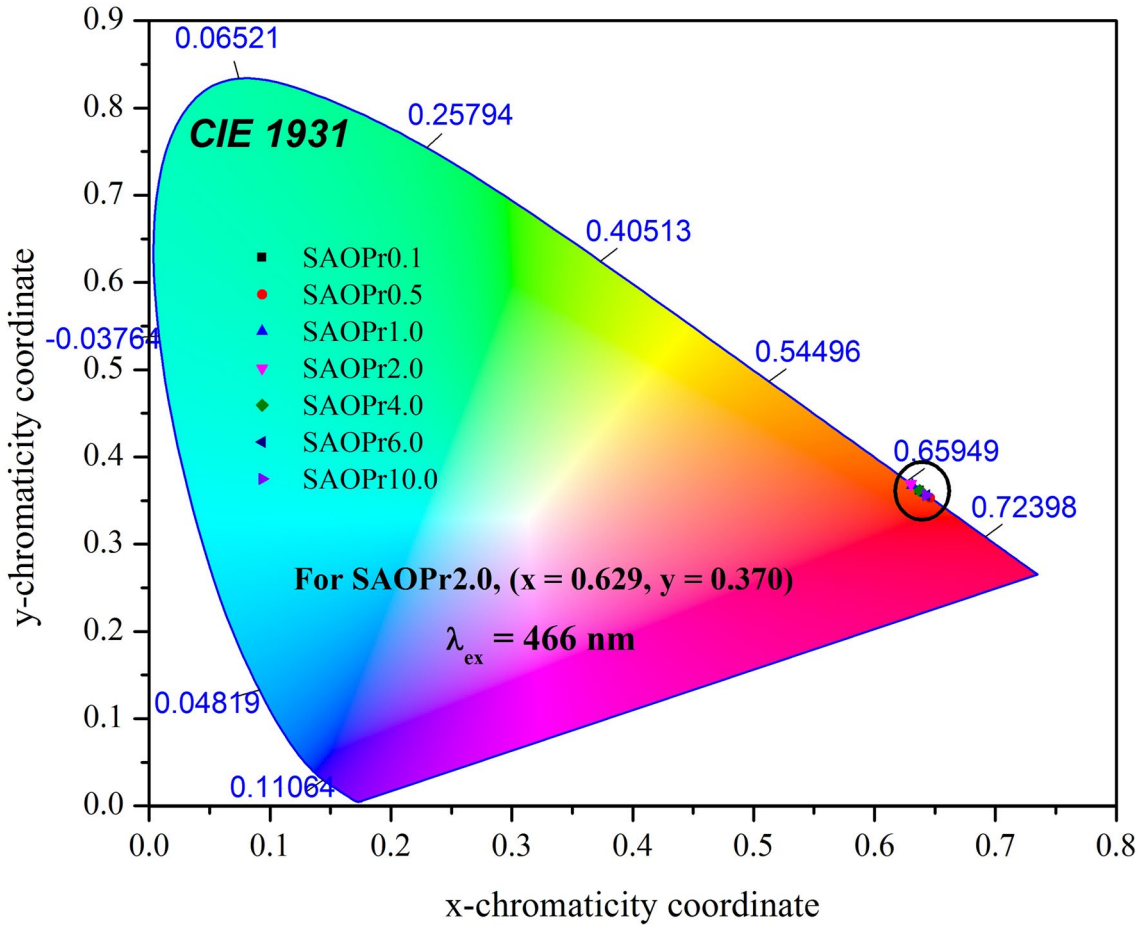


Fig. 10 CIE chromaticity diagram of SAOPrx phosphors at 466 nm excitation.

When excited at 482 nm, the slope of $\ln\left(\frac{I}{C}\right)$ vs. $\ln(C)$ plot for $^1D_2 \rightarrow {}^3H_4$ (612 nm) transition (see Fig. 11b) is noted as 1.82 ± 0.13 and the corresponding value of S is on the order of 5.37 confirming the d-d interaction mechanism type of ET among the excited Pr^{3+} ions. Similarly, when excited at 441-nm radiation, the slope of $\ln\left(\frac{I}{C}\right)$ vs. $\ln(C)$ plot for $^1D_2 \rightarrow {}^3H_4$ (609 nm) transition (see Fig. 13c) is noted as 1.68 ± 0.10 and the corresponding S value is of the order of 5.04 confirming the d-d interaction mechanism type of ET among the excited Pr^{3+} ions. From this discussion it can be concluded that the d-d interaction mechanism plays a significant role for ET among the excited Pr^{3+} ions.

Lifetime Analysis

The luminescence decay profiles of Pr^{3+}: $^1D_2$, $^3P_0$ and $^3P_1$ emission states in SAOPrx phosphors have been studied by exciting the phosphors at 466-nm wavelength monitoring the emission at 609 nm, 648 nm and 598 nm, respectively, and they are shown in Fig. 17. The decay times of SAOPr0.1, SAOPr0.5 and SAOPr1.0 are found to be almost the same. Therefore, the decay profiles of SAOPr0.1 and SAOPr0.5 are

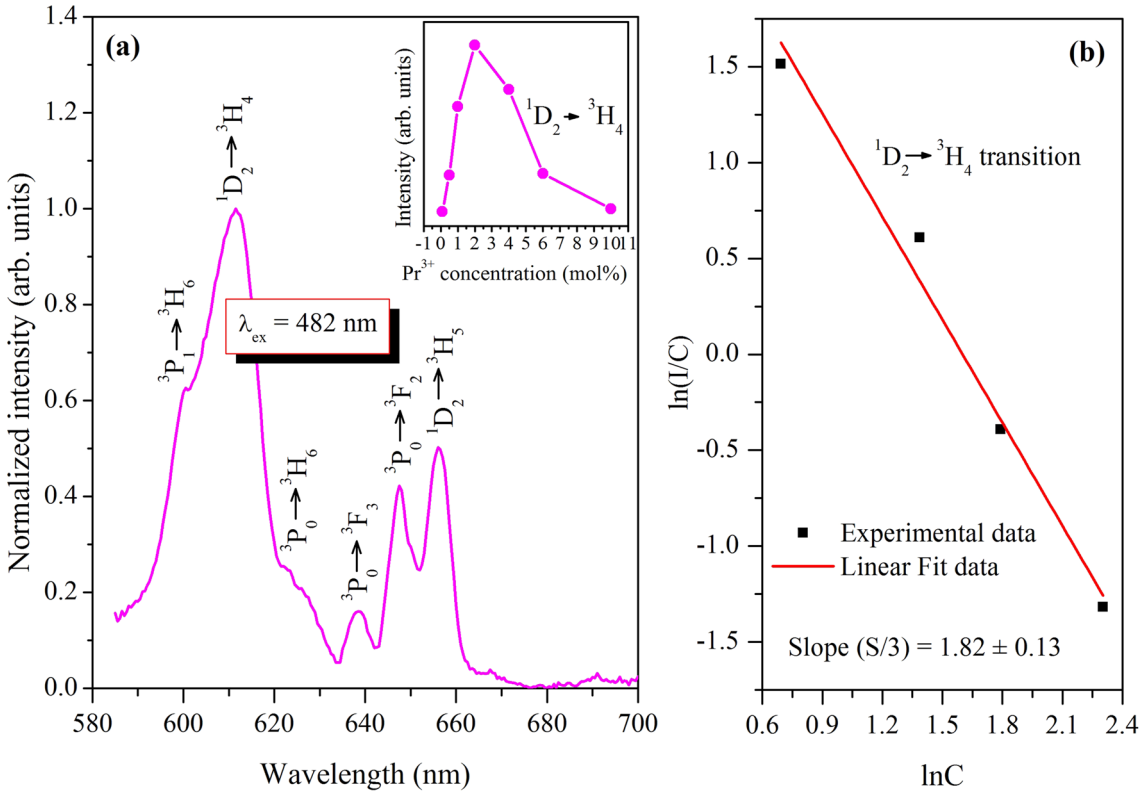


Fig. 11 Emission spectrum of SAOPr2.0 phosphor at $\lambda_{ex} = 482$ nm (a). Inset shows the variation of intensity of $^1D_2 \rightarrow {}^3H_4$ transition as a function of Pr^{3+} concentration. The ln (I/C) vs. ln(C) plot for $^1D_2 \rightarrow {}^3H_4$ transition (b).

not shown. All the decay profiles of Pr^{3+}: $^1D_2$, $^3P_0$ and $^3P_1$ emission states are well fitted to a two-exponential function, $I = I_0 + A_1.\exp(-t/\tau_1) + A_2.\exp(-t/\tau_2)$, where I describes the intensity as function of time t and I_0 is the intensity at $t = 0$ s. τ_1 and designates the long and short decay time components. The average decay time has been evaluated using the equation, $\langle\tau\rangle = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2}$ where A_1 and A_2 are constants corresponding to $\tau_1$and, respectively.[47,48] The lifetime of a particular emission state at different concentrations is summarized in Table III. As seen Table III, one can notice that the lifetime of Pr^{3+}: $^1D_2$, $^3P_0$ and $^3P_1$ emission states decrease with increase of concentration of Pr^{3+} ions as illustrated in Fig. 18. The values of decay time are comparable to those reported for Pr:$SrAl_2O_4$ crystals.[27]

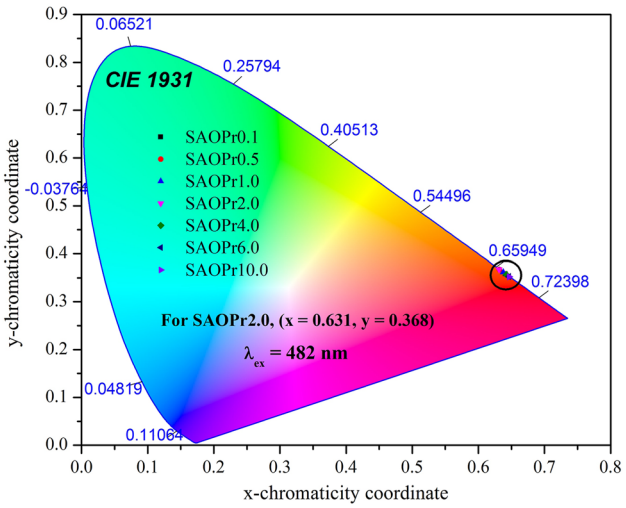


Fig. 12 CIE chromaticity diagram of SAOPrx phosphors at 482 nm excitation.

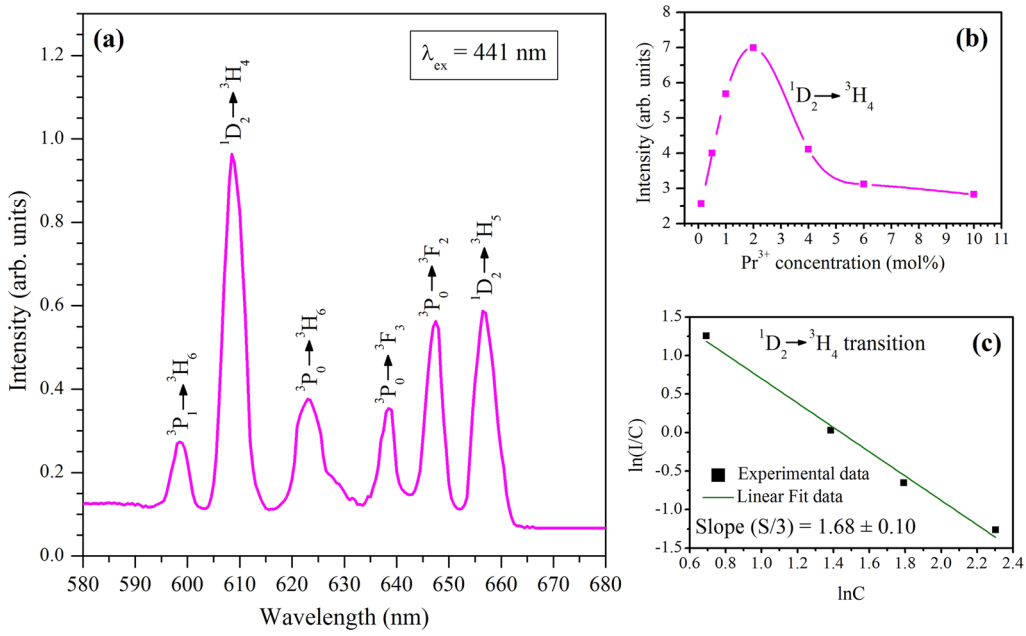


Fig. 13 Emission spectrum of SAOPr2.0 phosphor at λ_{ex} = 441 nm (a) the variation of intensity of $^1D_2 \rightarrow {}^3H_4$ transition as a function of Pr^{3+} concentration (b) the ln (I/C) vs. ln C plot for $^1D_2 \rightarrow {}^3H_4$ transition (c).

The self-absorption and the non-radiative ET among the excited Pr^{3+} ions cause a monotonic decrease in lifetime and hence luminescence quenching. The self-absorption and non-radiative ET can be ascribed to the following possible cross-relaxation (CR) and resonant energy transfer (RET) channels which are clearly illustrated in Fig. 9b.

$$\mathrm{CR}-1:\ \left({}^3P_0, {}^3H_4\right) \rightarrow \left({}^1D_2, {}^3H_6\right)$$

$$\mathrm{CR}-2:\ \left({}^3P_0, {}^3H_4\right) \rightarrow \left({}^3H_6, {}^1D_2\right)$$

$$\mathrm{RET}:\ \left({}^1D_2, {}^3H_4\right) \rightarrow \left({}^3H_4, {}^1D_2\right)$$

The photoluminescence emission studies at visible excitation (441, 466 nm and 482 nm) show that the Pr^{3+} ions emit efficient red luminescence when $x = 2.0$ mol.%. It can be concluded that the optimum concentration for intense and efficient luminescence is 2.0 mol.%. The studied phosphors can suitably excite either at 441 nm or 466 nm or 482 nm to generate red luminescence while they produce feeble bluish-white luminescence at 255 nm excitation. From the literature, it is clear that LEDs coated with phosphor enhances their working efficiency and hence lowers the surface temperature.[49,50] Therefore, the SAOPr*x* phosphors would be the best choice to fabricate red light emitting solid-state light sources.

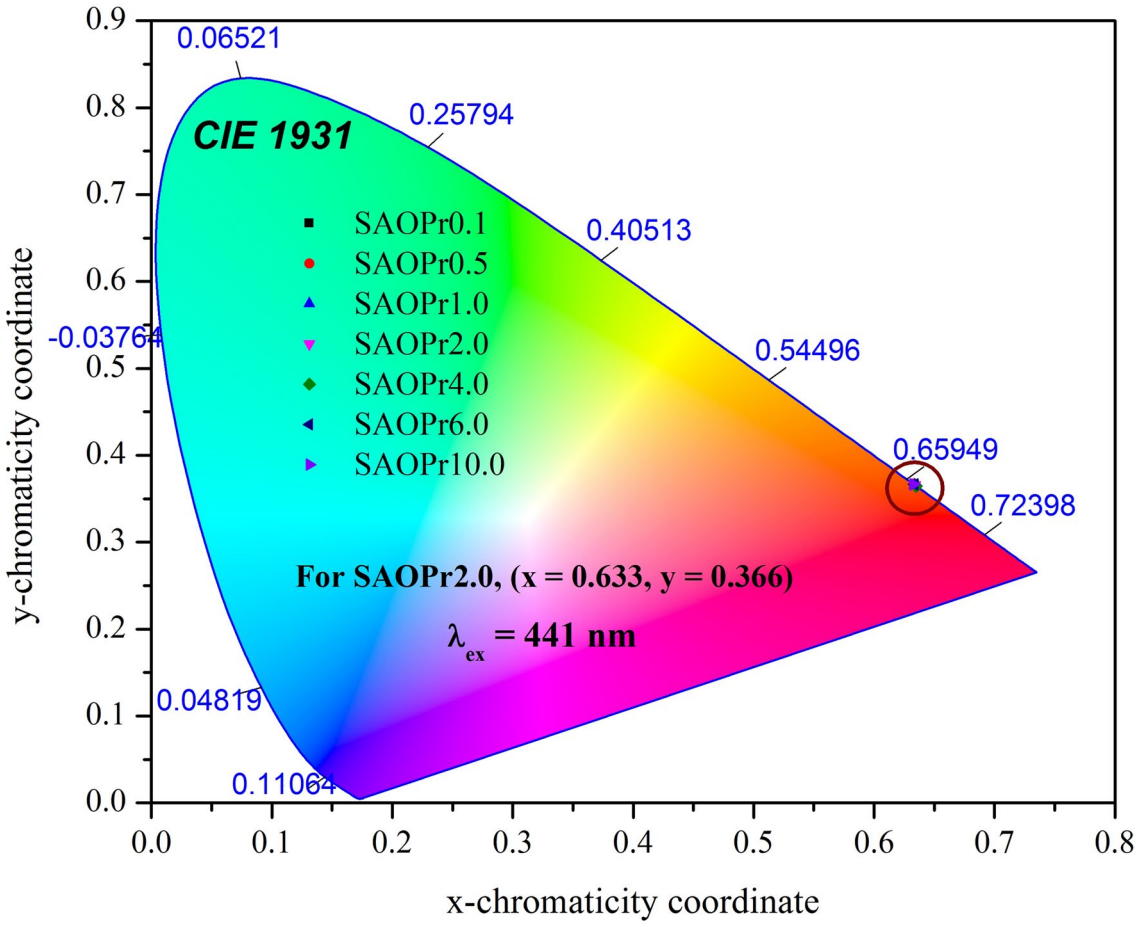


Fig. 14 CIE chromaticity diagram of SAOPrx phosphors at 441-nm excitation.

Conclusions

The $SrAl_2O_4$:$x$$Pr^{3+}$ phosphors have been prepared by conventional solid-state technique sintering at 1050°C for 3 h with average crystallite size of 34–36 nm. The SEM image establishes homogeneous distribution of particles of spherical shape with different sizes. The adjacent spacing of the lattice fringes corresponding to (310) crystal plane is estimated as 0.28 nm from the HRTEM image. The concentration of Pr^{3+} ions has been optimized to be 2.0 mol.% for efficient luminescence. The quenching in luminescence and lifetime has been ascribed to the ET among the excited Pr^{3+} ions through d-d and q-q multipolar interaction mechanism. The colour perception of emitted luminescence has been investigated by evaluating chromaticity coordinates. The studied phosphors generate strong red luminescence at 441, 466 and 482 nm excitations, while they produce feeble bluish-white luminescence at 255 nm excitation. Based on the observed results it can be concluded that the SAOPr2.0 phosphor could be the best choice for solid state red light emitting sources.

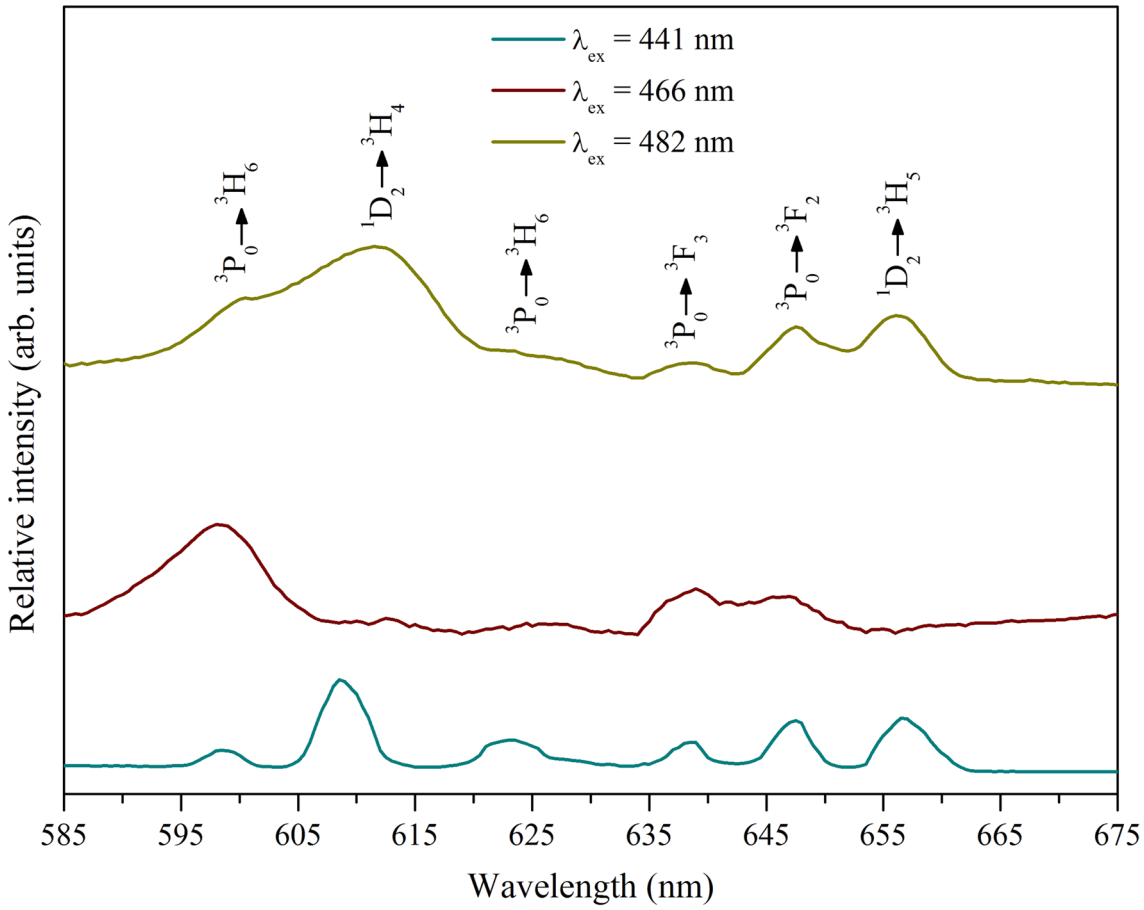


Fig. 15 Comparative emission spectra of SAOPr2.0 phosphor at different excitations.

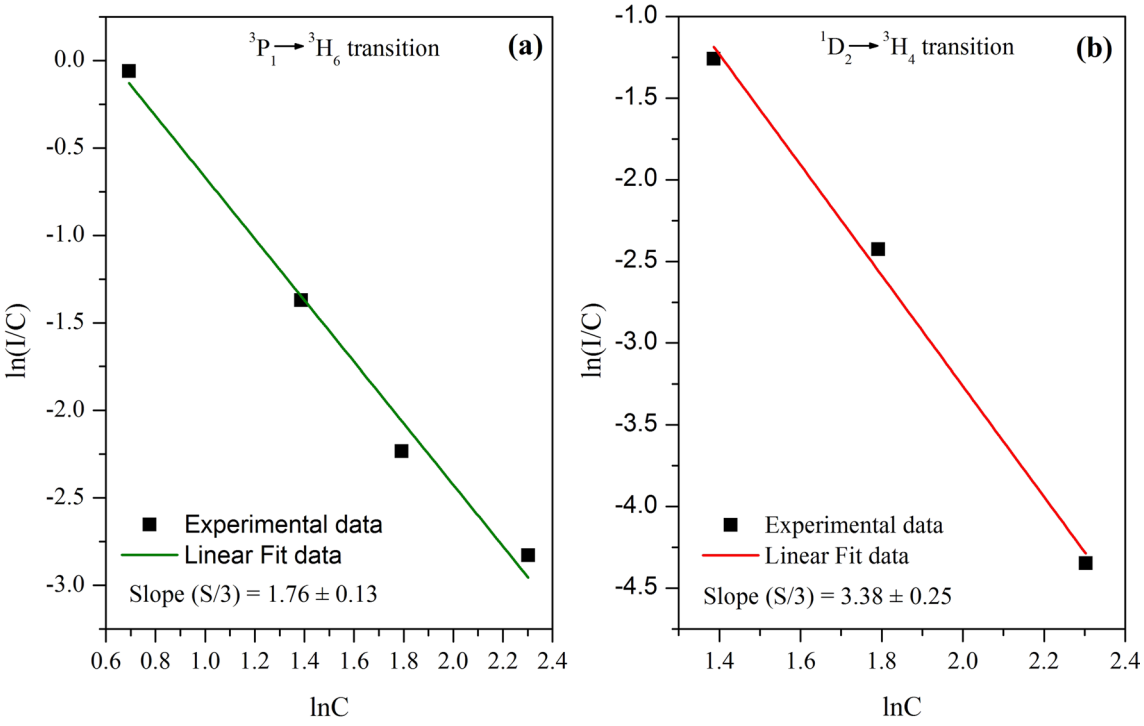


Fig. 16 The ln (I/C) vs. ln C plots for $^3P_1 \rightarrow {}^3H_6$ (598 nm) transition (a) and $^1D_2 \rightarrow {}^3H_4$ (609 nm) transition (b).

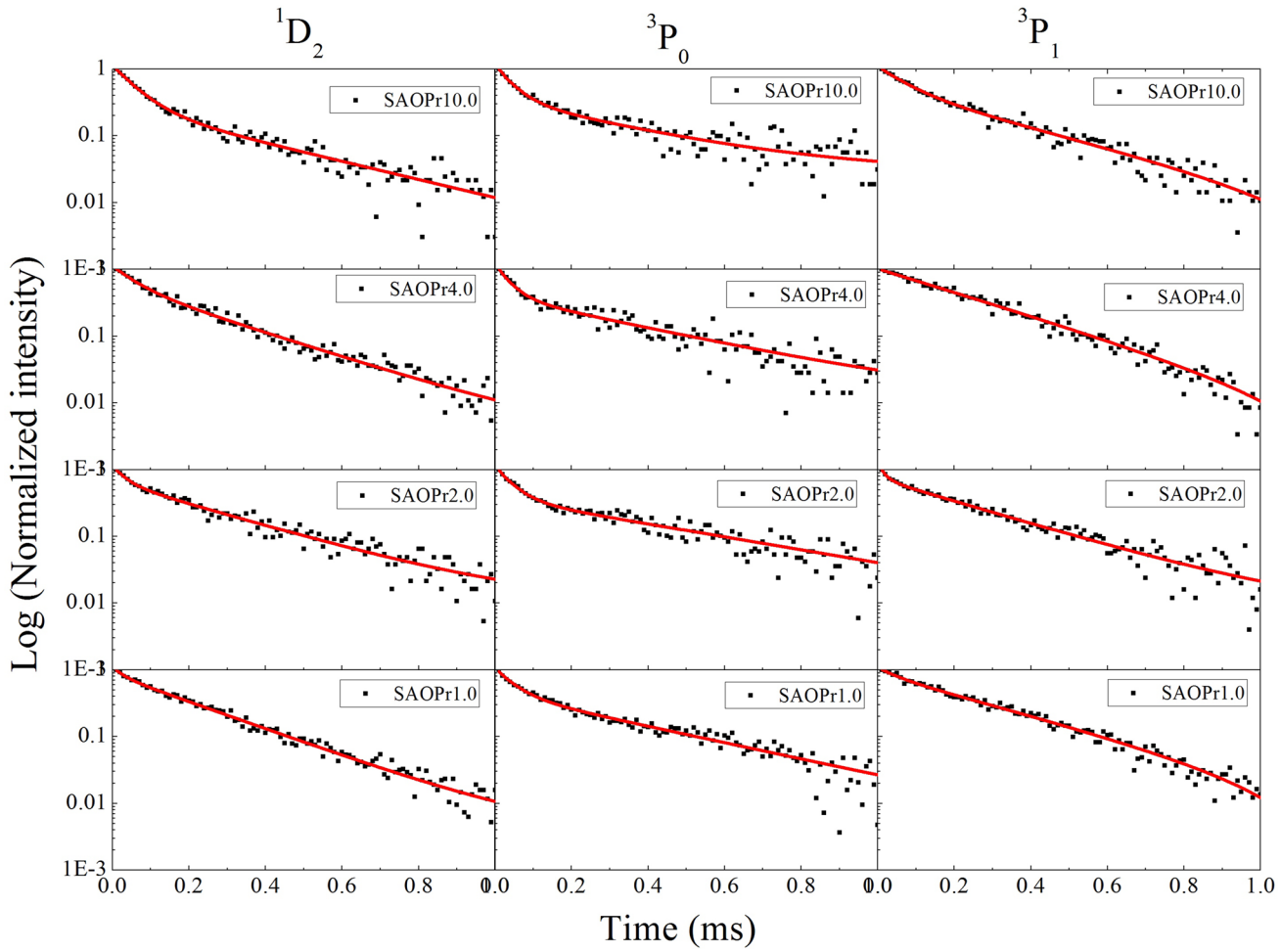


Fig. 17 Decay curves of the Pr^{3+}: $^1D_2$ [λ_{ex} = 466 nm; λ_{em} = 609 nm] (a), Pr^{3+}: $^3P_0$ [λ_{ex} = 466 nm; λ_{em} = 648 nm] (b) and Pr^{3+}: $^3P_1$ [λ_{ex} = 466 nm; λ_{em} = 598 nm] (c) emission states.

Table III Comparison of lifetime of Pr^{3+}: $^1D_2$, $^3P_0$ and $^3P_1$ emission states in SAOPrx phosphors

Sample	Lifetime (τ, ms)		
	Pr^{3+}:$^1D_2$	Pr^{3+}:$^3P_0$	Pr^{3+}:$^3P_1$
SAOPr1.0	0.219 ± 0.004	0.154 ± 0.006	0.252 ± 0.005
SAOPr2.0	0.188 ± 0.004	0.120 ± 0.006	0.228 ± 0.005
SAOPr4.0	0.139 ± 0.004	0.098 ± 0.006	0.203 ± 0.005
SAOPr10.0	0.108 ± 0.004	0.074 ± 0.006	0.158 ± 0.005

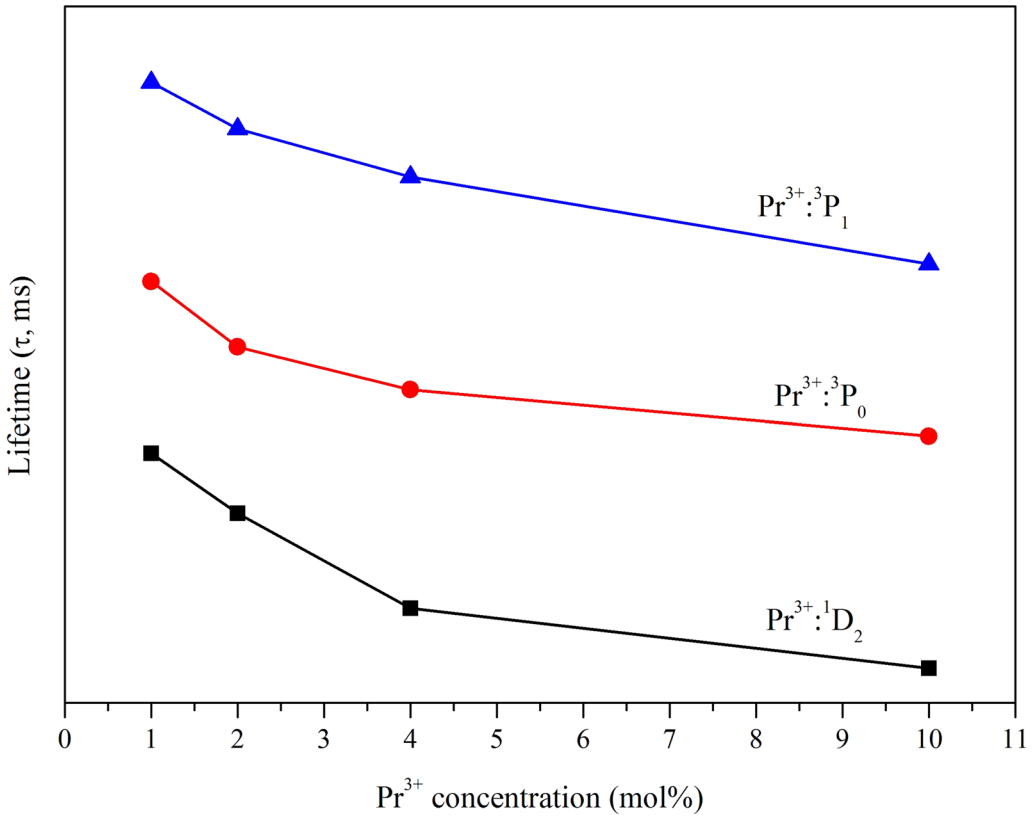


Fig. 18 Variation of lifetime as a function of Pr^{3+} concentration in SAOPrx phosphors.

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Author contributions B.C. Jamalaiah: Conceptualization, Software, Investigation, Writing-Original draft preparation, Writing-Reviewing and Editing, N. Madhu: Investigation, Writing-Reviewing and Editing, K. Pavani: Conceptualization, Data curation, Writing-Reviewing and Editing, A.J. Neves: Conceptualization, Validation, Writing-Reviewing and Editing

Data availability Data will be made available on reasonable request.

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Bi_2O_3-B_2O_3-CaF_2-EuF_3 glass–ceramics for lighting applications

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ABSTRACT

The Bi_2O_3-B_2O_3-CaF_2-EuF_3 (BiBCEu) glass and glass–ceramics were prepared by controlled heat treatment method for orange-red laser sources and characterized through X-ray diffraction, Fourier transform infrared, Raman, transmission electron microscopy, photoluminescence excitation, emission and luminescence decay studies. Up on 396 nm excitation, the BiBCEu glass–ceramics containing $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites exhibit an enhanced orange-red luminescence through Eu^{3+}:$^5D_0 \rightarrow {}^7F_2$ (616 nm) transition. The radiative parameters such as radiative emission probability rate (A_R), luminescence branching ratio (β_R) and radiative decay time (τ_R) were determined using the intensities of Eu^{3+}: $^5D_0 \rightarrow {}^7F_J$ (J = 1, 2, 4) emission transitions following the Judd–Ofelt theory. The chromaticity coordinates of BiBCEu glass–ceramic heat treated at 575 °C for 10 h are situated in the orange-red region of the CIE diagram. The BiBCEu glass–ceramic synthesized at 575 °C for 10 h has an excellent proficiency for solid state orange-red laser sources.

1 Introduction

The oxyfluoride glass–ceramics (GCs) doped with certain rare earth (RE) ions and having one or more crystalline phases distributed uniformly within the glassy phase have been the significant materials for various optical applications owing to their intense and narrow emission lines, high luminescence efficiency and long decay time [1–3]. The transparent GCs having low optical absorption and scattering losses with relative refractive index difference between the glassy and crystalline phases of the order 0.1 can be fabricated by modifying the experimental conditions. The refractive index is found higher in GCs compared to that of bared glasses and it increase with the increase of annealing time [4]. The

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importance of borate based host matrix containing Bi_2O_3 heavy metal oxide, the formation of $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites have been clearly discussed in our earlier research work [5]. It is well known that GCs are polycrystalline materials in which the crystallites are dispersed uniformly throughout the glassy matrix. The GCs are characterized through their high strength, high impact resistance, considerably low thermal expansion coefficient, excellent translucent properties and thermal shock resistance. They can be synthesized through controlled crystallization of base glass. They are resistant to surface damage due to its improved tensile strength and they have a low thermal coefficient of expansion. These qualities favour GCs to be used in many industrial, scientific, defense and bio-medical fields. The GCs find applications in solar panels, liquid crystal display devices, high-temperature lamp envelops, magnetic disc substrates and smart electronic devices.

Trivalent europium (Eu^{3+}) is one of the best and efficient RE ions used for efficient red and/or orange-red colour center in optical devices due to its dominant emission through $^5D_0 \rightarrow {}^7F_2$ transition. The Eu^{3+} ions have been used to probe the site symmetry as well as inhomogeneity of the ligand environment due to the non-degenerate $^7F_0$ ground and $^5D_0$ excited states and relatively simple energy level system [6, 7]. The laser characteristic parameters such as radiative emission probability (A_R), luminescence branching ratio (β_R) and radiative decay time (τ_R) have been evaluated using the intensities of Eu^{3+}: $^5D_0 \rightarrow {}^7F_J$ (J = 1, 2, 4) emission transitions following the Judd–Ofelt (J-O) theory [8, 9]. In literature, the Eu^{3+} -doped various multi-composition GCs have been reported for different optical, sensor and photonic applications [10–12]. This research work reports the synthesis, morphological, structural and optical analysis of 1.0 mol% Eu^{3+} activated Bi_2O_3-B_2O_3-CaF_2 glass and GCs. The process of finding the three phenomenological J-O intensity parameters (Ω_2, Ω_4 and Ω_6) using the Eu^{3+}: $^5D_0 \rightarrow {}^7F_{J\,=\,1,2,4,6}$ emission transitions is also presented.

2 Experiments

2.1 Preparation

The glass samples of composition, 71.25 Bi_2O_3 + 17.75 B_2O_3 + 10.00 CaF_2 + 1.00 EuF_3 (in mol%) were prepared by conventional melt quench method. High purity Bi_2O_3 (99%), H_3BO_3 (99.5%), CaF_2 (99.99%) and EuF_3 (99.99%) were used as precursors. A batch composition of about 20.0 g homogeneous powder with an additional amount of 5.0 wt% of H_3BO_3 (to compensate its loss at higher temperatures) was taken and heat treated at 300 °C for 5 h, cooled to room temperature and then grinded into a fine powder in dust free environment. These heat treated powders were melted for 30 min using a pre-heated muffle furnace at 1000 °C at ambient pressure and air-quenched in a clean atmosphere, then annealed at 400 °C for 20 h to eliminate thermal strains developed during the process of quenching. The as-prepared glasses were heat treated at 575 °C for 5 h and 10 h to continue the crystallization process forming $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites and hence to obtain transparent GCs. The prepared samples were labeled as BiBCEu-G (as prepared glass), BiBCEu-GC1 (heat treated at 575 °C for 5 h) and BiBCEu-GC2 (heat treated at 575 °C for 10 h).

2.2 Characterization

The powder X-ray diffraction (PXRD) patterns were recorded using Rigaku Miniflex 600 X-ray Diffractometer ($\lambda_{CuK\alpha}$ = 1.5406 Å). The Fourier transform infrared (FT-IR) and Raman analysis were carried out with Perkin Elmer Spectrum One Spectrometer (KBr pellet) and Horiba Micro-Raman Spectrometer provided with 532 nm laser, respectively. The formation of $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites was examined with a JEM 2100 plus Hi-Resolution Transmission Electron Microscope (HR-TEM). The room temperature absorption studies were carried out on Perkin Elmer Lambda 950 Spectrophotometer. The photoluminescence excitation, visible emission and fluorescence decay studies were carried out with Jobin YVON Fluorolog-3 Spectrofluorimeter. All the characterizations were done at normal conditions only.

3 Results and discussion

3.1 Structure and morphology

The PXRD patterns of BiBCEu-G and GCs are illustrated in Fig. 1. The PXRD profile of BiBCEu-G reveals pure amorphous phase while BiBCEu-GC1 and BiBCEu-GC2 samples show a combined phase of

glass and crystalline. From these profiles, one can know that the PXRD profile of BiBCEu-GC2 is similar to the previous work on BiBCEr-GCs [5] and it contains crystalline peaks corresponding to the $Bi_3B_5O_{12}$ (JCPDS No. 00–025-1089) and CaF_2 (JCPDS No. 00-001-1274) nanocrystallites. The PXRD peaks noticed at around 2θ = 27.86, 47.02 and 55.61 have been ascribed to (111), (220) and (311) crystal planes of CaF_2.

The FT-IR spectral profiles of BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2 samples shown in Fig. 2 are almost identical and they reveal seven IR absorption bands with considerably small shift towards longer wavenumber region with increase of crystallanity due to heat treatment. The FTIR bands positioned at around (3465.2–3466.2), (1339.1–1340.4), (1222.2–1223.1), (1084.9–1086.0), (921.6–922.6), (684.1–685.2) and (532.9–534.1) cm^{-1} have been ascribed to symmetric stretching vibrations of OH^- group, asymmetric stretching vibrations of B-O bonds, B-O stretching vibrations in BO_3 units, B-O stretching vibrations in BO_4 units, bending vibrations of B-O-B linkage and Bi-O bending vibration in BiO_6 units, respectively [13–17]. These results are identical to those reported for Er^{3+} doped Bi_2O_3-B_2O_3 glass–ceramics [5].

The Raman spectral profiles of BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2 samples in the Raman shift range 50–950 cm^{-1} are also identical and they are shown in Fig. 3. Like FTIR bands, the Raman band positions are also shift towards higher wavenumber region with increase of crystallanity due to heat treatment. The Raman bands are positioned at around (128.1–129.2), (154.0–155.6), (277.9–279.0), (336.0–337.5), (691.2–692.3) and (757.6–758.4) cm^{-1}.

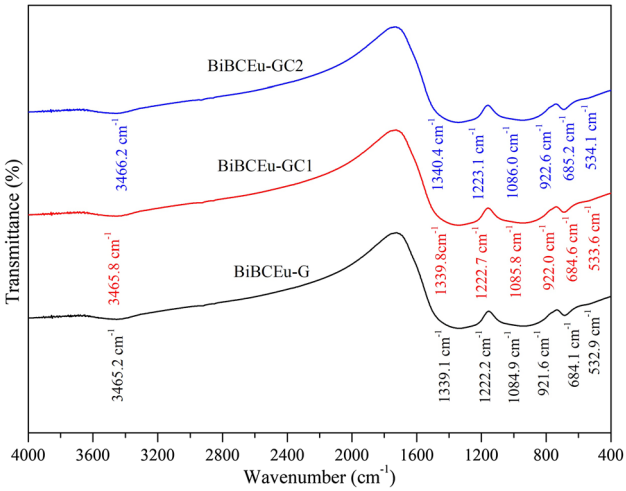

Fig. 2 FT-IR spectral profiles of BiBCEu-G and GCs

The Raman band positions in the region 128.1–337.5 cm^{-1} confirm the formation of CaF_2 nanocrystallites [18, 19], while the Raman bands noticed in the region 691.2–758.4 cm^{-1} corresponds to $Bi_3B_5O_{12}$ nanocrystallites [20, 21].

The realization of nanocrystallites alongside the glassy phase has also been examined from the TEM image of BiBCEu-GC2 and it is shown in Fig. 4a. This image shows a uniform distribution of nanocrystallites. The lattice spacing corresponding to (111) crystal plane [$d_{(111)}$] is estimated to be 0.318 nm using HR-TEM micrograph shown in Fig. 4b and it is close to CaF_2 nanoparticles [22]. The selected area electron diffraction (SAED) image has been used to find the (h k l) crystal planes. It is well known that the $d_{(hkl)}$ is equal to the reciprocal of radius of SAED ring. The

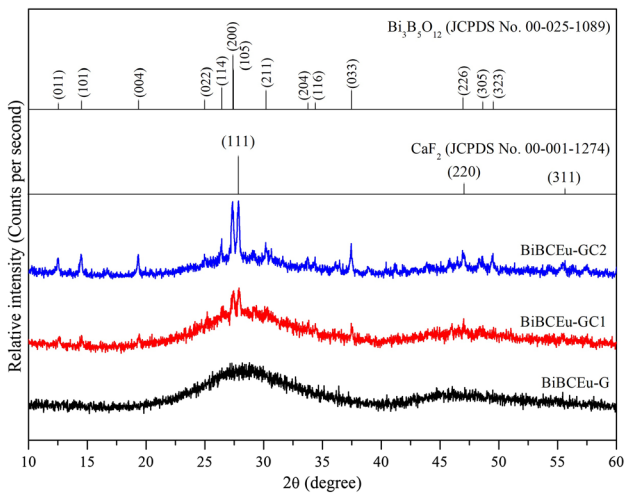

Fig. 1 PXRD profiles of BiBCEu-G and GCs

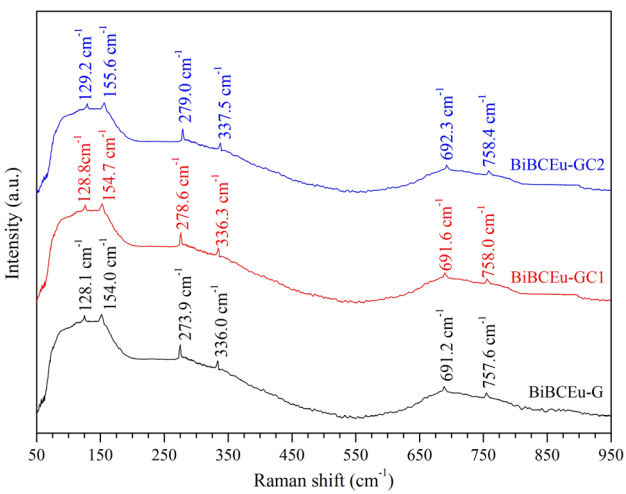

Fig. 3 Raman spectral profiles of BiBCEu-G and GCs

observed SAED rings have been ascribed to (111) (220) and (311) lattice planes of CaF_2 nanoparticles and they are described in Fig. 4c.

It is well known that the fluoride nanocrystals facilitate low phonon energy environments for RE ions and the glasses and GCs with good transparency and relatively low phonon energy could be an attractive material for numerous photonic appliances. The addition of F^- reduce the phonon energy and hence the OH^- content resulting to enhance the luminescence properties of RE ions [23]. J.L Adam et al. [24] reported that the thermal stability of amorphous materials improves with the addition of F^-. The channel waveguide materials based on fluoride and oxyfluoride glasses were prepared with 0.3 dB/cm background loss and 1 dB/cm net gain at 1.5 μm for integrated optical components [25]. These reports reveal that the presence of F^- content can enhance the thermal and optical properties by minimizing background losses. The formation and characteristic behaviour of CaF_2 nanocrystals in oxyfluoride glasses for various photonic applications were reported [3]. The phonon energy of oxide based bismuth borate glass is about 1466 cm^{-1} [26]. In the

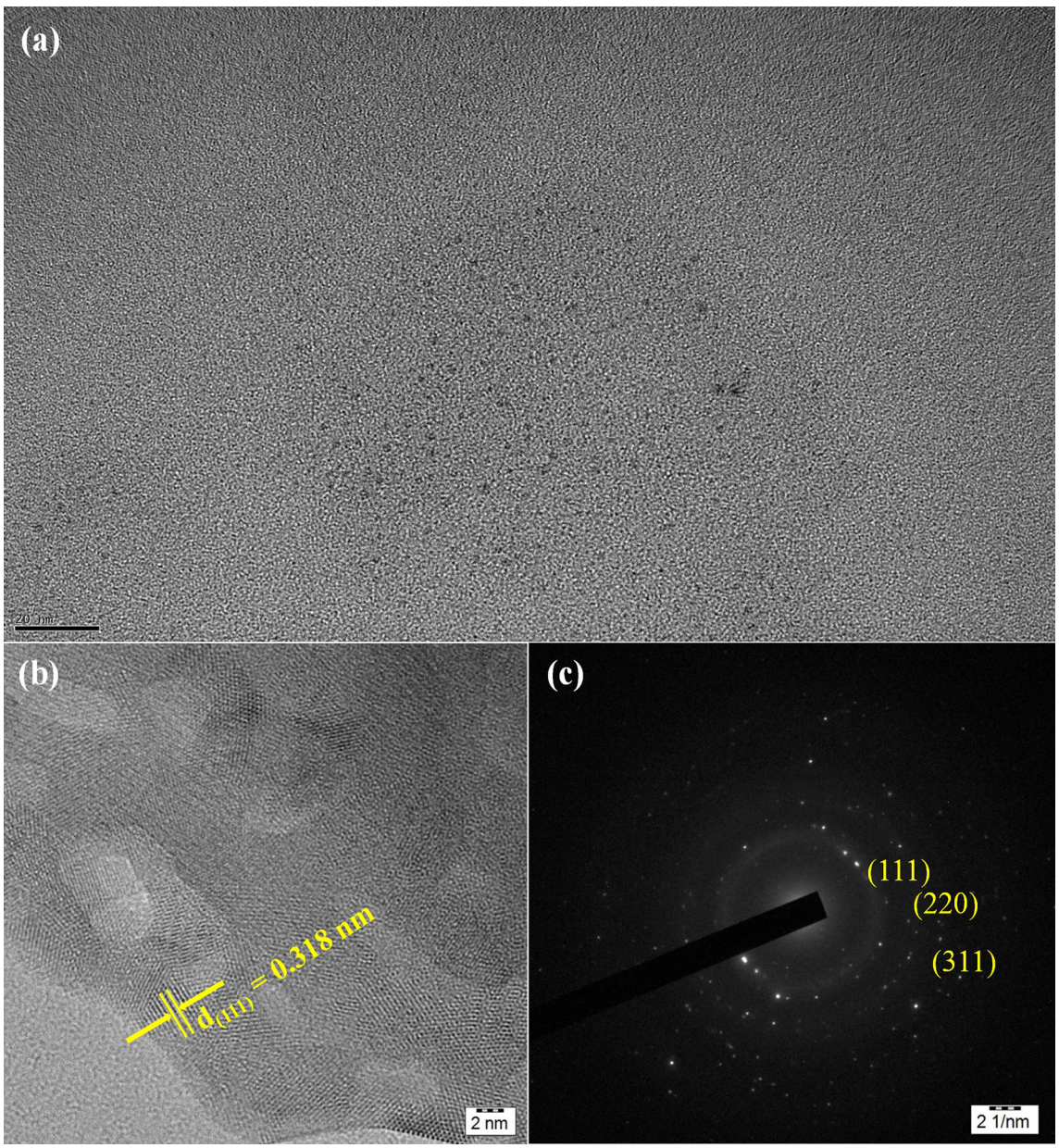

Fig. 4 TEM (**a**), HR-TEM (**b**) and SAED (**c**) images of BiBCEu-GC2

present investigation, the inclusion of fluorine amount significantly lowers the phonon energy of Bi_2O_3-B_2O_3 glass system and it is obtained as 1334.67 ± 0.93 cm^{-1} [5].

3.2 Near UV–VIS absorption spectra

The near UV–VIS optical absorption spectra of BiBCEu-G and GCs in the wavelength range from 375 to 600 nm are shown in Fig. 5. The inset-(a) of Fig. 5 illustrates the magnified spectral region from 500 to 600 nm. These spectra reveal two groups of very weak absorption bands originating from the $^7F_0$ ground energy state and the $^7F_1$ first excited energy state. The observed absorption bands have been attributed to the $^7F_0 \rightarrow {}^5L_6$ (about 395 nm), $^7F_0 \rightarrow {}^5D_3$ (about 420 nm), $^7F_0 \rightarrow {}^5D_2$ (about 465 nm), $^7F_0 \rightarrow {}^5D_1$ (about 525 nm), $^7F_1 \rightarrow {}^5D_1$ (about 537 nm), $^7F_0 \rightarrow {}^5D_0$ (about 579 nm) and $^7F_1 \rightarrow {}^5D_0$ (about 590 nm) transitions [27]. The presence of these absorption transitions is an indication for the uniform distribution of Eu^{3+} ions in BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2 samples. Usually, the presence of surface defects (if any) results a broadening of absorption band. In the present research work, the broadening of absorption bands is almost negligible indicating fever surface defects due to the crystallization effect by the heat treatment. From these spectra it is obvious that the absorbance of the samples reduce with the enhancement of crystallization of $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites. The inset-(b) of Fig. 5 illustrates the variation of absorbance of $^7F_0 \rightarrow {}^5D_2$ transition with crystallization. In general, Eu^{3+} ions in any host matrix exhibit very weak absorption transitions in near UV–VIS region [4, 11, 28]. In case of Eu^{3+} ions, the estimation of oscillator strengths and hence the J-O intensity parameters become difficult using near UV–VIS absorption spectra. However, the absorption spectral profiles have been used to evaluate optical band gap energies of BiBCEu-G and GCs.

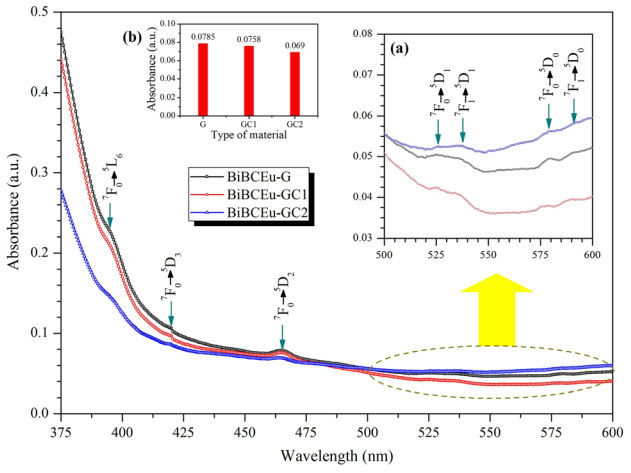

Fig. 5 UV–VIS absorption spectra of BiBCEu-G and GCs. Insets show the magnified spectral region from 500–600 nm (**a**) and the variation of absorbance of $^7F_0 \rightarrow {}^5D_2$ transition with crystallization (**b**)

In order to know the suitability of a luminescent material for an optical application, the knowledge of its optical band gap energy (E_g) is essential and it is expressed in terms of photon energy (hν) and the fundamental absorption edge coefficient (α) as $\alpha . (h\nu) = B(h\nu - E_g)^r$, where B is the energy independent parameter. The parameter r is 1/2 for direct allowed, 1/3 for direct forbidden, 2 for indirect allowed and 3 for indirect forbidden transitions [29]. The $(\alpha.h\upsilon)^2$ vs $h\upsilon$ plots are known as the Tauc's curves. When the linear region of Tauc's curve is extrapolated to $(\alpha.h\upsilon)^2 = 0$, then the corresponding energy is known as direct allowed E_g. The values of E_g are obtained as 2.820, 2.444 and 2.183 (± 0.01) eV for BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2, respectively from the Tauc's plots for $r = 1/2$ illustrated in Fig. 6. Similar kind of results have been reported for Eu^{3+}-doped $BaBi_2Ta_2O_9$ based glass–ceramics in which the value of direct band gap energy decreases from 2.82 eV for as prepared glass to 1.62 eV for GC heat treated at 550 °C for 12 h [30]. For Eu^{3+} -doped zinc silicate derived from waste rice husks, the direct band gap energy value varies from 4.14 eV for a glass to 3.71 eV for GC heat treated at 1000 °C for 3 h [31]. For Er^{3+} -doped GBANZ glass–ceramics, the direct band gap energy value changes from 3.7750 eV for glass to 3.5220 eV for GC heat treated at 595 °C for 1 h [32]. The gradual reduction in E_g value with increase of crystallization time could be due to the presence of unoccupied energy states below the conduction band edge which causes a structural modification around the Eu^{3+} ions.

3.3 Luminescence analysis

The photoluminescence excitation (PLE) spectra of BiBCEu-G and GCs monitoring the emission corresponding to Eu^{3+}: $^5D_0 \rightarrow {}^7F_2$ (616 nm) transition are shown in Fig. 7. These spectra reveal a total of nine PLE bands due to $^7F_0 \rightarrow {}^5D_4$ (about 363 nm),

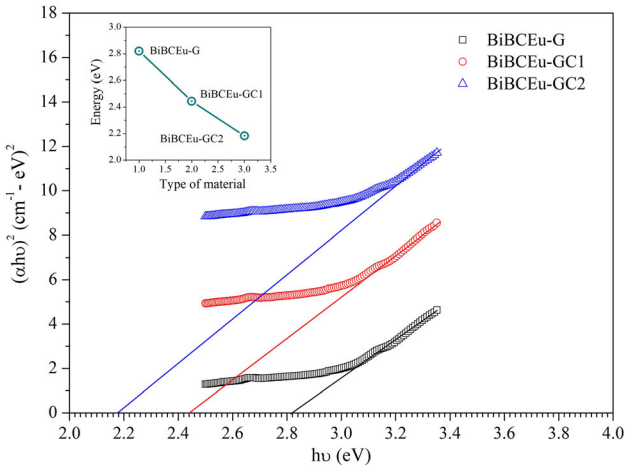

Fig. 6 Tauc's plots for direct allowed transitions for BiBCEu-G and GCs. Inset shows the variation of band gap energy as a function of crystallization

$^7F_0 \rightarrow {}^5G_2$ (about 383 nm), $^7F_0 \rightarrow {}^5L_6$ (about 396 nm), $^7F_0 \rightarrow {}^5D_3$ (about 416 nm), $^7F_0 \rightarrow {}^5D_2$ (about 466 nm), $^7F_0 \rightarrow {}^5D_1$ (about 527 nm), $^7F_1 \rightarrow {}^5D_1$ (about 534 nm), $^7F_0 \rightarrow {}^5D_0$ (about 579 nm) and $^7F_1 \rightarrow {}^5D_0$ (about 589 nm) transitions [27]. The imperfections caused by the crystallization process with heat treatment have no considerable influence on peak maxima of observed PLE bands due to the availability of number of vibrational levels. However, the strength and/or intensity of PLE bands improve with the enhancement of crystallization without causing any shift in their peak maxima. The inset of Fig. 7 describes the variation of strength of $^7F_0 \rightarrow {}^5L_6$ (396 nm) transition as a function of crystallization. From the PLE spectra one can notice that the BiBCEu-GC2 exhibits prominent and high intensity emission bands than the other glass and GC samples. It could be due to the more crystalline atmosphere around the Eu^{3+} ions. The prominent excitation band noticed at 396 nm (Eu^{3+}: $^7F_0 \rightarrow {}^5L_6$) indicates that the studied BiBCEu-G and GCs can show an efficient luminescence when excited at 396 nm radiation.

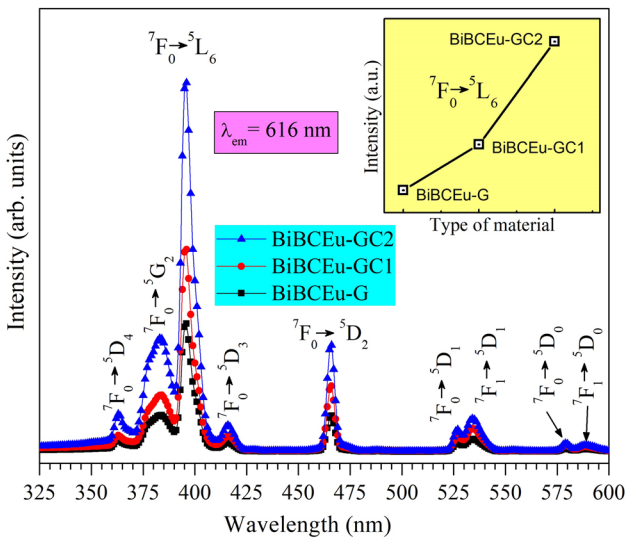

Fig. 7 Photoluminescence excitation spectra (λ_{em} = 616 nm) of BiBCEu-G and GCs. Inset shows the variation of intensity of $^7F_0 \rightarrow {}^5L_6$ (396 nm) transition as a function of crystallization

Upon 396 nm excitation, the emission spectra of BiBCEu-G and GCs in the spectral range from 550 to 750 nm are presented in Fig. 8. The emission spectra displayed a total of five emission bands at 580, 594, 616, 653 and 700 nm and they are assigned to Eu^{3+}: $^5D_0 \rightarrow {}^7F_0$, Eu^{3+}: $^5D_0 \rightarrow {}^7F_1$, Eu^{3+}: $^5D_0 \rightarrow {}^7F_2$, Eu^{3+}: $^5D_0 \rightarrow {}^7F_3$ and Eu^{3+}: $^5D_0 \rightarrow {}^7F_4$ transitions, respectively. From these spectra it is known that the BiBCEu-GC2 sample exhibits intense luminescence when excited at 396 nm radiation. From the emission spectral profiles one can notice a negligible red shift in emission band positions towards longer wavelength regions. This red shift is mainly due to the presence of crystal imperfections caused by the different heat treatment processes. The observed E_g values of 2.820, 2.444 and 2.183 (± 0.01) eV for BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2, respectively support these results. The inset of Fig. 8 illustrates the emission mechanism of Eu^{3+} in BiBCEu-G and GCs. The process of emission of Eu^{3+} ions in BiBCEu-G and GCs takes place as follows.

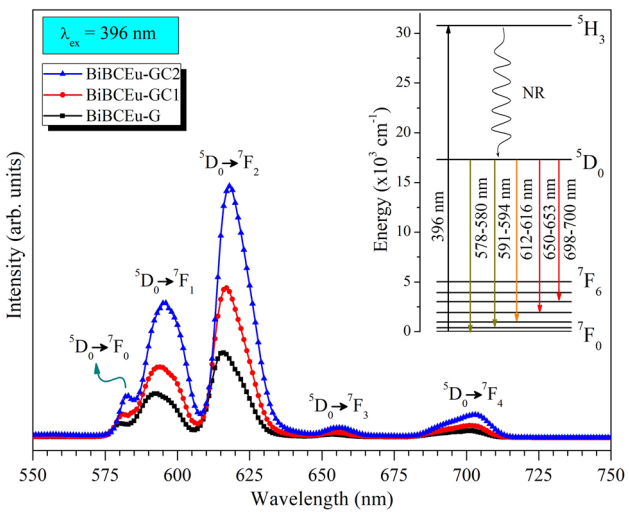

Fig. 8 Emission spectra (λ_{ex} = 396 nm) of BiBCEu-G and GCs. Inset shows the partial energy level diagram of Eu^{3+} ions

Up on pumping at 396 nm wavelength, the Eu^{3+} ions have been excited to higher energy level that lies above the $^5D_0$, then a quick non-radiative (NR) multi-phonon transition occurs to $^5D_0$ emission level ensuing $^5D_0 \rightarrow {}^7F_J$ radiative relaxations by quenching the other emission transitions through ($^5D_3$,$^5D_2$,$^5D_1$) $\rightarrow {}^7F_J$ transitions (not shown in Fig. 8). Thus, the emitted luminescence can be assumed as the cumulative emission of $\Sigma^5D_0 \rightarrow {}^7F_J$ transitions. The multi-phonon relaxation from $^5D_0$ level to its lower lying $^7F_6$ level is disparate due to the huge energy gap of ~ 12,282 cm^{-1}. Since the maximum phonon energy of BiBCEu-G and GCs is of the order ~ 1334.67 cm^{-1}, nearly nine phonons are necessary to bridge the energy gap between $^5D_0$ emission level and $^7F_6$ lower lying level.

The studied samples display intense orange-red emission with CIE (Commission-International de I'Eclairagein) colour coordinates nearly (x = 0.64, y = 0.35), and they are appropriately situated in the orange-red region of CIE diagram described in Fig. 9. These CIE colour coordinates are close to Y_2O_3: Eu^{3+} (x = 0.64, y = 0.34) (KX-YOX, Kasei-Optonix Ltd. Japan), (Y,Gd)BO_3: Eu^{3+} (x = 0.65, y = 0.34) (KX-504, Kasei-Optonix Ltd. Japan) and ideal red emitting source (x = 0.67, y = 0.33) (National Television Standard Committee). They are also close to GC610 (x = 0.625, y = 0.374) [12], $Na_3Gd(PO_4)_2$: Eu^{3+} GCs (x = 0.6233, y = 0.3701) [33] and Al_2O_3: Eu^{3+} ceramic (x = 0.645, y = 0.355) [34].

It is familiar that the intensity of electric dipole (E-D) transition is more responsive and affected by the site symmetry of host where as the intensity of magnetic dipole (M-D) transition is not responsive and do not varies with the site symmetry of host. In case of Eu^{3+}, the $^5D_0 \rightarrow {}^7F_2$ transition is an electric dipole ($\Delta J = 2$) and $^5D_0 \rightarrow {}^7F_1$ is a magnetic dipole ($\Delta J = 1$) in nature. The intensity ratio, $I_R = I_{E-D}/I_{M-D} = I_{(^5D_0 \rightarrow {}^7F_2)}/I_{(^5D_0 \rightarrow {}^7F_1)}$ represents the site symmetry around the Eu^{3+} ions in the given host matrix. Thus, the Eu^{3+} ion can acts as a spectroscopic probe to investigate the local site symmetry of host matrix. Lower the value of I_R higher will be the symmetry around the Eu^{3+} ion in a given host matrix [35]. The value of I_R is found to be 1.80, 1.75 and 1.68 for BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2, respectively. These results indicate that the site symmetry of the host around the Eu^{3+} ions increases with the increase of crystallization due to the controlled heat treatment. Symmetric environment around the Eu^{3+} ions has been reported for different host matrices in the literature [28, 36–39].

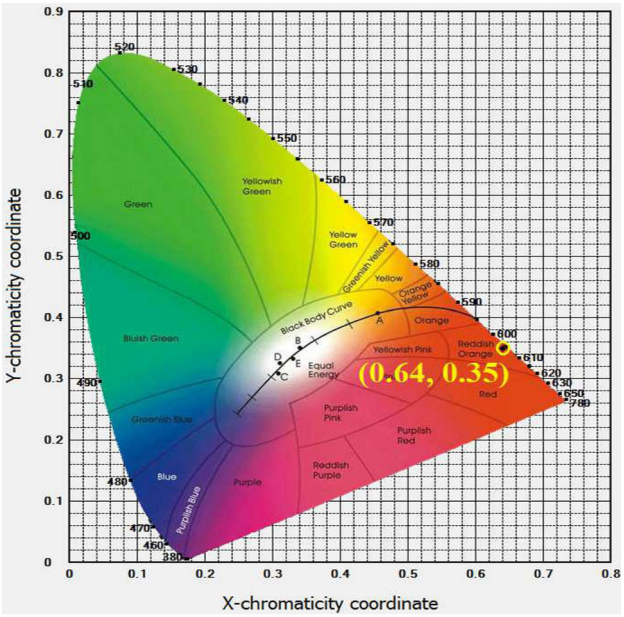

Fig. 9 CIE diagram of BiBCEu-G and GCs

3.4 Judd–Ofelt and radiative parameters

The three phenomenological J-O parameters are the characteristics of RE ions in a specified host and they correspond to the radial wave functions of the $4f^N$ energy levels, the admixing $4f^{N-1}5d$ energy levels and the ligand field parameters that describe the host matrix. In case of Eu^{3+} ion, the J-O parameters have been determined using the relation among $^5D_0 \rightarrow {}^7F_J$ emission transitions and they are used to evaluate the radiative parameters such as radiative emission probability (A_R), radiative luminescence branching ratio (β_R) and radiative decay time (τ_R). The radiative emission rate from the $^5D_0$ upper level to the.^7F_J lower lying levels is given as [8, 9]

$$A_R = \frac{64\,\pi^4\,v^3}{3h\,(2J+1)}\left[\frac{n\,(n^2+2)^2}{9}\right]\sum_{\lambda}\left|\left\langle {}^5D_0\left\|U^{(\lambda)}\right\|{}^7F_J\right\rangle\right|^2 \quad (1)$$

where n is the index of refraction, $\left\|U^{(\lambda)}\right\|$ represents the reduced matrix elements and J is the sum of spin and orbital angular momenta of ground energy level. Though the index of refraction is wavelength dependent parameter, a constant value has been taken to evaluate the various spectroscopic

parameters which cause a negligible deviation in the obtained Ω_λ values. This deviation is mainly due to the small variation in the value of index of refraction in the studied wavelength region, i.e., 580–720 nm. The Ω_λ values have been estimated using the intensities of $^5D_0 \rightarrow {}^7F_J$ emission transitions of Eu^{3+} ion using the relation.

$$\frac{\int I_\lambda \, dv}{\int I_1 \, dv} = \frac{e^2}{S_{md}} \left(\frac{v_\lambda}{v_1}\right)^3 \frac{n\,(n^2+2)^2}{9\,n^3} \Omega_\lambda \left|\left\langle {}^5D_0 \left\| U^{(\lambda)} \right\| {}^7F_\lambda \right\rangle\right|^2 \quad (2)$$

where, $\int I_\lambda \, dv$ and $\int I_1 \, dv$ illustrate the intensity of emission leap at 396 nm excitation (i.e., $^5D_0 \rightarrow {}^7F_\lambda$ (λ = 2,4,6) and $^5D_0 \rightarrow {}^7F_1$ emission transitions, respectively). The parameter S_{md} represents the magnetic dipole line strength of $^5D_0 \rightarrow {}^7F_1$ emission transition. v_1 and v_λ are the energies corresponding to the $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_\lambda$ transitions, respectively. The Ω_λ values have been evaluated considering $\left\|U^{(6)}\right\|^2 \approx 0.0003$ for $^5D_0 \rightarrow {}^7F_6$ transition and zero for other transitions such as $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_4$. The $^5D_0 \rightarrow {}^7F_6$ emission band has peak maximum in near infrared (− 800 nm) and it is not obtained owing to the limitations of measuring instruments. Thus, the Ω_2 and Ω_4 values related to $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_4$ transitions, respectively have been determined using the matrix elements of $\left|\left\langle {}^5D_0 \left\| U^{(2)} \right\| {}^7F_2 \right\rangle\right|^2 = 0.0032$ and $\left|\left\langle {}^5D_0 \left\| U^{(4)} \right\| {}^7F_4 \right\rangle\right|^2 = 0.0023$ [40, 41]. The luminescence branching ratio ($\beta_R = \tau_R \times A_R$) and the radiative decay time ($\tau_R = 1/\Sigma A_R$) have also been obtained using the J-O theory. The values of J-O parameters and some significant radiative parameters of BiBCEu-G and GCs are also summarized in Table 1.

The obtained Ω_2 intensity parameter of BiBCEu-GC2 is comparable to GCLSCAS [42], Eu^{3+}: NAT ceramic [43] and oxyfluoroborate GC [44]. It is found higher than GC400 [45] and lower than Eu^{3+}: Tellurite Ceramic [46], GC24h [11] and TBBS: 2Eu (GC) [47]. A comparison of J-O intensity parameters in various glass–ceramics is given in Table 2. The measured values of branching ratios (β_m) have been obtained using the relative areas covered by emission bands are found close to the β_R (see Table 1). The branching ratio values have been utilized to study the strength of a stimulated emission transition. An emission transition with $\beta_m > 0.50$ possess strong stimulated emission. The measured and predicted values of branching ratios of the order − 0.60 advise that the BiBCEu-GC2 is suitable for intense orange-red laser sources.

The stimulated emission cross-section (σ_e) which is used to identify a laser active media have been evaluated using the following equation.

$$\sigma_e = \frac{\lambda_P^4 \times A_R}{8\,\pi\, c\, n^2\, \Delta\lambda_P} \quad (3)$$

where λ_P represents the peak emission wavelength, c represents the light speed and $\Delta\lambda_P$ represents the effective line-width (or) full width at half maximum (FWHM) of an emission transition. The gain parameters such as band width gain ($\sigma_e \times \Delta\lambda_P$) and optical gain ($\sigma_e \times \tau_m$) have been used to study the amplification of gain medium where RE ions are situated. In current research work, the optical gain is found almost constant, while the band width gain decreases with increase of crystalline environment around the Eu^{3+} ions. The evaluated values of $\Delta\lambda_P$, σ_e, ($\sigma_e \times \Delta\lambda_P$) and ($\sigma_e \times \tau_m$) are also summarized in Table 1. The decrease in σ_e with increase of heat treatment results an intense luminescence in a narrow region. The value of σ_e obtained for BiBCEu-GC2 is comparable to PTBEu glass [48] and higher than RLTB [49] glass.

3.5 Decay time analysis

The luminescence decay profiles of Eu^{3+}: $^5D_0$ metastable energy state in BiBCEu-G and GCs obtained by exciting at 396 nm monitoring the emission at 616 nm ($^5D_0 \rightarrow {}^7F_2$) are displayed in Fig. 10. From the decay profiles one can notice that all the decay curves are well suited for single exponential nature which reveals insignificant non-radiative (NR) losses. The magnitudes of decay time have been determined to be 1.527, 1.534 and 1.594 ms for BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2, respectively by suitably fitting to single exponential function.

The luminescence quantum efficiency (η) which is the ratio of measured decay time (τ_m) to the radiative decay time (τ_R) is one of the most significant laser characteristic parameter and it is the measure of number of photons emitted per excited ion. The quantum efficiency for Eu^{3+}: $^5D_0 \rightarrow {}^7F_2$ (616 nm) emission transition is found to be 39.97, 40.05 and 41.51% for BiBCEu-G, BiBCEu-GC1 and BiBCEu-GC2, respectively. The value of η obtained for BiBCEu-GC2 (η = 41.51%) is comparable to $BaMoO_4$:-Eu^{3+} GC (46.3%) [10], GC24h (48%) [11] and PTBEu glass (40%) [48]. Based on the experimental results it

Table 1 J-O intensity parameters and radiative properties for $^5D_0 \rightarrow {}^7F_2$ (616 nm) emission transition of Eu^{3+} in BiBCEu-G and GCs

Parameter	BiBCEu-G	BiBCEu-GC1	BiBCEu-GC2
Ω_2 ($\pm$ 0.02 $\times$ 10^{-20}) cm^2	2.66	2.59	2.46
Ω_4 ($\pm$ 0.02 $\times$ 10^{-20}) cm^2	0.35	0.36	0.38
Ω_6 ($\pm$ 0.02 $\times$ 10^{-20}) cm^2	− 0	− 0	− 0
FWHM ($\pm$ 0.05 nm)	10.93	10.60	10.80
A_R ($\pm$ 0.08 s^{-1})	163.80	159.86	157.47
β_R ($\pm$ 0.01)	0.62	0.62	0.61
β_m ($\pm$ 0.03)	0.61	0.62	0.60
τ_R ($\pm$ 0.01 ms)	3.82	3.83	3.84
τ_m ($\pm$ 0.006 ms)	1.527	1.534	1.594
η ($\pm$ 0.01%)	39.97	40.05	41.51
σ_e ($\pm$ 0.01 $\times$ 10^{-22} cm^2)	8.37	8.34	7.94
($\sigma_e \times \Delta\lambda_P$) ($\pm$ 0.01 $\times$ 10^{-28} cm^3)	9.15	8.84	8.57
($\sigma_e \times \tau_m$) ($\pm$ 0.01 $\times$ 10^{-24} $cm^2 s$)	1.27	1.27	1.26

Table 2 Comparison of J-O intensity parameters (Ω_λ) in various GCs

Host matrix		$\Omega_\lambda \times 10^{-20}$ cm^2		Trend
		Ω_2	Ω_4	
BiBCEu-GC2	[This work]	2.46	0.38	$\Omega_2 > \Omega_4$
GCLSCAS	[42]	1.85	4.67	$\Omega_2 < \Omega_4$
Eu^{3+}: NAT ceramics	[43]	2.95	0.39	$\Omega_2 > \Omega_4$
Oxyfluoroborate GC	[44]	2.10	4.05	$\Omega_2 < \Omega_4$
GC400	[45]	1.38	0.84	$\Omega_2 > \Omega_4$
Eu^{3+}: Tellurite Ceramic	[46]	4.30	3.23	$\Omega_2 > \Omega_4$
GC24h	[11]	4.17	2.37	$\Omega_2 > \Omega_4$
TBBS: 2Eu (GC)	[47]	3.21	1.37	$\Omega_2 > \Omega_4$

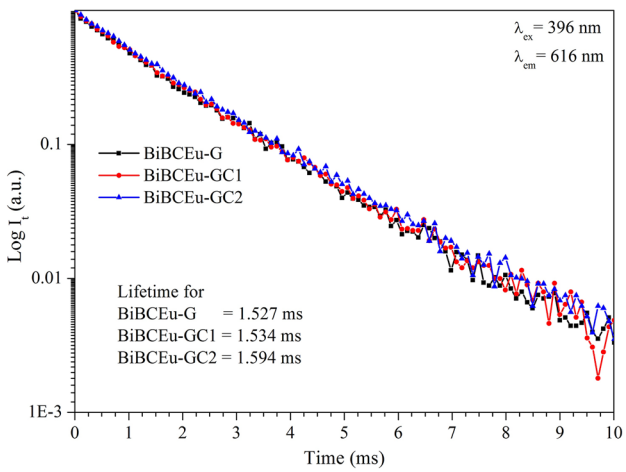

Fig. 10 Luminescence decay profiles of BiBCEu-G and GCs

can be suggestible that the BiBCEu-GC2 has high potentiality to produce intense orange-red luminescence (− 616 nm) when excited at 396 nm radiation.

4 Conclusions

The BiBCEu-G and GCs have been prepared to investigate the crystallization effect on orange-red emission of Eu^{3+} ions. After appropriate heat treatment at 575 °C for 10 h, the $Bi_3B_5O_{12}$ and the CaF_2 nanocrystallites distributed homogeneously among the glassy matrix. Observed intensity ratios of $^5D_0 \rightarrow {}^7F_2$ to $^5D_0 \rightarrow {}^7F_1$ transitions indicate that the symmetry of the host environment around the Eu^{3+} ions improve with the enhancement of duration of heat treatment and hence the crystallization of $Bi_3B_5O_{12}$ and CaF_2 nanocrystallites. Upon 396 nm excitation, the 616 nm emission (Eu^{3+}: $^5D_0 \rightarrow {}^7F_2$) significantly enhanced by the improved crystallization. Obtained results showed that the BiBCEu-GC2 containing 1.0 mol% of Eu^{3+} ions is advisable to design orange-red laser sources.

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Author contributions

BCJ: Conceptualization, Methodology, Validation, Investigation, Writing-Original draft preparation, Writing-Review & Editing, Visualization, Supervision, Project administration, NM: Conceptualization, Investigation, Resources, Writing-Review & Editing, SA: Conceptualization, Investigation, Resources, Writing-Review & Editing, KVR: Conceptualization, Investigation, Resources, Writing-Review & Editing, KP: Conceptualization, Investigation, Writing-Review & Editing, Visualization, Project administration.

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Data availability

Data will be made available on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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Free Radical Scavenging Activity and Phytochemical Screening of *Trigonella foenum-graecum*: A Study with Reference to Drug Discovery

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Trigonella foenum-graecum **(TG) is one of the important medicinal plant which have antioxidant, antidiabetic, antilipidemic and antimicrobial properties. The main aim of this study was to evaluate the free radical scavenging properties, the total phenolic, total flavonoid content and phytochemical screening of *Trigonella foenum-graecum*. The antioxidant activities like 2,2-diphenylpicrylhydrazyl (DPPH), hydroxyl activity, hydrogen peroxide, superoxide and reducing power activities are investigated in the methanolic extract and aqueous extract of TG. Results of this study showed that TG possess good free radical scavenging activity in methanolic extract than aqueous extract. Significant levels of phenolic and flavonoid contents were found in TG. The phytochemicals like alkaloids, tannins, phenolic compounds etc. are present in methanolic extract and aqueous extract of TG. Our investigation indicates that free radical scavenging activities, phenolic and flavonoid compounds in TG may play an important role in reducing the oxidative stress in diseases like diabetes, cancer and heart attack.**

Keywords: Free radical scavenging activity, Phytochemicals, *Trigonella foenum-graecum.*

INTRODUCTION

Free radicals are highly reactive and unstable molecules that are made by the body naturally as a byproduct of normal metabolism. It is well known that reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) play a major role in the development of oxidative stress that can lead to many diseases like diabetes, anaemia, cancer and ischemia. Many allopathic drugs have been used to treat these diseases, but in long run they have side effects on liver, kidney, heart and lungs. These drugs are costly, less available and they have more side effects. In this connection natural antioxidants received a prominence as they are often free from side effects, less expensive and abundant in nature i.e. found in medicinal plants.

Medicinal plants like ginger, ocimum, phyllanthus and trigonella etc have many bioactive compounds, pharmacological compounds, phytochemicals, vitamins and minerals. These compounds have many pharmacological properties, these are responsible for various beneficial effect against diseases. They promote human health through their antioxidant properties and their ability to modulate the activity of various enzymes. The main characteristic of an antioxidant in the body is its ability to trap free radicals which may oxidize nucleic acids, proteins, lipids or DNA and protect the cells from free radical toxicity.

Trigonella foenum-graecum is well known for its pharmacological activities like antidiabetic, antioxidative, hypocholesterolemic, antineoplastic, anti-inflammatory, antiulcerogenic, antipyretic, immunomodulatory and antitumor (Ramesh et al., 2021). Trigoenella have many active components like disogenin, gitogenin, neogitogenin, neogigogenin, trigogenin, fibers, flavonoids, polysaccharides, fixed oils and alkaloids, like trigonelline and choline. Hence, screening of herbal plant extracts for compounds with antioxidant and free radical scavenging activities may yield new leads to target and combat diseases.

Thus, the present investigation was undertaken to evaluate the free radical scavenging effect,

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total phenolic content and flavonoid content and phytochemical screening in methanolic extract and aqueous extract of TG.

MATERIALS AND METHODS

Collection of Plant Material

Trigonella foenum-graecum was collected locally and identification was confirmed by Dr. Madhav Chetty, Department of Botany, S.V. University, Tirupati, Andhra Pradesh. *Trigonella foenum-graecum* seeds are air dried in the shade, coarsely powdered and soaked in methanol for three days at room temperature. The extract was filtered and the solvent was removed using rotary evaporator at 65°C. The yield was 10% (w/w). The same procedure was followed for aqueous extract also. The yield for aqueous extract was 8% (w/w). The methanolic extract and aqueous extract of TG was used for free radical scavenging activities, quantitative estimation of phenolic compounds and flavonoids in *Trigonella foenum-graecum*. Screening of phytochemicals are also performed in methanolic extract and aqueous extract TG.

Chemicals

All the chemicals used in this study are of analytical grade and obtained from Sigma-Aldrich (St. Louis, USA), Merck, qualigens, fischer, etc.

Determination of Free Radical Scavenging Activity

DPPH, H_2O_2, hydroxyl radical, reducing power and superoxide radical activities are estimated in methanolic extract and aqueous extract of *Trigonella foenum-graecum* by the methods of Brand-Williams et al., (1995), Ruch et al., (1989), Klein et al., (1981), Hyland (1983) and Oyaizu (1986).

Quantitative Estimation of Total Phenols and Flavonoids

The total phenolic content of *Trigonella foenum-graecum* seeds was determined by Folin Ciocalteu method as described by Sarikurkcu et al., (2008). The total flavonoid content was determined by the method as described by Dahmoune et al., (2015).

Screening of Phytochemicals

In the present study, methanolic extract and aqueous extract was used to screen the phytochemicals in trigonella by using standard protocols.

RESULTS

DPPH, hydroxyl radical, hydrogen peroxide, reducing power and superoxide showed dose depended free radical activity in the methanolic extract and aqueous extract of TG. However, methanolic extract showed more free radical scavenging activity than aqueous extract (Figure 1).

In our study, total phenol (22.4 µg/100 g extract) and flavonoid (11.2 µg/100 g extract) in the methanol extract and total phenol (16.6µg/100g extract) and flavonoid (9.8µg/100g extract) in aqueous extract of TG was reported (Table 1).

Phytochemical screening of methanolic extract showed the presence of flavonoids, saponins glycosides, alkaloids, tannins, terpenoids, steroids and phenolic compounds, cardiac glycosides, anthraquinones, reducing sugars and quinines. Where as in aqueous extract all phytochemicals are seen except steroids and anthraquinones (Table 2).

DISCUSSION

Since, the dawn of civilization medicinal plants are used to treat many diseases. They have long been known to promote human health and wellness. Indian, Chinese and many traditional cultures in the world are using plant based treatment to treat many diseases. The present study aimed to investigate the free radical scavenging effect of TG and evaluation of total phenolic and flavonoid contents in methanolic extract and aqueous extract.

Free radicals are produced as byproducts in biological systems during metabolic processes that can cause damage the tissues and biomolecules leading to various severe diseases like diabetes, cancer, inflammation, hepatitis and liver diseases. DPPH is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH assay is a common antioxidant assay and is a one of the important and well-known radical. DPPH is a deep violet color in solution, and it becomes colourless, when neutralized and converted into DPPH-H. Many medicinal plant

extracts have been reported to possess DPPH free radical scavenging activity. In our study, methanolic extract and aqueous extract of TG scavenged DPPH radicals in a concentration-dependent manner. However, methanolic extract showed more activity than aqueous extract. Similarly, ginger, phyllanthus, etc also containing a number of polyphenols which are responsible for scavenging the DPPH free radicals. (Kedare and Singh, 2011) (Figure 1).

Hydroxyl radicals are highly reactive and are short lived. They have detrimental effects on the important macromolecules like proteins and nucleic acids. In the Haber-Weiss/Fenton reaction in the presence of iron ions, hydroxyl radicals are generated from hydrogen peroxide. The high reactivity of hydroxyl radicals lead to tremendous damage to the cell and its components and subsequently to the organisms as a whole. Therefore, it is very important to remove hydroxyl radicals which cause detrimental effects. In our study, we reported that TG methanloic extract showed more hydroxyl scavenging effect than aqueous extract (Sies, 1997; Lipinski, 2011) (Figure 1).

Superoxide anion, a reduced form of molecular oxygen. Superoxide is one of the important radical generated in the cell. It has been implicated in begin the process of oxidation associated with aging. Superoxide anions play an important role in the formation of other reactive oxygen species such as singlet oxygen, hydrogen peroxide and hydroxyl radical, which induce oxidative damage in DNA, lipids and proteins (Pietta, 2000). The reducing power of the plant extract components might serve as a significant indicator of its potential antioxidant activity. Higher reducing powers in medicinal plants imply that it has higher amounts of total phenolic and flavonoid compounds. In our study, we reported that methanolic extract showed more superoxide activity and reducing power activity than aqueous extract (Figure 1).

In our present study it was observed that methanolic extract and aqueous extract of TG showed maximum activity towards scavenging of DPPH, superoxide radical, hydroxyl, hydrogen peroxide and reducing power.

Medicinal plants have phenolic compounds in fruits, leaves, stem and roots, because they possess potential biological activity. Phenolic compounds such as flavonoids, phenolic acid, and tannins show diverse biological activities like anti-inflammatory, anti-carcinogenic, and antiatherosclerotic activities. These activities might be related to their antioxidant activity (Cai et al., 2004). The yield percent, total phenolic and flavonoid content of the extracts obtained from *Trigonella foenum-graecum* are shown in Table 1.Total phenolic content estimation indicated higher concentration of polyphenols in methanolic extract and aqueous extract. Our results are concomitant with previous findings, where high content of phenols in methanolic extract and aqueous extract of TG was compared to methanolic extract and aqueous extract of many plants like ginger, ocimum, etc.

Our study has revealed the presence of phytochemicals in methanolic extract and aqueous extract of *Trigonella foenum-graecum*. The phytochemicals like reducing sugar, flavonoids, alkaloids, terpenoids, cardiac glycosides, phenols, and steroids are present in trigonella. These phytochemicals are biologically active (Table 2). These phytochemicals have many pharmacological actions against different diseases.

The presence of these phytochemicals has been attributed to the bioactive principles responsible for ethnopharmacological activities of medicinal plants. The medicinal value of plants are dictated by their phytochemicals and other chemical constituents (Snoussi et al., 2015). The importance of alkaloids, saponins and tannins in various medicinal plants used in treat many diseases like diabetes, bronchitis and many more diseases have been reported.

From our study, we reported that methanolic extract and aqueous extract of TG have good free radical scavenging activities. It has phenolic compounds and flavonoid compounds which are responsible for their beneficial effect against many diseases. TG also have many phytochemicals which are reported to possess pharmacological activities. Hence, *Trigonella foenum-graecum* is beneficial to treat free radical related diseases.

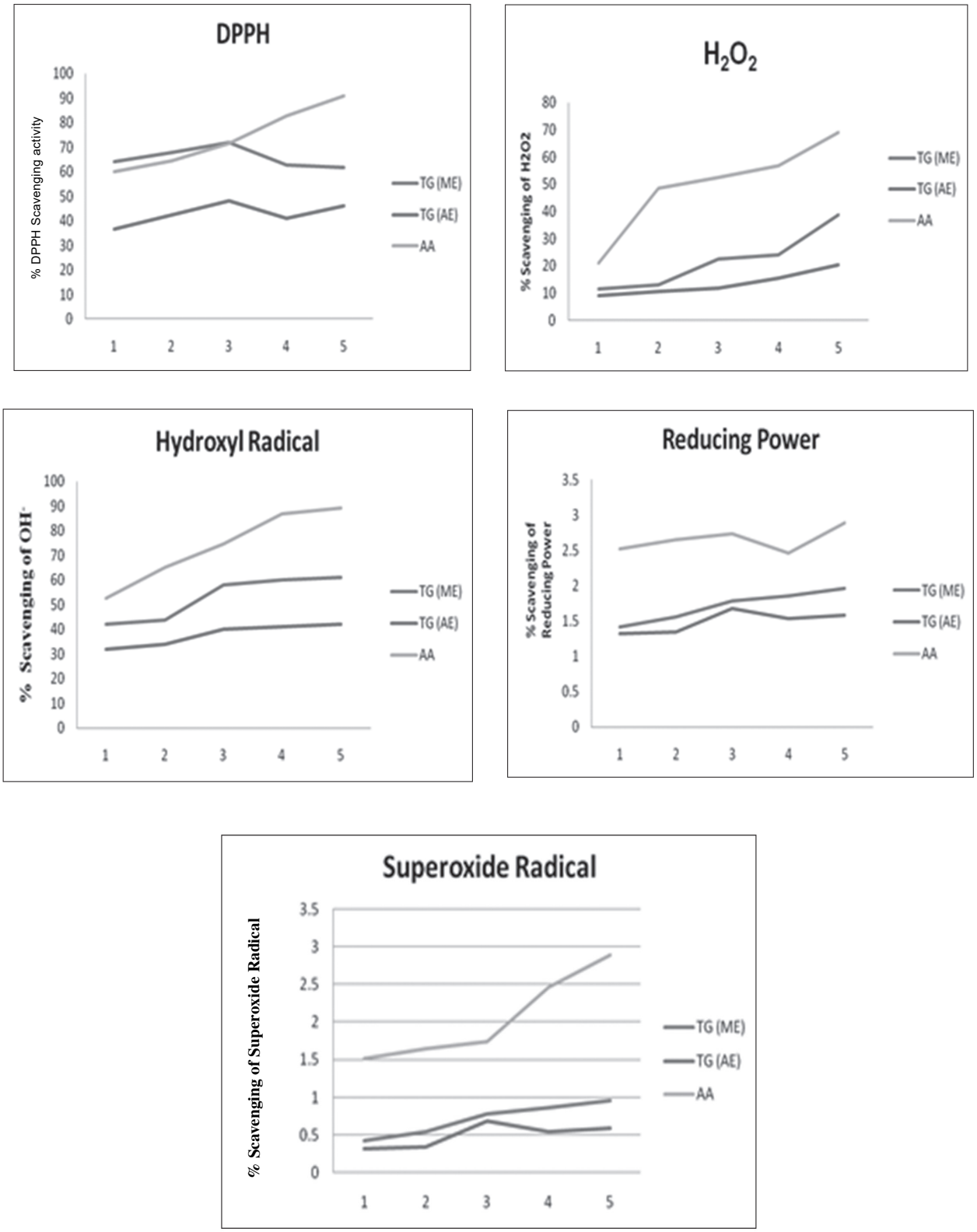

Fig. 1: Free radical scavenging activities of methanolic extract (TG ME) and aqueous extract (TG AE) of *Trigonella foenum-graecum*

Table 1: Total phenolic content (µg/mL) and Total flavonoid content(µg/mL) in Methanolic extract and Aqueous extract of *Trigonella foenum-graecum*

S.No	*Trigonella foenum-graecum*			
	Methanolic Extract		***Aqueous Extract***	
	Total phenolic content (µg/mL)	**Total flavonoid content(µg/mL)**	**Total phenolic content (µg/mL)**	**Total flavonoid content(µg/mL)**
1.	22.4	11.2	16.6	9.8

Table 2: Phytochemical screening of *Trigonella foenum-graecum*

S.No	Phytochemicals	*Trigonella foenum-graecum*	
		Methanolic Extract	**Aqueous Extract**
1.	Saponins	Present	Present
2.	Flavonoids	Present	Present
3.	Alkaloids	Present	Present
4.	Galactomannans	Present	Present
5.	Tannins	Present	Present
6.	Terpenoids	Present	Present
7.	Steroids	Present	Negative
8.	Phenolic compounds	Present	Present
9.	Cardiac glycosides	Present	Present
10.	Anthraquinones	Present	Negative
11.	Reducing Sugars	Present	Present
12.	Quinones	Present	Present

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Cardioprotective Effects of 6-Gingerol against Alcohol-Induced ROS-Mediated Tissue Injury and Apoptosis in Rats

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Abstract: The present study investigated the cardioprotective properties of 6-gingerol against alcohol-induced ROS-mediated cardiac tissue damage in rats. Experiments were conducted on 4 groups of rats, orally treated with control, 6-gingerol (10 mg/kg body weight), alcohol (6 g/kg body weight) and combination of 6-gingerol plus alcohol for two-month. In the results, we found 6-ginger treatment to alcohol-fed rats substantially suppressed ROS production in cardiac tissue. Alcohol-induced elevated 8-OHDG and protein carbonyls which represent oxidative modification of DNA and proteins were completely reversed by 6-gingerol. This was further endorsed by restored superoxide dismutase and catalase activities with 6-gingerol against alcohol-induced loss. The elevated cardiac biomarkers (CK-MB, cTn-T, cTn-I) and dyslipidemia in alcohol-intoxicated rats was significantly reversed by 6-gingerol. Furthermore, alcohol-induced apoptosis characterized by overexpression of cytochrome C, caspase-8 and caspase-9 was diminished with 6-gingerol treatment. Transmission electron microscope images conferred the cardioprotective properties of 6-gingerol as we have seen less structural derangements in mitochondria and reappearance of myofilaments. Our findings conclude that 6-ginger effectively protect alcohol-induced ROS-mediated cardiac tissue damage, which may be due to its potent antioxidant efficacy. Therefore, 6-gingerol could be a potential therapeutic molecule that can be used in the treatment of alcohol-induced myocardial injury.

Keywords: apoptosis; ROS; oxidative stress; protein carbonyl; gingerol; ethanol

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1. Introduction

Chronic alcohol consumption is implicated in risk of developing various diseases, including cardiovascular diseases (CVD), and leads to alcohol-related morbidity and mortality [1]. Alcohol and its metabolites can cause mild to severe tissue injuries, mainly due to impaired antioxidant status and oxidative stress [2,3]. The highly unstable free radicals or reactive oxygen species (ROS) that are produced during alcohol metabolism ruins the defensive antioxidant status, and cause destructive damage to cardiac tissue [4]. Excessive accumulation of ROS disrupts the redox homeostasis and leads to the oxidative modification of vital biomolecules, including proteins, lipids and DNA [5–7]. Oxidative stress is considered to be an important factor to promote cell death in response to a variety of signals and pathophysiological situations. Excessive alcohol consumption is linked with abrupt apoptosis of cardiomyocytes via various signaling pathways [8,9]. A study found that alcohol induced mitochondrial apoptosis in cardiomyocytes through stimulated oxidative stress [5,10]. Alcohol-induced cardiac tissue damage was further witnessed by elevated cardiac biomarkers, such as creatine kinase-MB (CK-MB), cardiac troponin-T (cTn-T) and

cardiac troponin-I (cTn-I) in rats [11]. Although ROS scavengers have been shown to inhibit alcohol-induced cardiac tissue damage [11], the mechanism and involved molecules behind this phenomenon remain elusive.

The rhizome of *Zingiber officinale*, known as 'ginger', has been consumed worldwide as a spice and herbal medicine, and cultivated in most tropical regions of the world. Ginger has vital non-volatile pungent phytochemicals, such as gingerols, shogaols, paradols and zingerone, which have shown biological activities [12]. Among them, 6-gingerol (Figure 1A), a phenol phytochemical constituent of fresh ginger, has been reported to possess several pharmacological efficacies, including anticancer, antioxidant, anti-inflammation, anti-platelet aggregation and antifungal [11,13,14]. Sampath et al. reported that 6-gingerol prevents atherosclerosis by inhibiting the oxidative stress biomarkers [15]. Another study showed that 6-gingerol treatment improves antioxidant status and prevents myocardial injury induced by doxorubicin [16]. The protective effects of 6-gingerol against myocardial fibrosis possibly associated with increased antioxidant capacity, decreased inflammation and apoptosis in mice [17].

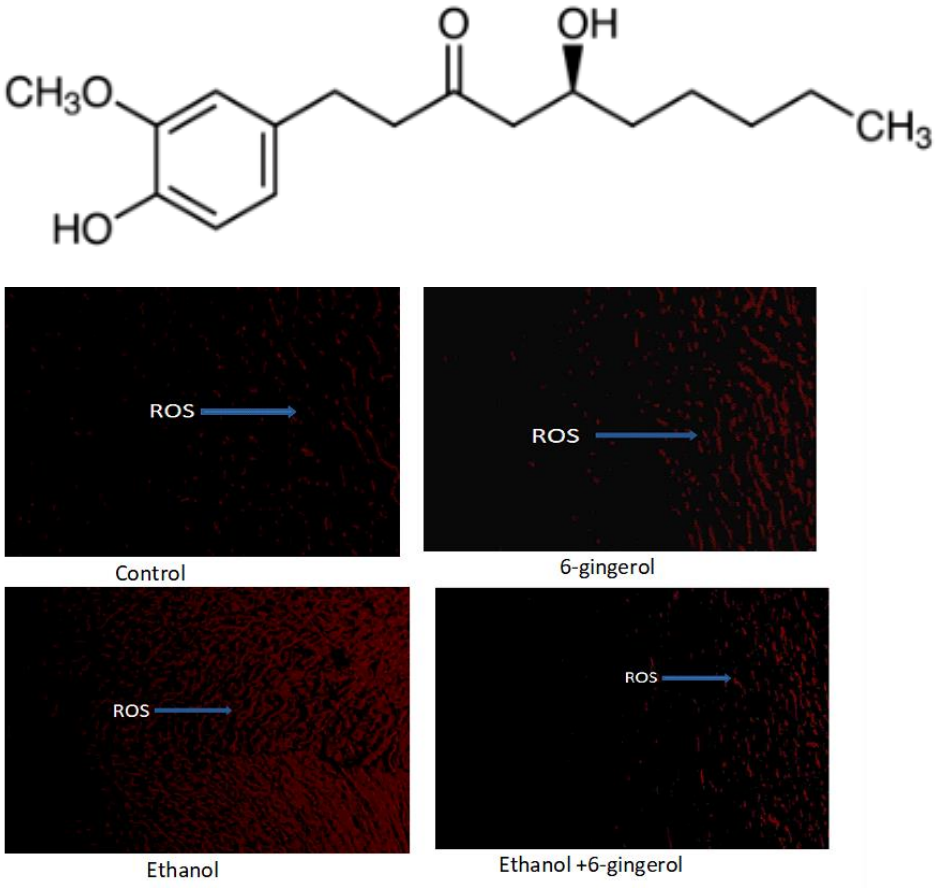

Figure 1. (**A**) Chemical structure of 6-gingerol. (**B**) Changes in intracellular ROS production in control, 6-gingerol, ethanol and 6-gingerol plus alcohol-fed rats.

Although previous studies have revealed the pharmacological efficacies of 6-gingerol against oxidative stress and inflammation, the function of 6-gingerol against alcohol-induced myocardial injury and oxidative stress has not yet been established. Therefore, in this study, we investigated the cardioprotective properties of 6-gingerol against alcohol-induced myocardial damage in rats. We examined the changes in key biomarkers that are involved in regulation of antioxidant status, oxidative stress and apoptosis with or without 6-gingerol treatment in alcohol-fed rats.

2. Results

2.1. Six-Gingerol Suppress ROS Production in Alcohol-Fed Rats

ROS, which play a crucial role in provocation of oxidative stress, were determined in cardiac tissues of all experimental groups by dihydroethidium (DHE) fluorescent dye. We found ROS distribution in a small area of cardiac tissue in control and 6-gingerol-treated rats (Figure 1B). By contrast, ROS were found in large areas of cardiac tissue in alcohol-treated rats, detected by red fluorescent dye (DHE) under the fluorescent microscope. However, 6-gingerol treatment in combination with alcohol substantially suppressed the ROS generation in cardiac tissue (Figure 1B). These findings reveal that occurrence of ROS-mediated myocardial damage in alcohol-fed rats could be attenuated by 6-gingerol treatment.

2.2. Treatment of 6-Gingerol Restores Antioxidant Enzymes against Alcohol-Induced Loss

The concentrations of intracellular ROS typically dictate the tissue antioxidant status. Therefore, we examined the changes of primary antioxidant enzyme activities in response to alcohol and gingerol treatments. In the results, we found a significant decrease of both superoxide dismutase (SOD) and catalase (CAT) activities in cardiac tissue of alcohol-fed rats (Figure 2A,B). Nevertheless, gingerol treatment considerably restored the SOD ($p < 0.05$) and CAT ($p < 0.05$) activities against alcohol-induced loss. The restored antioxidant enzyme activities with gingerol treatment were further witnessed in the inhibition of excessive ROS production.

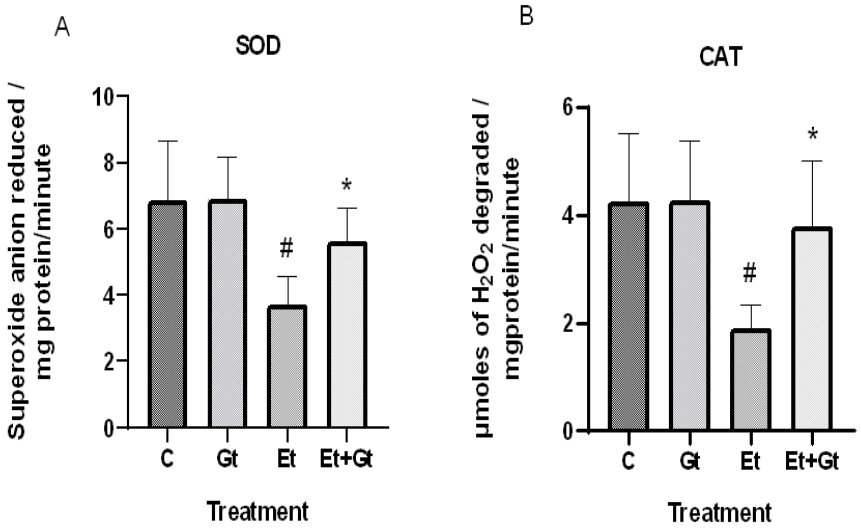

Figure 2. Changes in superoxide dismutase (**A**) and catalase (**B**) activities in cardiac tissue of control (C), 6-gingerol (Gt)-, ethanol (Et)- and ethanol + 6-gingerol (Et + Gt)-treated rats. The data were expressed as mean ± standard deviation (SD). $^{\#}$ $p < 0.05$ compared with control and * $p < 0.05$ compared with ethanol treated groups.

2.3. Gingerol Treatment Reverses Elevated 8-Hydroxyguanosine (8-OHDG) and Protein Carbonyls

We next determined the levels of oxidative damage to DNA and proteins in alcohol- and gingerol-treated groups. Our results showed a considerable DNA oxidative damage in cardiac tissue by alcohol intoxication, which was represented by elevated 8-OHDG levels. This was about 3-fold higher compared with normal control (Figure 3A). It is worth noting that gingerol treatment substantially ($p < 0.05$) inhibited the elevated 8-OHDG levels, indicating the protective effects of gingerol against DNA damage.

Furthermore, we found that alcohol intoxication led to oxidative modification of cardiac proteins, which was evidenced by increased protein carbonyl levels. However, gingerol treatment to alcohol-fed rats significantly ($p < 0.05$) suppressed protein carbonyl content in the myocardial tissue (Figure 3B). Taken together, gingerol is able to protect the cardiac tissue by inhibiting the oxidative modification of DNA and proteins against alcohol intoxication.

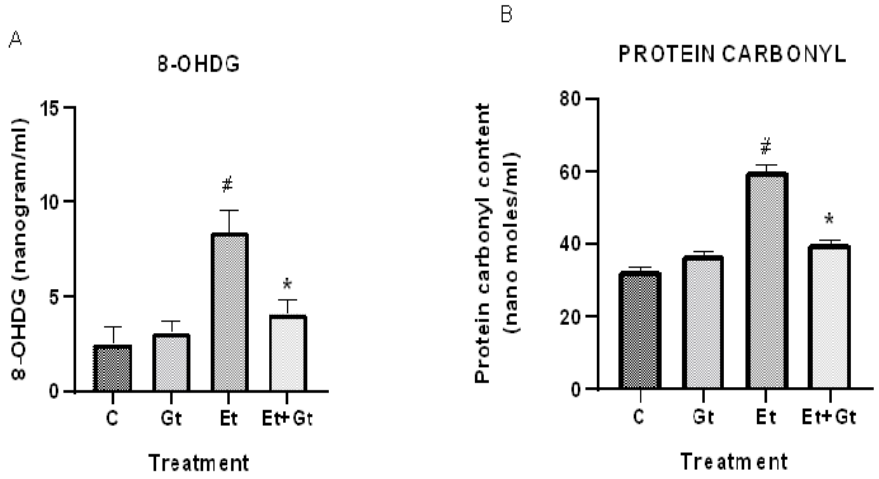

Figure 3. Gingerol treatment suppressed 8-OHDG (**A**) and protein carbonyls (**B**). Assessments were done in control (C), 6-gingerol (Gt)-, ethanol (Et)- and ethanol plus 6-gingerol (Et + Gt)-treated rats. The data were expressed as mean ± SD. # $p < 0.05$ compared with control and * $p < 0.05$ compared with ethanol-treated groups.

2.4. Antilipidemic Properties of 6-Gingerol in Alcohol-Fed Rats

To elucidate the antilipidemic effects of gingerol, we measured the changes of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (LDL) levels in all experimental groups. We found that circulating total cholesterol, TG and LDL were significantly increased, while HDL levels were significantly decreased following alcohol drinking. In contrast to these, gingerol treatment to alcohol-fed rats significantly decreased the total cholesterol, TG and LDL levels and restored the HDL levels (Table 1).

Table 1. Alcohol-induced dyslipidemia reversed by 6-gingerol. Data were expressed as mean ± SD (N = 6). # $p < 0.005$ compared with control * $p < 0.05$ compared with ethanol groups. Values were shown in control (C), 6-gingerol (Gt)-, ethanol (Et)- and ethanol plus 6-gingerol (Et + Gt)-treated groups.

Lipid Profile (mg/dL)	Groups			
	C	Gt	Et	Et + Gt
TG	38.83 ± 5.05	39.38 ± 4.17	89.20 ± 6.08 #	44.17 ± 4.98 *
TC	66.94 ± 4.12	64.24 ± 5.13	119.86 ± 5.45 #	72.45 ± 6.13 *
HDL	25.13 ± 2.36	24.51 ± 2.08	12.62 ± 3.06 #	21.33 ± 1.94 *
LDL	40.48 ± 2.11	38.73 ± 1.91	109.11 ± 2.37 #	56.77 ± 1.98 *

2.5. Gingerol Prevents Alcohol-Induced Elevation of Cardiac Biomarkers

The protective effects of gingerol against alcohol-induced cardiac tissue injury was determined by measuring the important cardiac biomarkers, including CK-MB, cTn-T and cTn-I. As we assumed, alcohol intoxication significantly elevated the circulating levels of CK-MB ($p < 0.05$), cTn-T ($p < 0.05$) and cTn-I ($p < 0.05$), which indicates the cardiac tissue damage (Figure 4A,B,C). Six-gingerol treatment prevents the alcohol-induced cardiac damage through suppressed cardiac biomarkers ($p < 0.05$). The suppressed levels of cTn-T, cTn-I and CK-MB with gingerol treatment were 41.5%, 40.26% and 42.38%, respectively, compared to alcohol-induced elevation (Figure 4). These results revealed the potential cardioprotective effects of 6-gingerol against alcohol intoxication.

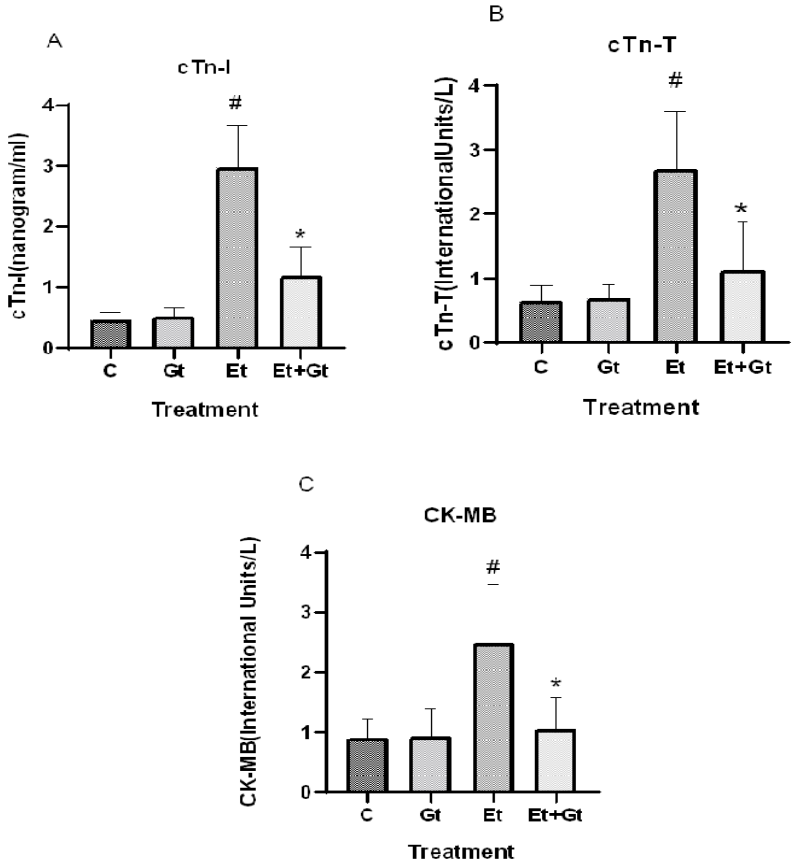

Figure 4. Changes in cTn-I (**A**), cTn-T (**B**) and CK-MB (**C**) levels in plasma of control (C), 6-gingerol (Gt)-, ethanol (Et)- and ethanol plus 6-gingerol (Et + Gt)-treated groups. The data are expressed as mean ± SD. # $p < 0.05$ compared with control and * $p < 0.05$ compared with ethanol-treated groups.

2.6. Gingerol Treatment Attenuates Upregulated Apoptotic Markers in Alcohol-Fed Cardiac Tissue

We extended our studies to evaluate the anti-apoptotic properties of 6-gingerol against alcohol-induced induction of apoptosis in cardiac tissue. The studied key apoptotic makers through immunohistochemistry visualized that alcohol drinking triggered the expression of cytochrome C, caspase-8 and caspase-9 in cardiac tissue. Interestingly, 6-gingerole treatment for 60 days along with alcohol diminished the overexpression of cytochrome C, caspase-8 and caspase-9 in cardiac tissue. These findings evidenced that alcohol-induced initiation of apoptosis could be attenuated by 6-ginferol treatment (Figure 5). Similar to antioxidant enzyme activities, the immunohistochemistry images also showed restored expression of SOD and CAT with 6-gingerol co-administration with alcohol (Figure 6A,B).

2.7. Transmission Electron Microscopy (TEM) Demonstrates Cardioprotective Effects of Gingerol

Subsequently, TEM was performed to assess the cardioprotective efficacies of 6-gingerole. As shown in the images, control heart was characterized by typical symmetric myofibrils comprised of Z lines with sarcomeres. Furthermore, packed mitochondria adjacent to the fibers were evidenced in the control group. Gingerol alone treated rats shown mitochondria slightly deviated from the fibers remaining myofibril structure intact. However, alcohol alone treated group represented with altered myofibril structure, disappearance of Z lines, derangement of sarcomeres and mitochondrial swelling. All these destructive features imply the mitochondrial apoptosis with alcohol intoxication. It should be noted that gingerol treatment prominently attenuated these structural derangements. These protective effects were evidenced by less structural alterations in mitochondria, reappearance of myofilaments and Z lines (Figure 7).

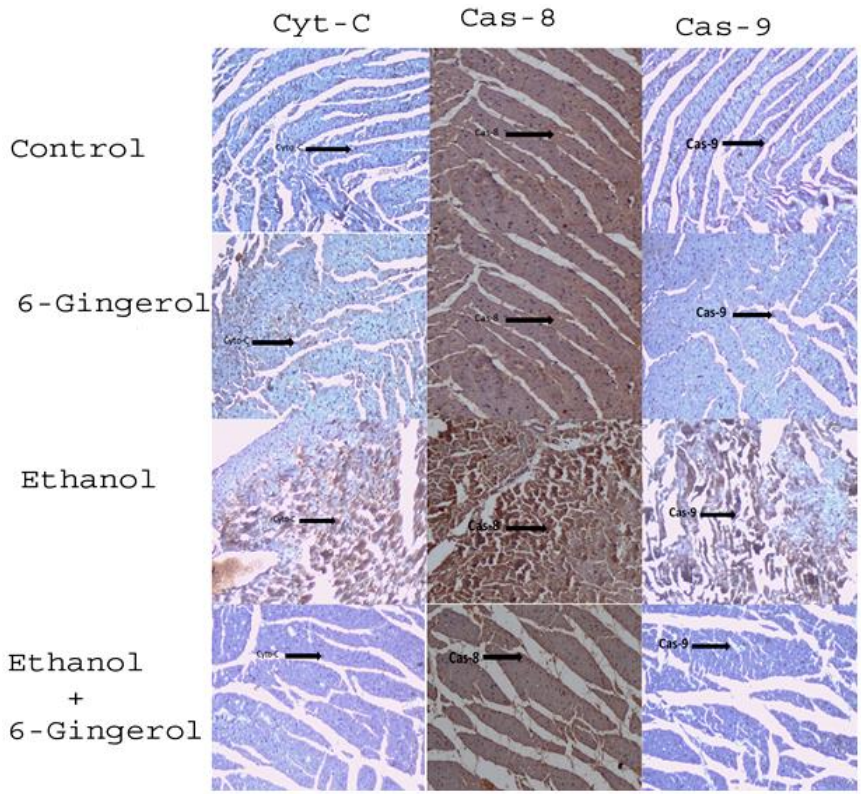

Figure 5. Six-gingerol regulates ethanol induced ROS-mediated apoptosis in cardiac tissue. Expressions of mitochondrial cytochrome C, caspase-8 and caspase-9 in cardiac tissues of control, 6-gingerol, ethanol and ethanol plus 6-gingerol groups. The arrow in each panel indicates the expressions of cytochrome C, caspase-8 and caspase-9 in respective groups.

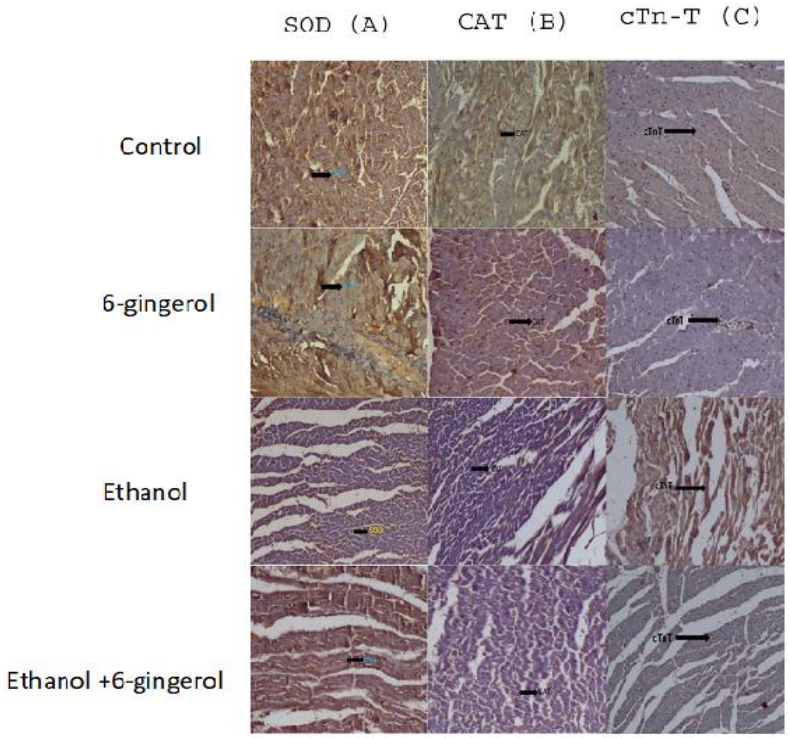

Figure 6. Immunohistochemistry images of SOD (**A**), CAT (**B**) and cTn-T (**C**) in all experimental groups. The arrow in each panel indicates the expressions of SOD, CAT and cTn-T in respective groups.

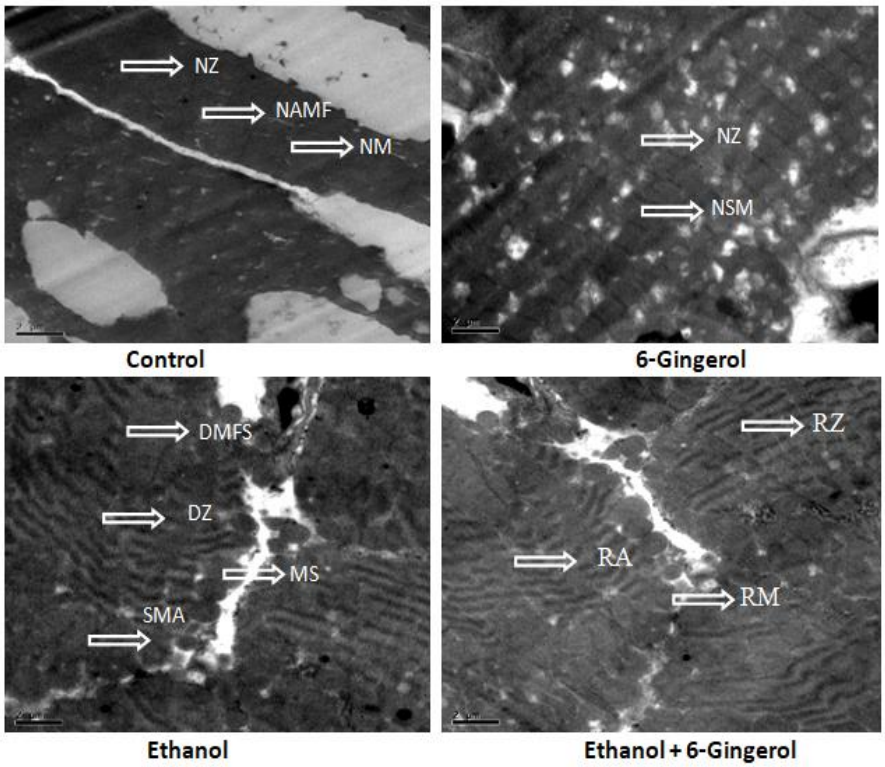

Figure 7. Transmission electron microscopy images of cardiac tissue display alcohol-induced tissue damage and 6-gingerol mediated recovery. The arrow indicates normal structure of Z line (NZ), normal architecture of myofibril (NAMF), normal mitochondria (NM) and normal sarcomere (NSM) in control or 6-gingerol groups. The arrow indicates, degeneration of myofibril structure (DMFS), degeneration of Z lines (DZ), sarcomere alteration (SMA) and mitochondria swelling (MS) in ethanol group. The arrows in ethanol plus 6-gingerol group indicate regeneration of Z line (RZ), regeneration of mitochondria (RM) and reappearance of sarcomere (RA).

3. Discussion

In this study, for the first time we have demonstrated that 6-gingerol substantially alleviated the alcohol-induced oxidative damage, myocardial injury and apoptosis. Experimental evidence shows that 6-gingerol treatment suppressed alcohol-induced ROS production in cardiac tissue. This therapeutic effect was accompanied by restored myocardial antioxidant status, and decreased DNA oxidative damage and protein carbonyls. In addition, alcohol-induced elevated cardiac biomarkers (CK-MB, cTn-T, cTn-I) and dyslipidemia were significantly reversed by gingerol. Histopathological evidence further confirmed the tissue protective effects of 6-gingerol against alcohol-induced myocardial injury and apoptosis. Since alcohol is typically involved in impairing of antioxidant homeostasis and myocardial injuries, supplementation of antioxidant rich molecules like 6-gingerol could possibly alleviate the myocardial damage. The calculated 6-gingerol dose from our rat model (10 mg/kg) is ~97 mg per adult with an average weight of 60 kg [18]. This dose might be feasible to humans, as a previous study reported no serious adverse effects of 6-gingerol with dose ranges from 100 mg to 2000 mg per adult [19].

It has been documented that alcohol intake, either chronic or acute, alters the cardiomyocyte and whole organ functioning of the heart [20]. ROS or free radicals that are generated during alcohol metabolism are crucially involved in the onset of myocardial disturbances possibly through ruining of intracellular antioxidant homeostasis [21,22]. Accumulations of highly reactive ROS destabilize the myocardial contractility, cause myocardial tissue injury, and induce myocyte apoptosis [23]. In agreement with these findings, we also noticed excessive production of ROS and subsequent myocardial injury in alcohol-fed rats. However, 6-gingerol treatment to alcohol-fed rats for a period of 2 months considerably inhibited

the ROS production in cardiac tissue. Previous studies have shown that 6-gingerol is able to decrease ROS-mediated oxidative stress in the liver [24], and its antioxidant property reduces acute renal toxicity [25]. Our previous in vitro findings also demonstrated the free radical scavenging ability of 6-gingerol [26]. A recent study reported that only 7 days of 6-gingerol treatment to arsenic trioxide injected mice significantly decreased ROS production in heart [27]. These findings indicate that 6-gingerole is able to inhibit intracellular ROS production during ethanol metabolism.

Oxidative stress is an imbalance between the amount of intracellular ROS and antioxidant defense status. Alcohol intoxication is characterized by elevated oxidative stress and decreased antioxidant enzyme status in various tissues, including liver and heart of rodents [6,11,28]. Here, we found alcohol-induced oxidative damage of DNA and proteins as reported by elevated 8-OHDG and protein carbonyl levels, respectively. Chronic ethanol consumption in rats has been shown to increase protein oxidation in parallel with increased free radicals [29]. The uncontrolled-highly unstable ROS that are generated during alcohol metabolism eventually affect the function of cardiomyocytes/heart through irreversible damage of mitochondria, DNA and proteins. These events are clinically associated with pathogenesis of cardiomyopathy [5,30]. Therefore, controlling alcohol-induced oxidative stress by known antioxidants is necessary to prevent the progression of cardiomyopathy. We found that gingerol treatment along with alcohol tremendously inhibited the formation of 8-OHDG and protein carbonyls. Due to their highly reactive nature, hydroxyl radicals or singlet oxygen hydroxylate the deoxyguanosine and formed 8-hydroxy-2′deoxyguanosine. Decreased 8-OHDG levels with 6-gingerol explains that gingerol molecules possibly inhibit the production of intracellular hydroxyl radical and/or singlet oxygen against alcohol toxicity and thereby prevent the oxidative damage to DNA and proteins. Our present findings and previous reports also confirmed the potent free radical scavenging activity or ROS inhibitory efficacy of 6-gingerol [26]. An in vitro study reported that 6-gingerol possess a strong protective ability against the DNA damage caused by mono (2-ethylhexyl) phthalate in human endothelial cells, and the mechanism may be due to its antioxidant activity [31].

Decreased intracellular ROS production or oxidative stress in any context could possibly explained by the antioxidant status of a tissue. Therefore, we determined the primary antioxidant enzyme activities in the heart of alcohol and 6-gingerol-treated rats. In accordance with elevated oxidative stress and ROS production, the primary radical scavenging enzymes SOD and CAT were significantly decreased following alcohol intoxication. Decreased antioxidant enzyme activities with alcohol drinking were repeatedly confirmed by several previous studies [3,5]. Persistent lower grade antioxidant capacity and subsequent oxidative stress perhaps contributes to morphological changes and malfunctioning of cardiac tissue [5]. The important finding in our study is that the decreased SOD and CAT activities were restored with 6-gingerol treatment. In a rat model, 6-gingerol was reported to increase antioxidant enzyme activities and protect the intestinal barrier from ischemia/reperfusion-induced damage [32]. Various doses of gingerol fraction was reported to improve the renal antioxidant enzyme activities and decrease the lipid peroxidation against gentamicin-induced nephrotoxicity in rats [25]. Another study demonstrated that cotreatment of 6-gingerol and ethanol significantly restored the antioxidant enzyme activities against ethanol-induced loss in cultured mouse embryos [33]. Taken together, our findings provided additional knowledge that 6-gingerol treatment for a period of 2 months could protect the cardiac tissue against alcohol-induced oxidative damage through improved antioxidant status.

Chronic alcohol consumption not only impairs the redox homeostasis, but also causes dyslipidemia, a major risk factor for developing cardiovascular diseases. Dyslipidemia is typically represented by abnormal increase of circulating lipid profiles; including TG, TC and LDL, while HDL levels are decreased. Alcohol administration to rats resulted in increased TG, TC and LDL levels and decreased HDL, which confirmed the dyslipidemia status. In our study, 6-gingerol treatment to alcohol-fed rats reversed the dyslipidemia as shown by decreased TG, LDL, and TC levels along with increased HDL levels. Clinical

studies have shown that increasing TG, LDL and TC increases the risk of non-ischemic heart failure, while decreasing serum lipids can reverse the heart dysfunction [34]. In this context, decreased levels of TG, LDL and TC with 6-gingerol treatment perhaps improved the heart function which was deteriorated by alcohol intoxication in rats. Saravanan and colleagues also reported that oral administration of gingerol for 30 days contributed to a significant decrease of lipid profile in obese rats [35]. Another study reported that 6-gingerol treatment for 12 days significantly decreased plasma TG, LDL, and free fatty acid concentrations in db/db mice. This lipid-lowering effect of 6-gingerol was accompanied by an increased antioxidant enzyme activities and suppressed ROS generation in db/db mice [36]. These findings reveal that 6-gingerol is a considerable molecule to lower the lipid profile and prevent the lipid-associated cardiac dysfunction.

Circulating cardiac biomarkers are novel quantitative measures which shed light on occurrence of cardiac pathophysiology [37]. The biomarker troponin I (cTn-I/cTn-T) is a sensitive and specific marker for myocardial injury, which is widely used to diagnose the myocardial infarction [38]. In our study, alcohol-induced elevation of clinical cardiac biomarkers in plasma is another evidence of cardiac tissue damage in alcohol-fed rats. There was a substantial correlation between elevated lipid profile and increased release of cardiac biomarkers in plasma by damaged cardiac cell under ethanol intoxication [11]. In agreement with previous findings, our results also revealed that ethanol intoxication increase the cardiac biomarkers, including CK-MB, cTn-T and cTn-I in plasma. A previous study reported a several-fold rise in serum troponin, myoglobin and CK-MB concentrations after alcohol septal ablation for the treatment of hypertrophic cardiomyopathy [39]. Based on the cardiac biomarker data, a community-based cohort study reported even modest habitual alcohol intake was associated with an increased risk of atrial fibrillation [37]. These results indicate that controlling of cardiac biomarkers is essential in treating the myocardial injuries. Nevertheless, 6-gingerol treatment along with alcohol exerts the cardioprotective effects by diminishing the elevated CK-MB, cTn-T and cTn-I levels. A study by Ren et al., demonstrated that various concentrations of 6-gingerol protects cardiomyocytes from hypoxia-induced injury by suppressing the release of lactate dehydrogenase (LDH) and apoptosis [40]. Cotreatment of ginger to alcohol-fed rats also reported decreased concentrations of cTn-T, LDH and CK-MB in rats [11]. Pretreatment of 6-gingerol has been shown to improve the cardiac function by decreasing the cTnT and CK-MB expressions in ischemia/reperfusion (I/R)-induced myocardial injury model [41]. Lipid-lowering effect and/or improved antioxidant status by gingerol possibly contributed to attenuate the alcohol-induced elevation of cardiac biomarkers.

It has been speculated that overwhelming production of ROS intrinsically involved in initiation and progression of cardiac myocytes apoptosis under chronic ethanol intoxication [42]. Several studies have shown that chronic alcohol consumption promptly associated with occurrence of cardiomyocytes apoptosis by alterations in mitochondria membrane potential [43,44]. Although we haven't detected the mitochondrial membrane potential, the apoptotic proteins in implicated in mitochondrial apoptosis was determined through immunohistochemistry. Our results showed that the apoptotic biomarkers, such as cytochrome C, caspase-9 and caspase-8, were highly expressed in alcohol-intoxicated hearts. Furthermore, elevation of apoptotic proteins were accompanied by decreased cardiac SOD and CAT expressions with alcohol. At molecular levels, ethanol may initiate the apoptosis in cardiac tissue by both intrinsic and extrinsic pathways. In the cascades of apoptosis, ethanol-induced ROS accumulation and decreased antioxidant defense mechanism may play a major role in the expression of caspase-8. Triggering of cytochrome C release by alteration in mitochondrial membrane permeability led to activation of caspase-9 [45,46]. Meanwhile, 6-gingerol treatment profoundly inhibited the apoptosis in cardiac tissue by suppressing the overexpressed cytochrome C, caspase-9 and caspase-8, while restoring the tissue antioxidant status. The earlier reports have shown that ROS scavengers or inhibitors can control the alcohol-induced apoptosis in cardio myocytes [47]. In this context, 6-gingerol was reported to decrease the formation of apoptotic proteins that reserved the myocardial

apoptosis under hypoxia condition [40]. 6-gingerol administration to isoproterenol-treated mice (14-day) resulted in a significant reduction of caspase-3, Bax protein expressions and Bax/Bcl-2 ratio, indicating attenuation of cardiac apoptosis. This anti-apoptotic property of gingerol was accompanied by inhibition of oxidative stress and inflammatory biomarkers [17]. It has been reported that 6-gingerol metabolites may stay about 12 h in the body after oral administration [48]; therefore, we assume that the bioavailability of gingerol may be responsible for its therapeutic efficacies under stress. Taken together, our results suggest that 6-gingerol can inhibit the apoptosis under ethanol intoxication through reduction of ROS production and improvement of antioxidant defense mechanism.

Another important finding of this study is that TEM analyses provided additional convincing evidences that 6-gingerol is a potent tissue protective molecule. TEM visuals demonstrated that ultra-structural derangement in cardiac tissue following ethanol intoxication. We further noticed intense lysis of myofibrils with disruption of Z lines and sarcomere structure, mitochondrial swelling and damage, which collectively results in myocardium apoptosis in ethanol-intoxicated rats. It is worth noting that 6-gingerol attenuate all these destructive changes and retained the ultra-structure of myocardium against cardiotoxic effect of ethanol. In I/R-induced myocardial injury model, 6-gingerol pretreatment to rats reduce myocardial infarction area and degree of cardiac pathological injury followed by lower levels of myocardial enzyme and inflammatory mediators [41]. Some studies have shown that food-based formulations that contain ginger could promote alcohol metabolism and alleviate the alcohol hangover effects [49]. In such case, we assume that 6-gingerol may be involved in rapid alcohol metabolism, which facilitates lower accumulation of toxic alcohol metabolites and then results in less cardiac tissue damage. Despite the lack of experimental evidence to reveal this phenomenon, this is the first report to demonstrate the cardioprotective effects of 6-gingerol against ethanol-induced cardiac tissue damage in rats.

4. Materials and Methods

4.1. Chemicals and Reagents

The phytochemical compound, 6-gingerol (purity: >98.0%) was purchased from TCI chemicals (India) Pvt. Ltd. Both dihydroethidium fluorescent dye and SOD primary antibody were obtained from the Sigma-Aldrich (St. Louis, MO, USA). Catalase, caspase-9 and -8, cytochrome C primary antibodies and secondary antibody horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG were purchased from Invitrogen (Waltham, Thermo Scientific, MA, USA). Troponin-T primary antibody was obtained from Abcam (Cambridge, UK). Creatine kinase–MB (CK-MB), cardiac troponin-T (cTn-T) and cardiac troponin-I (cTn-I) ELISA kits were purchased from the Biocheck, South San Francisco, USA. Triglyceride (TGs), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) colorimetric kits were obtained from the Merck (Rathway, NJ, USA). All other chemicals and reagents were analytical reagent grade and purchased from Sigma-Aldrich.

4.2. Experimental Animals

A total of 24 Wister strain male albino rats, weighing 200 ± 30 g, were obtained from the Indian Institute of Science, Bangalore, India. All rats were housed in standard polypropylene transparent cages at a controlled temperature (23–27 °C) and humidity (50% ± 5%) with a 12 h light/dark cycle. Rats had free access to food and water. The experimental design and protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Sri Venkateswara University, India, with (10/i/a/CPCSEA/IAEC/SVU/KSR-GVS/dt 15 November 2010).

4.3. Animal Grouping and Treatment

After a week of adaptation to the laboratory conditions, the rats were randomly assigned into 4 groups, control (C), 6-gingerol (Gt), ethanol (Et) and combination of both ethanol plus 6-gingerol (Et + Gt) groups, with 6 rats in each. Rats in the control group received 0.9% saline solution, rats in the Gt group received 6-gingerol by gavage (10 mg/kg

bodyweight, oral administration) and rats in the Et group received ethanol by gavage (20%, 6 g/kg bodyweight, oral administration). The remaining rats in the Et + Gt group received both ethanol and 6-gingerol as described in respective groups. The treatment was given once a day for all groups, and the whole treatment lasted for 2 months. The selective dosage of alcohol and 6-gingerol used in this study was well established by previous studies [11,16]. Blood samples were collected at 10 am into a sterile heparinized tube from the retro-orbital plexus of rats at the end of the treatment period, and plasma was collected by centrifugation at 2000 rpm for 10 min at 4 °C and then stored at −20 °C for further analysis. The heart tissues were isolated from rats after cervical dislocation and immediately washed with ice cold saline and kept on a chilled glass plate. One part of the heart tissue was stored at −20 °C for further biochemical assays, and the remaining was fixed in 4% formaldehyde for histopathological studies.

4.4. ROS Detection by Dihydroethidium (DHE) Fluorescent Dye

ROS in the cardiac tissue was detected using DHE dye as described by Carter et al., (1994) method [50]. Cryostat sections of cardiac tissue was cut into 10 µm thick and incubated with 5M DHE dye at 37 °C for 30 min. The DHE is oxidized by ROS to produce fluorescent ethidium that subsequently binds to nucleic acids, further staining the nucleus a bright fluorescent red. The red fluorescent from DHE was detected by fluorescent microscope.

4.5. Determination of 8-Hydroxydeoxyguanosine (8-OHDG)

Wash solution (20×) in a volume of 15 mL was diluted with 285 mL of distilled water and prepared 1x assay buffer by 14 mL of assay buffer was added to 56 mL of distilled water. To each well, cardiac tissue samples or standard 50 µL and 75 µL of conjugate was added. With the exception of NSB wells, 25 µL of 8-hydroxydeoxyguanosine antibody was added, and then the side of the plate was tapped to mix and incubated for 2 h at room temperature and 100 µL of TMB substrate was added to each well. The substrate solution began to turn blue, was incubated for 30 min at room temperature without shaking, 50 µL of stop solution was added to each well and the absorbance was read at 450 nm. The plate reading was completed within 10 min after adding the stop solution.

4.6. Estimation of Protein Carbonyl Content

Protein carbonyl content in the cardiac tissue was estimated by commercial kit (Sigma-Aldrich). Briefly, 200 mg of tissue was homogenized in 1 mL of tris buffer and centrifuge at 10,000× *g* for 15 min at 4 °C, and the supernatant was stored on ice. A 200 µL sample was added to 800 µL of DNPH, incubated for 1 h in dark at room temperature, and each tube was vortexed briefly for every 15 min during incubation, 1 mL of 20% TCA was added to each tube, and the tubes were vortexed, placed on ice, incubated for 15 min and centrifuged at 10,000× *g* for 10 mint at 4 °C. The supernatant was discarded, the pellet was re-suspended in 1 mL of TCA and the tubes were placed on ice for 5 min and centrifuged at 10,000× *g* for 10 min. The supernatant was discarded, and the pellet was resuspended in 1 mL of (1:1) ethanol and ethyl acetate mixture, then centrifuged at 10,000× *g* for 10 min at 4 °C (Remi C-24 BL/CPR 24 Cooling centrifuge, Mumbai, India). A 220 µL sample was added to each well, and the absorbance was measured at 360–385 nm (Simadzu, UV1800 Spectrophotometer, Kyoto, Japan).

4.7. Assessment of Superoxide Dismutase and Catalase Activities

Changes in SOD activity in the mitochondrial fraction was assessed by the method of Misra and Fridovich (1972) [51] at 480 nm for 4 min on a Simadzu, UV1800 Spectrophotometer (Kyoto, Japan). Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein. We then determined the CAT activity at room temperature using the modified version of Aebi [52]. The absorbance of the sample was measured at 240 nm for 1 min in a UV-Spectrophotometer

(Simadzu, UV-Spectrophotometer, Kyoto, Japan). One-unit activity is equal to the moles of H_2O_2 degraded/mg protein/min.

4.8. Estimation Cardiovascular Risk Factors

Widely considered lipid based cardiovascular risk factors, including triglyceride (TGs), total cholesterol (TC), high density lipoprotein (HDL) and low-density lipoprotein (LDL) levels, were measured in plasma by commercially available kits (Merck, Rathway, NJ, USA).

4.9. Evaluation of Cardiac Biomarkers

The isoenzyme creatine kinase–MB (CK-MB) levels in plasma were estimated using a commercially available kit (Biocheck, ELISA kit, South San Francisco, CA, USA) in all experimental groups. The absorbance of the sample was measured at 340 nm using iMark Microplate reader (Bio-Rad, Hercules, CA, USA). CK-MB levels were described as international units per liter. Next, changes in cardiac troponin–T (cTn-T) and cardiac troponin–I (cTn-I) in plasma were determined by commercially available kits (Biocheck ELISA kit, South San Francisco, CA, USA) in all experimental groups. The sample absorbance was measured at 450 nm in an iMark Microplate absorbance reader (Bio-Rad, Hercules, CA, USA). The units of cTn-T and cTn-I levels were described as grams per milliliter.

4.10. Immunohistochemistry

Immunohistochemistry study was performed according to the study by Liu et.al., [53]. Briefly, the sections were collected on 3-aminopropyltriethoxysilane (APES)-coated slides, and xylene was added and kept for 15 min. For each slide, alcohol was added in descending concentrations (100–70%), and after 1 min, the slides were washed in tap water for 10 min and distilled water for 5 min. Further, the slides were kept in a presser cooker in 1 mM of citrate buffer (pH 6). After leaving the pressure cooker, placed in sink water for cooling at room temperature, the samples were washed in distilled water for 5 min and with 5 mM of TBS buffer (pH 7.6) twice for 5 min each time. The samples were blocked with peroxidase for 10–15 min, washed three times with TBS buffer and drained. The section was covered with concerned primary antibodies (caspase-8, caspase-9 and cytochrome C) for 1 h (1:1000). Further, the samples were washed three times with TBS buffer for 5 min, andthen incubated with the HRP-conjugated secondary antibody (1:1000). The color was developed within 5–8 min with the use of 3,3′-diaminobenzidine. The samples were washed with TBS buffer three times for 5 min, then incubated in hematoxylin for 1 min. Finally, samples were kept in tap water for 5 min. In the same way, the antioxidants enzymes, such as SOD and CAT, and cardiac biomarkers, such as cTn-T were also assessed in the cardiac tissues of all experimental groups. A microscope (BX51; Olympus, Tokyo, Japan) was used to capture the images of the sections.

4.11. Transmission Electron Microscopy

Heart tissue excised from the rats was perfused with 0.1 M sodium phosphate buffer (pH 7.4) solution containing 2.5% glutaraldehyde and 2% formalin for 15 min at 48 °C according to the protocol described by Watanabe and Yamada [54]. Then the tissue was kept for 2 h at 48 °C in 1% osmium tetroxide contained buffer solution for post fixation. After dehydration, the heart tissue with a various concentration series of alcohol (70–100%), embedded in spurr1 resin. Reichert Ultracut 1 microtome was used for cut the heart tissue with 90 nm thickness. The grids were counterstained with 4% uranyl acetate and 0.4% lead citrate solutions and examined in a Jeol JEM 1010 transmission electron microscope (Peabody, MA, USA) at 80 kV. This assay was performed at the Centre for Cellular and Molecular Biology, Hyderabad, India.

4.12. Statistical Analysis

The experimental data was expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted followed by Duncan's test using SPSS version

19.0 software (Armonk, New York, NY, USA). Statistical significance was considered at $p < 0.05$. Graphs were prepared using GraphPad Prism (Version 5.00, GraphPad Software Inc., San Diego, CA, USA).

5. Conclusions

For the first time, our findings demonstrated the potential cardioprotective properties of 6-gingerol against alcohol-induced oxidative stress, apoptosis and architectural damage. The underlying phenomenon may be due to an effective suppression of alcohol-induced ROS protection and/or antioxidant efficacy of gingerol. Further evidence showed that gingerol cotreatment suppressed vital biomarkers of apoptosis and cardiac injury followed by recovered tissue architectural damages. Our findings suggest that 6-gingerol could be a potential therapeutic molecule to use in the treatment of alcohol-induced myocardial injury.

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Genetic polymorphisms and their role in incidence of Non-alcoholic fatty liver disease

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Keywords: Non-alcoholic fatty liver disease (NAFLD), Genetics, single nucleotide polymorphisms (SNPs), glucose homeostasis, lipid metabolism, nonalcoholic steatohepatitis (NASH), hepatocellular carcinoma (HCC)

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is one reason for chronic liver disease all over the world, be it the steatosis, nonalcoholic steatohepatitis (NASH), advanced hepatic fibrosis, cirrhosis, or hepatocellular carcinoma. The increasing prevalence of NAFLD is not clearly understood, but it is an established fact that metabolic abnormalities like diabetes, dyslipidemia, glucose impairment and

obesity contribute significantly in the onset and progression of NAFLD. Genetics, nutritional factors and life style habits contribute to this. It is not understood how genetic factors play a role in triggering the pathogenesis and progress of NAFLD. In the present review, we debate the recent facts of the genetic basis of NAFLD induced by glucose homeostasis, lipid metabolism, oxidative stress, and related cytokines.

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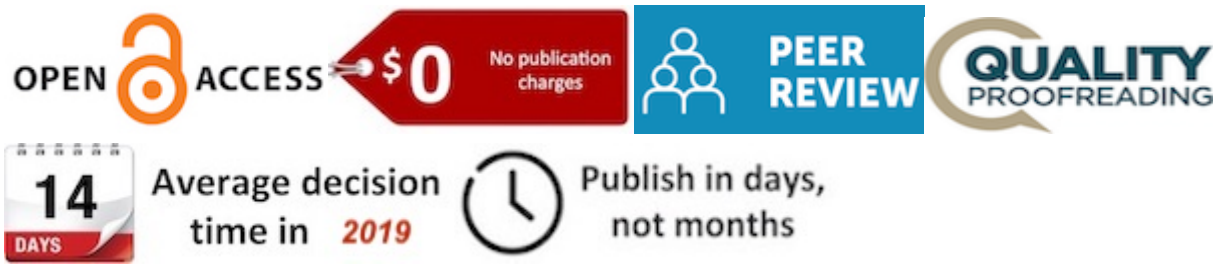

Polymophism education corner

What is a polymorphism?

Polymorphism are defined as common variations in anything around you. In biology, polymorphisms are defined as common variations in the shape, size, colour or texture of various organisms or their organs. With respect to genetics, polymorphisms are the variations in the DNA; for example, single nucleotide polymorphisms (SNPs), copy number variations (CNVs) and satellite variations (simple repeats). In molecular biology, polymorphisms are defined as variations in the level/expressions of various genes or proteins. Quantitatively, polymorphism is defined when it occurs commonly in the population, generally above 1% frequency.

What are restriction enzymes?

Restriction enzymes, also called as restriction endonucleases, are the enzymes employed by bacteria that cleave DNA at specific sites. These enzymes serve as a defence mechanism in the bacterial cells. They typically serve to cut the DNA of bacteriophages to restrict their invasion in the bacterial cells. Interestingly, these enzymes do not cut the DNA of the host, which is typically protected by methylation. This system in bacteria is called host restriction modification (HRM) system, which provides bacteria protection against bacteriophages.

Polymorphism health blog

Stay indoors, but stay fit !

It is important to stay active and fit during COVID-19 pandemic. In order to support individuals in eating healthy during self-quarantine and isolation, WHO/Europe has prepared a set of general tips, a list of "best food buys" and a few examples of recipes for inspiration.

COVID-19 vaccine - WHO

The world is in the midst of a COVID-19 pandemic. As WHO and partners work together on the response -- tracking the pandemic, advising on critical interventions, distributing vital medical supplies to those in need--- they are racing to develop and deploy safe and effective vaccines.

Vaccines save millions of lives each year. Vaccines work by training and preparing the body's natural defences --- the immune system--- to recognize and fight off the viruses and bacteria they target. If the body is exposed to those disease-causing germs later, the body is immediately ready to destroy them, preventing illness.

There are currently more than 50 COVID-19 vaccine candidates in trials. WHO is working in collaboration with scientists, business, and global health organizations through the ACT Accelerator to speed up the pandemic response. When a safe and effective vaccine is found, COVAX (led by WHO, GAVI and CEPI) will facilitate the equitable access and distribution of these vaccines to protect people in all countries. People most at risk will be prioritized. While we work towards rolling out a safe and effective vaccine fairly, we must continue the essential public health actions to suppress transmission and reduce mortality.

Read more here

Biomass Conversion and Biorefinery
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ORIGINAL ARTICLE

Extraction of novel biosilica from finger millet husk and its coconut rachilla-reinforced epoxy biocomposite: mechanical, thermal, and hydrophobic behaviour

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Abstract

High-toughness epoxy biocomposites are made in the current work employing coconut rachilla fibre and biosilica particles recovered from finger millet husk (FMH) for lightweight and affordable technological applications. The purpose of this study is to determine the impact of FMH biosilica particles added at various concentrations on the mechanical and wear properties, as well as the thermal and hydrophobic behaviour, of epoxy composites made from coconut rachilla fibre. The combination of 3 vol.% FMH biosilica particle with surface-treated palmyra sprout fibre as a reinforcing material has the highest tensile strength, impact strength, flexural strength, and hardness with 155 MPa, 6.17 J, 183 MPa, and 92 D-shore, respectively. The coconut rachilla fibre and FMH biosilica-generated epoxy composite have the lowest specific wear rate and coefficient of friction (COF) of 0.006 mm^3/Nm and 0.42, respectively, with the addition of 5 vol% FMH biosilica particles. These composites also exhibit excellent thermal properties having maximum first decomposition temperature of 342 °C and glass transition temperature of 102 °C. Additionally, these composites keep their hydrophobic properties indefinitely, and the ERB3 composite has the lowest contact angle at 81°. These epoxy composites having improved mechanical, thermal, and wear properties may be helpful in a range of engineering applications that can use for high load bearing capacity and biodegradability, such as sporting goods, automobiles, home furnishings, food packaging, transport, and aircraft.

Keywords Composites · Natural fibre, Biomass, Filler · Mechanical properties · Hydrophobicity

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1 Introduction

In recent years, demand for natural fibres and composites made from them has increased significantly for new advance materials for engineering. Natural fibres are employed primarily for the reason that they are affordable, lightweight, simple to produce, renewable, recyclable, need less energy from fossil fuels, and most importantly they have a high specific strength to weight ratio [1, 2]. Growing demand for environmentally friendly materials has caused a paradigm change in favour of natural fibres alternative to synthetic fibres and culminating in the creation of these materials [3, 4]. Being versatile, the natural fibre composites are particularly desirable in many sectors like automobile, drug or food storing, sporting goods, and light weight applications [5, 6].

However, due to the hydrophilic nature of natural fibres and hydrophobic nature of resin does not yield suitable outcomes in natural fibre polymer composites [7]. As a result, the surface of the natural fibres might be surface treated before being incorporated into the polymer as a main reinforcement. Natural fibres can have their loosely bound cellulose and hemicellulose removed through surface treatment. To improve the quality of the fibre-polymer matrix interface, a variety of chemical processes are used to modify the surface of natural fibres, including alkali treatment, benzoyl peroxide treatment, silane treatment potassium permanganate treatment, acetic acid treatment, isocyanate treatment, bleaching, and graft copolymerization treatment, which will improve the fibre's interface interaction with the matrix [8]. The most used chemical treatment for natural fibres is alkali treatment and silane treatment [9]. Alkali treatment removes the lignin, hemicellulose, wax, and other natural impurities, while during silane treatment, the silane molecules attached to the cellulose improves the interaction between the polymer matrix and fibre by increasing reaction sites and surface roughness [10]. In this research work, the combined alkali-silane treatment is used for the surface treatment of fibre. Researchers have looked into using natural plant fibres such as jute, sisal, bamboo, flax, cornhusk, banana, and ramie as a reinforcement material in composites [11, 12].

Murugan et al. [13] studied the silane-treated aloe vera/hemp/flax-reinforced epoxy composite. They demonstrated that the addition of silane-treated hybrid fibre has the highest normalised strength of 98% and low velocity impact damage. Arun Prakash et al. [14] examined the silane-treated *Caryota urens* fibre as a reinforced material in epoxy composite and study their mechanical, thermal, and fatigue behaviour. They found that the incorporation 30vol% fibre in the resin shows the highest fatigue cycle of 18,315 for 25% of maximum tensile stress and highest interlaminar shear strength of 28 MPa. Likewise, to these natural lignocellulosic fibres, coconut rachilla fibre is a new entry which basically obtained from stem of coconut plant and potentially used as reinforcement material in a polymer composite. Akter et al. [15] investigated the mechanical properties of coconut spathe fibre-reinforced HDPE composite. They revealed that tensile strength, flexural strength, impact strength, and hardness of HDPE are enhanced by 5% with the incorporation of coconut spathe fibre.

Furthermore, in order to achieve the appropriate properties for polymer composites, particle reinforcing may be just as important as fibre reinforcement. The particles might improve the composite distinctive properties such as mechanical, thermal, wear properties, and damping properties. Numerous ceramics, nanoparticles, and metallic particles are frequently employed to form composites; however, innovative bio-based particle types are gaining popularity for usage in multi-dimensional composites [16]. Bio-based particles produced from domestic, animal, or marine waste may also be used as filler in composites [17]. These agricultural wastes might be transformed into beneficial bioceramics, which would minimise pollution, increase process economy, and help with solid waste management [18]. Poomathi et al. [19] conducted a study on red matta rice husk ash-extracted biosilica particles' derived epoxy composites. They found that with incorporation of biosilica particles, there is improvement of mechanical and fatigue behaviour of composite. Jayabalakrishnan et al. [20] evaluated the mechanical, dielectric, and hydrophobic behaviour of epoxy composites fabricated with biochar particle extracted from coconut shell. They found that composites with 5vol.% biochar particles has maximum mechanical strength. While the composite with 7 vol.% has highest dielectric constant with 6.2 and high hydrophobic contact angle of 70°. Similarly, Gairola et al. [21] explore the crop residual waste extracted from finger millet husk (FMH) and barnyard millet husk (BMH) and its potential in thermoplastic composites. They demonstrated the FMH-reinforced composite exhibits better mechanical properties as compared to BMH-reinforced composites.

The objective of the current study is to determine the advantages of employing biosilica extracted from finger millet husk and the development of a unique fibre made from coconut rachilla as a reinforcement material. The main objective of the current study is to determine how the high-performance epoxy composite's mechanical, wear, thermal, and hydrophobic behaviour is affected by coconut rachilla fibre and FMH biosilica particles.

2 Material and methods

2.1 Materials

As reinforcements, coconut rachilla fibre with dimensions of 300–400 mm in length, 0.2 µm in diameter, and 0.94 g/cc is acquired from regional farms in Tamil Nadu, India. Fig. 1

shows how coconut rachilla fibre is prepared. Usually the green coconut rachilla was soaked in the water for 2 weeks. Then, it was dried in sun light for easy removal of fibre. The top thick lignin layer is removed in the soaking period and the fibre bundle alone remains. Finally the fine fibre was extracted using hammering process. The prepared fibre contains cellulosic content of 30–38%, semi-cellulosic content of 13–18%, holo-cellulose of 8–16%, lignin of 28–38%, and wax content of 1–3%. This fibre has tensile strength of 380 MPa, young's modulus of 9.3GPa, and density of 1.35 g/cc, whereas the other peer group natural fibres such as cotton, flax, hemp, jute, and kenaf have lesser physical properties than this fibre [22]. Finger millet husk is also collected from the local market for preparation of biosilica particles. Huntsman, India Pvt. Ltd. supplied the Araldite Epoxy Resin of grade LY556 and the hardener of grade HY591 used as the resin system. 3-Aminopropyletriethoxysilanesilane is used as a surface enhancer procured from Sigma-Aldrich by the USA (APTES). Another chemical like NaOH, HCl is provided by MERCK India Ltd.

2.2 Preparation of biosilica from finger millet husk

The finger millet husk (FMH) biosilica is synthesized using a thermo-chemical process. The FMH biosilica particles are prepared in two stages. The FMH are initially interlaced and lit using a separate air supply mechanism at 750 °C in a thermal reactor. The by-product of burning is FMH ash which has high biosilica content and very few contaminants. Cleaning the FMH ash contaminants is the second stage. To manufacture sodium silicate solution, the produced FMH ash is combined with various concentrations of NaOH solution at 80 °C and continuously agitated for 1 h. To remove sodium silicate, the solution is filtered using filter paper (Whatman grade 41). The sodium silicates are titrated once more in the 1 N (normality) HCl solution at a pH of 7. The silica gels are gathered when the mixing procedure is finished and aged for 24 h. Silica gels will be washed with distilled water to create a silica slurry. This silica slurry is heated in a beaker for roughly 20 h at 70 °C after being periodically rinsed. To obtain a finer particle size, the created xerogel silica is subsequently mashed in a mortar for a number of hours. The FMH biosilica that is obtained has a density of 2.7 g/cc with a simple cubic structure and a diameter of 1–3 µm. The prepared biosilica particles are then surface treated with silane as same as fibre treatment. SEM image of FMH biosilica and coconut rachilla fibre is shown in Fig. 2.

2.3 Surface treatment of coconut rachilla fibre

The palmyra sprout fibre is first given an alkali base treatment. Prior to the alkali treatment, the fibres are dried for 2 h at 100 °C in an oven. For alkali treatment, fibres are steeped for 3 h at room temperature in a 1 N NaOH solution using a 1:20 ML ratio. After the procedure, the samples are thoroughly neutralized by being rinsed with water to get rid of any remaining NaOH. The samples are dried for 24 h at room temperature before being oven dried for 2 h at 100 °C [23].

2.4 Composite preparation

The epoxy biocomposites are created utilising a rubber mould by hand lay-up approach. Prior to moulding, a consistent volume of epoxy resin is combined with three distinct FMH biosilica particle concentrations (1 vol. %, 3 vol. %, and 5 vol. %), and the mixtures are continually stirred until homogeneous. Then, hardener is added to the epoxy resin and biosilica particle mixture with ratio of 10:1. After precisely measuring out 20 vol. % and 40 vol. % of coconut rachilla fibres, the epoxy resin mixture is then poured into the mould. Cover the mould with a top plate to get rid of any leftover resin. The resin can then

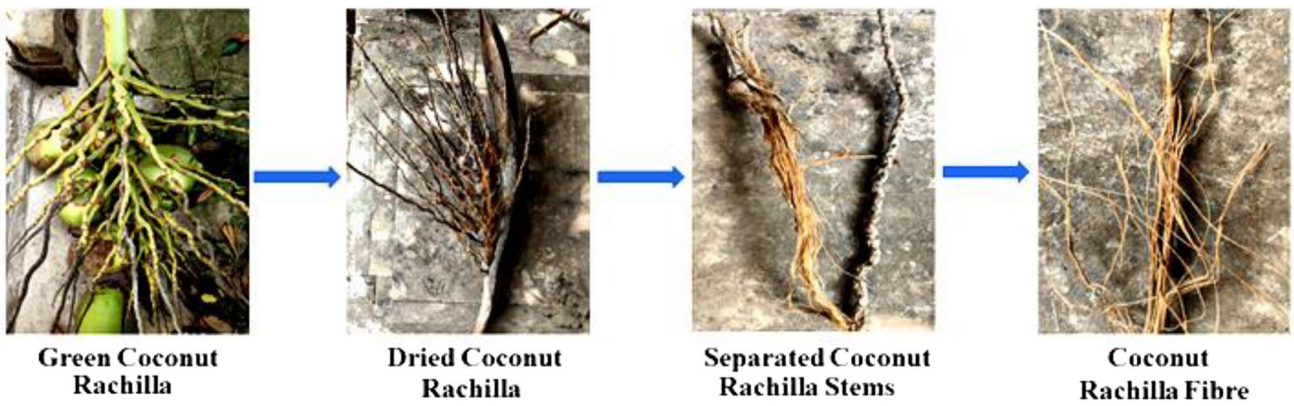

Fig. 1 Extraction step-by-step process of preparation of coconut rachilla fibre

Table 1 Composition and designation of composite fabricated

Composite designations	Epoxy resin (vol.%)	Rachilla fibre (vol.%)	Biosilica (vol.%)
E	100	-	-
ER1	80	20	-
ER2	60	40	-
ERB1	59	40	1
ERB2	57	40	3
ERB3	55	40	5

be post-cured for 12 h at 120 °C after curing for 24 h at room temperature [24]. Table 1 shows the composition and designation of composites fabricated.

3 Characterizations

According to ASTM D638 and D790, the tensile and flexural properties of composite materials are assessed using a universal testing machine (UTM), INSTRON 4855, UK, with a 10-ton loading capability. All samples are tested at a transverse speed of 1.1 mm/sec. A micro-impact tester made by Crystal Equipments India Pvt. Ltd. with a maximum energy reading scale of 20 J is used to assess impact toughness. The Shore-D durometer from Bluesteel India is used to measure the microhardness of composites in accordance with ASTM D 2240, 2016. The wear characteristics of epoxy and its composites are examined using the ASTM G99-compliant dry pin-on-disc technique (Magnum, India). The test conditions include a standard weight of 10 kg, a disc speed of 800 rpm, and a worn track diameter of 100 mm. The mass polymer composites lost with the temperature change are examined by thermogravimetric analysis. To conduct the thermogravimetry experiment on epoxy composite, TGA, thermo-scanner from NETZSCH STA Jupiter, 409 PL Luxx, Germany, is used. The temperature raise was controlled as 10 °C/min at N_2 gas environment. The glass transition behaviour and thermal stability of composites are investigated using differential temperature analysis. According to ASTM D 2578, contact angle measurement is used to assess the hydrophobicity (sessile drop test) of an epoxy composite coating (Holmarc, India). This included using a high-performance, aberration-corrected CMOS lens to place a water drop on the composite and determine the contact angle between the drop and the composite. Finally, a scanning electron microscope is used to study the epoxy composite micrographs (Hitachi-1500, Japan).

Table 2 Mechanical properties of epoxy and its derived composites

Composite designations	Tensile strength (MPa)	Tensile modulus (GPa)	Flexural strength (MPa)	Flexural modulus (GPa)	Izod impact (J)	Hardness (Shore-D)
E	61	2.361	94	3.832	0.38	86
ER1	94	3.845	136	5.384	3.74	86
ER2	128	4.872	152	6.175	4.28	87
ERB1	136	5.278	168	6.329	5.62	89
ERB2	155	6.335	183	6.819	6.17	92
ERB3	138	5.261	162	6.194	6.04	94

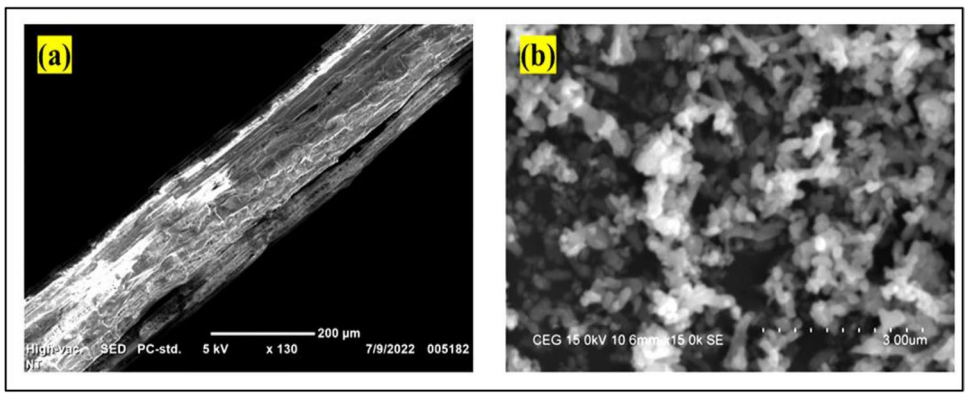

Fig. 2 SEM image of **a** coconut rachilla fibre and **b** FMH biosilica particles

4 Result and discussion

4.1 Mechanical properties

The mechanical characteristics of epoxy composites made with coconut rachilla fibre and FMH biosilica particles are listed in Table 2. The neat epoxy resin has the tensile strength, tensile modulus, flexural strength, flexural modulus, Izod impact toughness, and hardness of 61 MPa, 2.361 MPa, 94 MPa, 3.83 MPa, 0.38 J, and 86 Shore-D, respectively. According to ASTM standard test specimen, the values are almost closer and produced repeatability. Because of its brittle nature, epoxy matrix resin has poor mechanical qualities. The mechanical characteristics of composites improve by 54.1%, 62.8%, 44.6%, 26.1%, 884.2%, and 1.16%, respectively, when coconut rachilla fibre is added, as shown in Table 2. While there is further enhancement observed in mechanical properties when volume percentage of fibre is increased [25], on comparison with the previous article [25], the mechanical properties are improved significantly. The fibre acts as a load-bearing capacity in the composite and is equally disseminated throughout the epoxy matrix resin, which lowers the stress intensity factor and so improves mechanical qualities. Additionally, by lowering the fibre's moisture absorption, surface treatment of the palmyra fibre enhances the contact between the hydrophobic matrix and hydrophilic fibre. Alkali-silane-treated fibre thereby improves the interfacial interaction between the matrix and fibre at the interface, resulting in high adhesion to the resin system and the epoxy matrix. Additionally, it is clear from Fig. 3a that the silane group and epoxy matrix resin have a chemical link that makes the reactive phase of the alkali-silane-treated fibre more compatible with the matrix resin.

Additionally, the mechanical properties of coconut rachilla-reinforced epoxy composites are improved by the dispersion of FMH biosilica particles. The distribution of biosilica particles has a substantial impact on the initial and maximum debonding loads of palmyra sprout fibres from the epoxy matrix. Figure 3 illustrates the rather smooth pull-out behaviour of fibre from the epoxy matrix during the failure of ERB composites. This can be due to a strong chemical link between the alkali-silane-treated fibre and FMH biosilica particles that results in good adhesion between the epoxy matrix resin and coconut rachilla fibre. The composite with the designation ERB2 exhibits the greatest increase in mechanical characteristics. This betterment is due to strong bonding between matrix resin and fibre because of high concentration of biosilica particles as shown in Fig. 3c. It is also interesting to notice that, aside from hardness, the mechanical properties of the biocomposites drastically decrease as the concentration of FMH biosilica particles rises by 5vol%. This is due to the agglomeration that takes place at large biosilica particle volumes, and the voids the agglomerates create have a significant impact on tensile strength as shown in Fig. 3d.

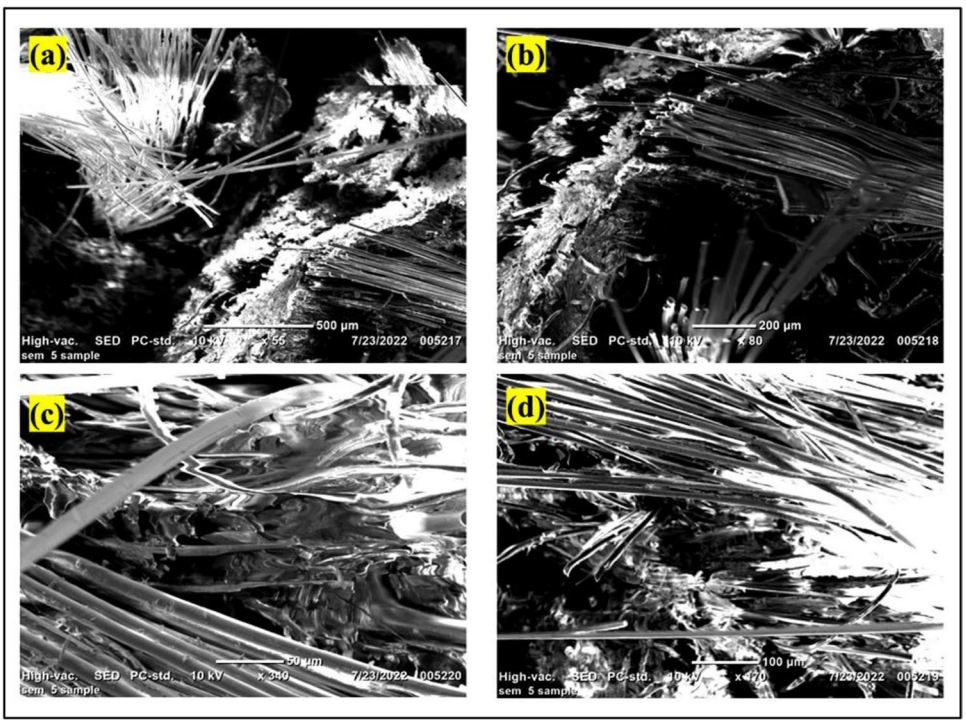

Fig. 3 SEM images of tensile fractured composite samples: **a** ER2, **b** ERB1, **c** ERB2, and **d** ERB3

4.2 Wear properties

Figure 4 demonstrates the wear characteristics of composites made from epoxy reinforced with coconut rachilla fibre and FMH biosilica particles. The increased wear attributes of coconut rachilla fibre-reinforced composites are comparable to the mechanical properties. The specific wear rate and coefficient of friction of pure epoxy resin are 0.028 mm^3/Nm and 0.74, respectively, higher than those of other composites. On comparison with the ASTM standard test specimen, the COF and specific wear rate are deviated with 0.02%. The high specific wear rate and coefficient of friction cause a rise in abrasion and adhesion wear loss. Epoxy resin's molecular structure is sticky and adheres to the abrasion disc, removing a lot of material from the work piece in the process. This substance re-adhered to the abrasion disc and provided increased two-body and three-body abrasion [26]. Additionally, a portion of the material is released from the work piece as a result of high frictional heat during a major run in the material, increasing wear loss.

By improving the epoxy system with coconut rachilla fibre, wear loss is reduced. However, wear loss is decreased even further when FMH biosilica particles are added to composites. Both the COF and the specific wear rate decrease as FMH biosilica particle concentration increases. The ERB3 composite has the lowest COF of 0.42 and the lowest specific wear rate of 0.006 mm^3/Nm. This significant improvement in wear characteristics results in the strong interfacial adhesion behaviour of the biosilica particles. By reducing adhesion with the abrasion disc, FMH biosilica particles improve the sliding behaviour of composite surfaces. The previous article reported lesser wear resistance of 0.014 mm^3/Nm for composite they prepared. However, the present composite holds higher wear resistance. The biosilica particles are converted into cellulose flakes when the biosilica dispersed epoxy composite is abraded in the friction disc. Acting as solid lubricant packets in between the abrasion discs, these flakes lessen wear loss. Additionally, because there is less friction between the surfaces, the rate of heat dissipation is higher [27]. The epoxy resin composite's biosilica particles work as a heat absorber to stop erosion and adhesion wear, lowering the wear rate and COF as a result.

4.3 Thermal properties

The TGA and relative DSC arrived Tg values of epoxy and its coconut rachilla fibre and FMH biosilica particles generated composite are shown in Fig. 5. It should be mentioned that the pure resin had a Tg value of 63 °C and breakdown temperatures of 295 °C, 374 °C, and 483 °C at the beginning, middle, and end of the process, respectively. The chemically reacted secondary side polymer branches and the highly cross-linked epoxy molecular structure are the reason for greater Tg and reasonable mass loss stability. Since the primary and secondary molecules of epoxy resin are very reactive, it takes a lot of heat energy for them to move around, which increases the heat flow and raises the Tg as well as mass loss stability [28]. However, it should be emphasised that the Tg and thermal stability at the beginning, middle, and final stages are only little altered by the addition of coconut rachilla fibre alone in to the resin. The Tg and thermal stability of an ER2 composite designated with high concentration of fibre are increased, which might prevent the early evaporation of large amount of fibre molecules when the temperature is raised, is a result of this enhancement.

Additionally, it should be noticed that the incorporation of FMH biosilica particles added to the resin improved the thermal stability as well as Tg. Due to the creation of nanocomposite materials, the softening temperature has

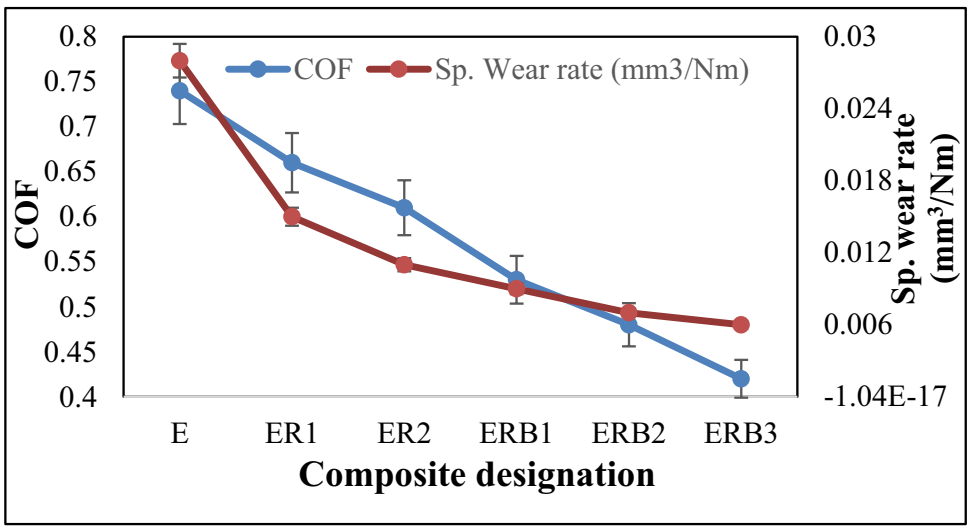

Fig. 4 Wear properties of epoxy and its derived composites

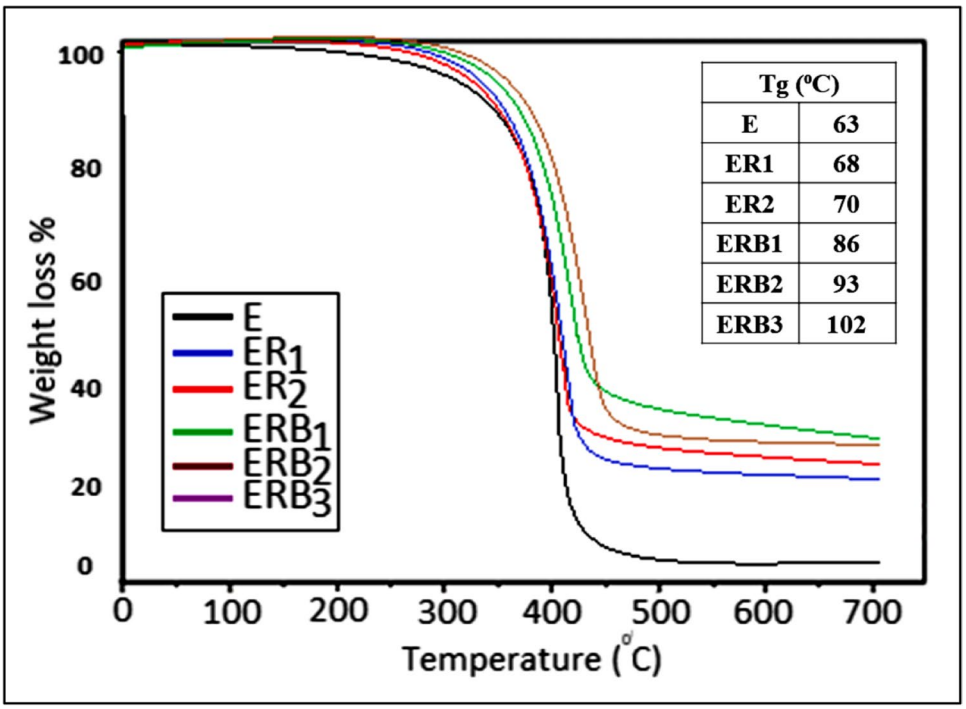

Fig. 5 Thermal behaviour of epoxy and its derived composites

increased, which has improved heat energy absorption. Biosilica additives neatly filled in the gaps left by the polymer and increased the heat capacity. The unit length and weight of the polymer chain has risen as a result of the additional FMH particle reacting with the multiple polymer chains. Therefore, weight gain in chain during heating may take a lot of energy to rotate itself around the core C–C chain [29]. As a result, the increased Tg is seen. The stability of mass loss is also increased by this improved Tg. The initial, middle, and final decomposition temperatures are raised for the FMH biosilica particle-added composite designations ERB1, ERB2, and ERB3. For EFP3 composite designation, a maximum first decomposition temperature of 336 °C is reported; this represents a 13.8% improvement over the neat epoxy resin system. Suganya et al. [29] reported the thermal stability of composites with an improvement of 8.4% on compare with neat epoxy resin. However, this present composite produced extra 5.4% of improvement.

4.4 Hydrophobic behaviour

Figure 6 shows the hydrophobic contact angle of composites made with FMH biosilica and coconut rachilla fibre-reinforced epoxy. Pure epoxy resin has been shown to have the largest contact angle, measuring 102°. High contact angle happens as a result of the free, unsaturated OH molecules in the epoxy primary chain's capacity to fend off water molecules and maintain low surface activation energy. Due to the addition of coconut rachilla fibre by 20 vol%, contact angle is drop by 3.9% because of high water permeability of

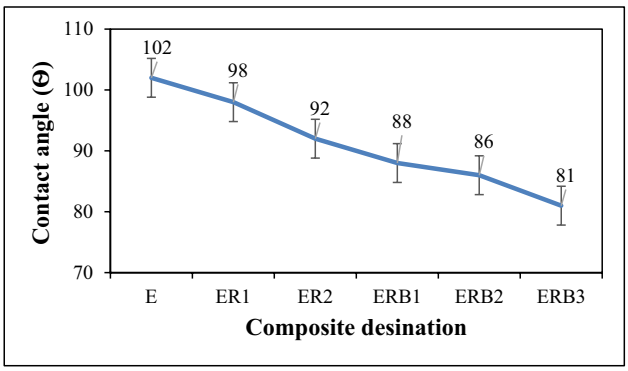

Fig. 6 Hydrophobic behaviour of epoxy and its derived composites

fibre and hence increases water absorption. Natural fibres are cellulosic; hence, coconut rachilla fibres are incredibly vulnerable to water absorption. With increasing concentration of fibre, contact angle decreases. Also, it is interesting to note that the contact angle continues to decrease with the addition of biosilica particles. Low contact angle is observed as a result of this behaviour, which is brought on by the FMH biosilica particle's high active surface energy and high water permeability. Alshahrani et al. [30] also reported similar effects when adding biosilica particle into the epoxy resin. With a contact angle of 81°, the composite with the designation ERB3 has the lowest value. As a result, despite the addition of natural fibre and biosilica particles, the composite retains its hydrophobic properties indefinitely and does not become more hydrophilic [31 and 32].

5 Conclusions

In this study, the role of FMH biosilica and coconut rachilla fibre in the creation of incredibly durable epoxy composites is examined. According to results it is noted that the composite containing 3vol% of FMH biosilica exhibits maximum enhancement and surpasses conventional composites in terms of tensile strength, tensile modulus, flexural strength, flexural modulus, Izod impact strength, and hardness. The mechanical properties of the biocomposite are diminished as a result of the agglomeration caused by the high concentration of biosilica particles (5 vol. %) in the material. The biocomposite contains 5 vol. % of biosilica particle, which has the lowest specific wear rate and COF of 0.003 mm^3/Nm and 0.42, respectively. Additionally, it also exhibits the greatest weight loss percentage with temperature and glass transition temperature with 102 °C; hence, it has outstanding thermal properties. These composites also do not become more hydrophilic when natural fibre and biosilica are added, thus maintaining their hydrophobic properties indefinitely. Therefore, for engineering applications, including high load bearing applications as well as in automobiles, the alkali-silane-treated coconut rachilla fibre-reinforced FMH biosilica particle-toughened epoxy composites could be helpful.

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P. Roshith: research and characterization
K. Ramesh: research and finance
B. Ramesh: research

Data availability All data within manuscript.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interest.

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Bioactivity, Chemical and Functional Characterization of Karaya Gum Sterculia

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Abstract:

Natural biopolymers have a wide range of uses and bioactivities. In this investigation, karaya gum was gathered and put to use for bioactivity tests. Thin layer chromatography (TLC), a UV-Vis spectrophotometer, and FTIR were used to characterise gum and screen it for phytochemicals. Additionally, it was tested for bioactivity in tests that looked at antioxidant activity and antibacterial activity against S. aureus and E. coli. The antioxidant and antibacterial properties of Karaya gum were effective against S. aureus and E. coli. The presence of sugars such sucrose, glucose, xylose, and other sugars was shown by UV-VIS spectroscopy examination. Alcohol, phenols, aldehydes, and ketones were among the functional groups that were detected using FTIR analysis.

Keywords: *Karaya gum, bioactivity, sustainability, karaya gum sterculia*

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1. Introduction

Plant gums have been used widely in several medical applications, as it is the cheapest and most available raw material for polysaccharide production[1]. These gums are usually formed after a wound in a superior plant as a result of their protection mechanisms. The ability of these materials to be bio-safe and biodegradable, makes it perfect to create a drug delivery system to enhance drug-delivery matrix due to their elevated water-produced swelling, dispersible in tablets, availability, low cost, and thickening characteristics in oral-administered liquids[2-5]. But, the chemical composition of the gum is important since it can affect the extraction technique and also can define the uses of the gum[6]. The karaya gum is an exudate from a big bushy tree known as *Sterculia urens*, this tree is originated from the family Sterculiaceae, that can be found in a dry forest located at the central and northern part of India. Another source for karaya gum is from *S.setigera* in Senegal and Mali, and minorsup- pliesform *S. villosa* in Sudan, India and Pakistan[7-8]. The production of these gums is so critical, the exudation will only begin after tapping the trunks by a manpower and then it continues for several days, the large exudes is dried in hot and dry weather, broken, cleaned to

take out the unwanted materials and the bark, Then it will be categorized based on the quality and stored. The Gums harvested during the hot climate (April, May and June) are the one characterized with the highest quality and they are exported internationally as grade one. Grade one gum is usually found as a powder or granules and used in pharmaceutical and food industries since they have food solubility, high viscosity, moisture retention, and transparent color. World production is around 3000 tons a year, half of it originated from India and the rest originated from NorthAfrica[7]. It has an acetic flavor and odor; it can create a soft film when it's plastified with glycols. The chemical structure has been found to contain D-glucuronic acid, D-galacturonic acid, D-galactose and L-rhamnose, but in different ratios depending on the type, source, and the quality of the gum[8-10]. The viscosity of Karaya gum is inversely proportional to the shear rate, where the viscosity in 0.5% dispersions has a value near the 120-400 centipoise[9]. Usually, the viscosity develops linearly in low-concentration solutions, until reaching 0.5% of karaya gum concentration. Viscosity reduces as the gum ages[11]. While regarding the pH stability, Karaya gum dispersion is resistant to acidic environments since it has a high uronic acid concentration. The dispersion stability can be only maintained at an acidic range [12]. The FDA categorized Karaya gum as safe after several teratogenic, toxicological tests and mutagenic studies[13], thus being used in drug delivering [14].

2. Materials and Methods

2.1. Materials used

Ethanol, Trichloroacetic acid,Acetone, Sulphuric acid , Phenol,Sodium Tri Meta Phosphate,Iodine, Potassium Iodide, Picric Acid, Chloroform, Ferric Chloride, Sodium Hydroxide, Ninhydrin reagent, Iodine Crystals, Nutrient agar, Nutrient broth, DPPH reagent, Methanol, Hydrochloric acid, *E.coli*, S.aureus. Centrifuge, Weighing balance, UV-Vis spectrophotometer, and FTIR.

2.2. Procurement of *Sterculia urens* gum exudate

Karaya gum was obtained from *Sterculia urens* trees found in the highlands of central and northern India. The gum granules were completely dried and powdered using pestle and mortar.

2.3. Defatting of Karaya gum

First, the Karaya gum granules were crushed into a powder by using pestle and mortar, then the powder was soaked in 100% ethanol overnight and dried using the hot air oven at 55–60 °C to obtain a defatted gum. 6g of the defatted gum powder was dissolved in 300 ml of water and stirred continuously for 2 hours using the magnetic stirrer, later the viscous solution was filtered by passing it through a cotton gauze to remove all the mucilage.

2.4. Characterisation of crude karaya gum:

Karaya gum powder was tested using various techniques to detect the characteristics of the polysaccharide. The crude gum was subjected to UV-Vis Spectrophotometer for characterization which was taken at 200-800nm. Additionally, the extract was then analyzed using FT-IR analysis at the frequent range of 450-4000 cm -1

2.5. Solubility tests

1 gram of the crude Karaya gum was weighed and dissolved in 20 ml of six various solvents which are water, Ethanol, Methanol, Acetone, Hydrochloric acid, and DMSO to check the solubility rate of the crude gum.

2.6. Moisture Content

An empty dish plate was first weighed on an Avery laboratory balance, and the weight was recorded, one gram of the crude gum was added to the dish and the weight was taken and recorded again. The sample was placed in an oven with a temperature of 50-55 °C. Later every 10 minutes the gum sample was taken out of the oven and weighed again. This procedure was repeated until the gum weight remained constant. The percentage of the gum moisture content was then calculated using the following formula:

Moisture Content (%) = *(amount of the moisture loss / Weight of bone dried sample) x 100*

2.7. Determination of Swelling index

The swelling capacity of the crude Karaya gum was measured using Meka et al[15]. First, one Gum granule was taken and weighted using an Avery laboratory balance and then transferred into a 100ml beaker. 50ml of distilled water was added and the beaker was shaken thoroughly and kept at room temperature for 6 hours. The weight of

the granules was taken every 30 min and recorded. The swelling index was determined using the following equation

Swelling Index = *(Final Volume - Initial Volume / Final Volume) x 100*

2.8. Phytochemical analysis

Several phytochemical analysis was conducted to detect the components of the gum extract and search for bioactive agents that can be applied in the synthesis of useful drugs and drug carriers, the first one was the detection of alkaloids by applying Wagner's test and Hager's Test. The gum extract was first dissolved in dilute Hydrochloric acid and filtered, later for Wagner's test the filtrate was mixed with a few drops of Wagner's reagent that is made of iodine in potassium iodide. While for Hager's test the filtrate was mixed with 1 or 2 drops of Hager's solution which is picric acid then the color was observed for both tests. Second test was Salkowski's test for the detection of Phytosterols, where the gum extract was mixed with 2ml of Chloroform and filtered and then the filtrate was mixed with 3 ml of concentrated sulphuric acid, later the mixture was allowed to set for a few minutes and the color change was observed. Third test was ferric chloride for detection of Phenols, where a few drops of ferric chloride solution was mixed with 2ml of the gum extract, and the color change was observed and recorded. Ninhydrin test to detect proteins and amino acids was conducted after, the gum extract was mixed with 0.25% w/v Ninhydrin reagent and boiled for 5 minutes till the color change was observed. Alkaline Reagent test was also done to detect flavonoids, 3-5 drops of Sodium Hydroxide solution was added to the gum extract and the yellow color observation was done. To add more, a Foam test to detect Saponins was carried on by mixing 2ml of water with 0.5ml of gum extract, the mixture was shaken for 2min and the foam persisting was observed for 10 minutes. Lastly, Benedict's test was conducted to test the sugar reduction; 1ml of the gum extract was placed into a clean test tube and 2ml of Benedict's reagent was added to the sample, the sample is then placed in a boiling water bath for few minutes, and the color change of the solution was detected. For all the test's, distilled water was used as the negative control[16].

3. Thin Layer Chromatography

Thin layer chromatography was performed on the Karaya gum extract to detect the number of components, and to determine the monosaccharide composition found in the Karaya gum extract. The procedure was placed using the solvent systems ethanol: water (1:1), methanol: water (1:1) and acetone:water (1:1). The TLC film was prepared first, and a room to load the sample was made by gently scratching the surface of the plate; by using a pipette a less than 1mm diameter sample was placed on the spot. Later the TLC films were placed at an angle of 60° in three different beakers (with an aluminum foil lid) which have the same volume but different types of solvents, and the films were allowed to sit in the beaker till the solvent and the sample almost reached the top of the film. The observation of the sample was done by first visualizing the films under the short length wavelength UV light, and the observed spots were outlined with a pencil, followed by placing the films in a jar containing iodine crystals for a few minutes. Lastly, the observed spots were also outlined and the R_f value was calculated.

4. Antibacterial activity

The Antibacterial activity of the gum extract was tested by using agar well diffusion technique. For this method, the Nutrient agar plates were prepared and the mother cultures of the *E.coli* and S.aureus were cultured for 24h in the nutrient broth. Later, six 10mm diameter wells were made in each plate by using a punching kit and needle. The broth culture of each bacteria was inoculated on each agar plate using cotton swab, then the gum extract were added on each well in different concentration (100%, 75%,50%, 25% and 0%), where in the last well an Ampicillin antibiotic disc were added as the positive control. Lasly, the *E.coli* and the S.aureus plates were incubated at 37° C for 18-24h. If the gum extract was able to inhibit the bacterial growth, it would form a clear zone around the well known as inhibition zone. The diameter of these zones was measured to determine the effectiveness of the gum extract against the bacteria. The larger the zone, indicates a strong antibiotic possessing.

5. Antioxidant activity

Determination of the antioxidant activity of the Karaya gum extract was tested by performing DPPH assay (Radical Scavenging assay) and Phosphomolybdenum assay. DPPH test is a cost effective and simple procedure to detect the antioxidant capacity of the extract, this test is done by the interaction of the hydrogen donors with the stable 2 2-diphenyl-1-picrylhydrazyl that leads to the decolorization of the purble DPPH. For this test, 0.1mM of DPPH solution was prepared in methanol. Later five different test tubes were prepared with different concentrations of the gum extract each containing 3ml; 1ml of the prepared DPPH solution was added to each tube and mixed well and allowed to sit in the dark at room temperature for 30min. The absorbance was recorded at 517 nm using a UV-spectrophotometer. Ascorbic acid was prepared as well in 5 different concentrations and used as the standard. Lastly the Scavenging activity was calculated based on the following formula[16]

"A0 - A1"/ A0 x 100, where A0 is the control absorbance and A1 is the sample absorbance

Phosphomolybdenum Assay was conducted to determine the presence of antioxidant components in the gum extract based on reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V). The reagent was prepared by mixing 0.04g of ammonium molybdate, 0.03g of sodium phosphate, 0.6ml of concentrated sulfuric Acid, and then the volume was made up to 5ml by adding distilled water. Later five tubes were prepared with the respective volume 100-500µl of the gum extract. 1ml of the reagent was added to each tube and then they were allowed to a set in a water bath at 95 °C for 90 minutes. Lastly, the absorbance at 625 nm was then taken and recorded.

6. Result and Discussion

6.1. Solubility Testing

The crude Karaya gum showed a low solubility, even by using different types of solvents (Table 1). The presence of insoluble matter, large and high weight molecules and impurities affect the solubility of gum, which reduces the solubility capacity of the crude Karaya gum. One gram of the crude karaya gum was able to dissolve partially in water, due to the acetyl group found in the gum structure, therefore it leads to the formation of viscous colloidal dispersion after the gum absorbs the water rapidly[17]. While for Hydrochloric acid, the crude Karaya gum was able to dissolve completely. On the other hand, Ethanol, Methanol, Acetone and DMSO were not able to dissolve the crude Karaya gum; and that because the physical characteristics of alcohols are based on the hydrogen bonding ability of the -OH group. When the gum was added to the ethanol,methanol or acetone solvents , which are less polar than water, it decreases the solubility and potential hydrogen bonds of the starch , since the water is more attracted to the alcohol than the starch, the starch is dehydrated by the alcohol and precipitates.

Table 1. Solubility of karaya gum in various solvents

Solvents	***Solubility***
Water	Partially Dissolvable
HCL	Dissolvable
DMSO	Non-Dissolvable
Ethanol	Non-Dissolvable
Acetone	Non-Dissolvable
Methanol	Non-Dissolvable

6.2. Determination of Swelling Index

The initial weight of the gum granule was 2.64g, then the granule weight was taken each 30 min for 6 hours. The weight was 5.28g, 6.20, 8.01 and 9.61g respectively. The swelling index then was calculated to be 50%, 57%, 67% 72.5% (Figure 1). This shows that the gum can be used as disintegrant which on swelling may explode and produce enough energy to free up drug contents; therefore, absorb enough moisture that allows it to swell and lead to the disintegrating of the tablets disintegrated. This test allows to discover the flow properties of the karaya gum, packaging and arrangement of particles and hence the compatibility of the granulation.

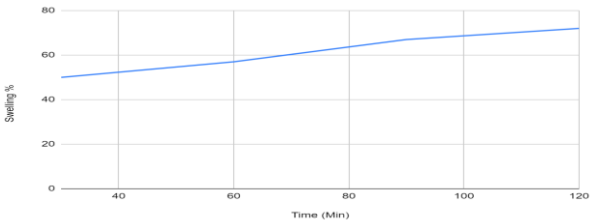

Figure 1. The swelling index percentage as the time increases

6.3. Moisture Content

The moisture content of the karaya gum versus drying time at 55 °C. It shows that the initial moisture content of 16% was reduced to 4%, 2%, and 1% every 10 minutes for 70 minutes till the moisture content became stable. More energy is required for it to be dried more, which means higher temperature can be used as well to reduce the moisture content further. Hence the drying time needed to decrease the moisture content to any certain level is based on the temperature (Figure not shown here).

6.4. Phytochemical Screening

The gum extract, shown to contain alkaloids after conduction Wagner's and Hager's Test. Positive results were observed for Wanger's test by detecting a brownish-reddish precipitate, while for Hager's test a yellow precipitate formed indicating the presence of alkaloids. To add more, the Ninhydrin test showed positive results, after the observation of the blue color, which indicates the presence of protein and amino acid in the gum extract. Phytosterols were detected in the gum extract after conducting Salkowski's test, a yellow golden color was observed indicating the presence of triterpenes. On the other hand, Negative results were observed for Phenols, Flavonoids, saponins and reducing sugar, where no color change were observed.

6.5. Thin Layer Chromatography

TLC was conducted for the extract Karaya gum; the separated bands showed the presence of monosaccharides. (R_f -0.75) indicates the presence of Rhamnose, (R_f -0.46) indicates the presence of glucose, lastly (R_f -0.67) indicates the presence of Xylose and Ribose (figure not shown here). The difference in the R_f values was detected because of the polar nature of the mobile phase towards the monosaccharides present in the Karaya gum.

6.6. UV-Vis Spectrophotometer

The aqueous Karaya gum extract was characterized using UV-Visible Spectrophotometer, absorbance maximum was found between the range of 270 – 280 nm which indicated the presence of sucrose and glucose (Figure 2). According to Sarazin et al.[18] the absorbance of sucrose is at 260 nm to 270 nm while glucose is from 260-270nm. Additionally, a peak at 210 nm and around 255 nm might indicate the presence of xylose. Similarly, Samrot et al.[19] have reported that the sharp peak at 254 nm indicates the presence of xylose in *Mangifera indica L* Gum.

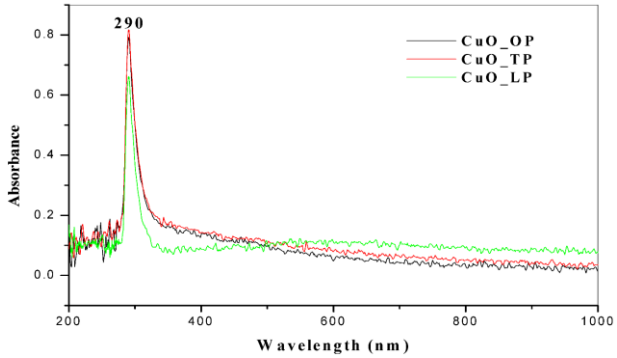

Figure 2. UV-Vis analysis of the Karaya gum extract

6.7. Fourier transform infra-red spectroscopic (FTIR)

FTIR analysis showed a peak at 3266 cm-1 indicates the hydrogen crosslinking of water molecules and shows O-H stretching, with compound type Alcohol and Phenols (Figure 3). 1721 cm-1 indicates the -C- stretching, with compound type Aldehydes and Ketones. 1601 cm-1 shows the C=C stretching, with compound type Alkenes. 1372 cm-1 represent C-H bending, with compound type Alkane. The peak at 1241 cm-1 indicates the stretching of the C-N, with compound type Amines. Lastly the peak 1036 cm-1 represents C-O stretching with compound type primary alcohol. Samrot et al.[20] have depicted that the *Terminalia Catappa L.* gum had a large peak at 3417 cm-1 for O-H stretching, 2940 cm-1, 1300-1450 cm-1 for alkenes and 1725 cm-1 for the aldehyde group, respectively.

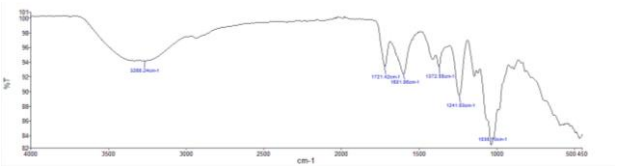

Figure 3. FTIR analysis of the Karaya gum extract

6.8. Antibacterial activity

For the negative control (water) there was no zone of inhibition observed. For The positive control (Ampicillin antibiotic discs), the zone of inhibition was observed for both *S.aureus*, but no zone of inhibition for *E.coli*. The Karaya gum extract inhibition zone was also observed against both *S.aureus* and *E.coli*. The zone of inhibition indicates that the gum extract was able to inhibit and kill the bacterial growth, which shows an antibacterial activity. Minimal inhibitory concentration of the Karaya gum extract was found to be 5µl/ml. while for 20µl/ml concentration it showed higher antibacterial activity than the positive control (ampicillin antibiotic discs) (Table 2). In a study it has been reported that the antibacterial activity of the greenly synthesized silver nanoparticles using gum ghatti showed zone of inhibition (ZOI) of around 12.25 mm diameter against S*. aureus* and nearly 9 mm ZOI for E*.coli*[21]

Table 2 Antibacterial Activity of Karaya gum extract against *E.coli* and *S.aureus*

Organism	Zone of inhibition (cm)					
	Positive Control	Negative Control	Concentration			
			20µg	15µg	10µg	5µg
Escherichia coli	10mm	-	23 mm	17 mm	-	10 mm
Staphylococcus aureus	10mm	-	18 mm	12 mm	9 mm	5 mm

6.9. Antioxidant Activity

Based on the calculation, the Karaya gum were found to have an increased radical scavenging activity against DPPH with the increase of the concentration. At 250 µl, the scavenging activity was 70% which indicates a good antioxidant activity by the gum extract (Table 3) (Figure 4). The absorbance value decreased as the concentration of the Karaya gum increased, which indicates an increased Antioxidant activity. Bouaziz et al.[20] have reported that the antioxidant activity of flaxseed gum was very higher with the IC50 value of 2.5 mg·mL^{-1}

Table 3 Antioxidant activity evaluation using DPPH assay

Concentration (µl/ml)	***Scavenged Activity of the sample (%)***	***Scavenged Activity of the standard (%)***
50	50%	71%
100	55%	76%
150	59%	82%
200	65%	87%
250	70%	90%

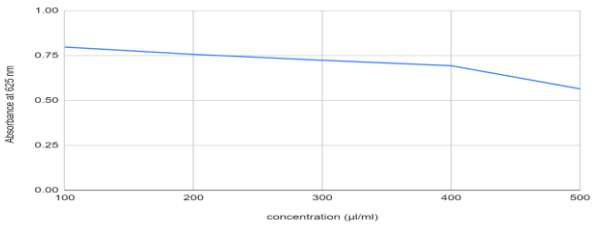

Figure 4. Phosphomolybdenum Assay of Karaya gum extract

Conclusion

In conclusion, the quality of well characterized materials is directly linked to their physical and chemical characters. In this study, Karaya gum extract was subjected to several characterization tests; Bioactivities were performed, Karaya gum were found to have a good antioxidant property and also good antibacterial activity against *E.coli* and *S.aureus*. On the other hand, Karaya gum extract was analyzed spectroscopically and found to have sugars like sucrose glucose and xylose etc. The gum was also characterized by TLC, Phytochemical Screening and FTIR, and it was found to contain several compounds such as Alcohol, Phenols, Aldehydes, Ketones, etc. Plant gum-based polysaccharides are natural components which are readily available, biocompatible and biodegradable. Therefore, production of nanocarriers from these non-toxic natural materials can be a convenient choice for drug delivery agents.

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Integrated network pharmacology and zebrafish model to investigate effects components of Origanum valgare for treating Alzheimers Disease

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Abstract

Origanum vulgare ssp. hirtum is a medicinal herb that promotes antioxidants, anti-inflammatory, antibacterial, and its neuroprotective effects. Here the research investigates the preventative impacts and the procedure of O. vulgare ssp. hirtum essential oil (OEO) on the impairment of cognitive and brain oxidative stress in a scopolaminedecreased uneasy-like behaviour and enhanced mental capacity. Our findings show that exposure to Sco (100 M) causes problems with anxiety, spatial memory, and novelty response. The OEO injection also lessened the impact of Sco on antioxidant stress and acetylcholinesterase (AChE).

The OEO mix was perceived by the gas chromatography-mass being thymol (27.83%), p-cymene (19.22%), and -terpinene (20.59%). These results point to OEO's potential to reverse the cognitive deficiencies caused by Sco by reactivating the cholinergic procedure and boosting brains antioxidant levels. OEO could therefore be employed as a possible source of bioactive chemicals that exhibit beneficial biological actions and have the potential for medicinal usage.

Keywords: Zebrafish model, Guiera senegalensis**,** Alzheimers Disease, Origanum valgare

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Overview

Aromatherapy is the supervise application of herbal essential oils that have been condensed, flowers, and other plant materials for therapeutic or preventative purposes. The three primary routes by which essential oils are delivered are orally, topically (aromatherapy massage), and olfactorily (direct and indirect inhalation). Essential oils' antifungal, antiviral, antibacterial, and antioxidant properties are their main pharmacological advantages.

Numerous authors have recently emphasised the advantages of aromatherapy for reducing stress, restoring mental well-being, and relieving anxiety and despair.

Origanum majorana L. (Lamiaceae), also known as marjoram, is a herb that is native to the Mediterranean region and is grown in many countries in, North Africa,Europe and Asia.

Popular spice marjoram has a powerful & alluring scent. Asthma, dyspepsia, headaches, and rheumatism are just a few of the diseases for which this herb has been used in folk medicine. Numerous authors have discussed marjoram's impact on various illnesses as well as its antibacterial, antiviral, antioxidant, anti-acetylcholinesterase (AChE), anti-metastatic, and anti-tumor growth properties. Recent research has revealed that Origanum majorana essential oil (OmEO) can mimic the effects of antidepressants by modulating the noradrenergic, dopaminergic, and serotonergic systems. Another group of researchers discovered that OmEO helped bruxism patients feel less anxious, and they hypothesised that this essential oil would be a safe alternative to benzodiazepines in the treatment of bruxism. O. majorana also possesses anti-inflammatory properties. [21].

The structural and functional similarity between the blood-brain & barrier (BBB) of the zebrafish and mammals, as well as the existence of many proteins that are related to human assisted neurodegenerative diseases in the zebrafish, highlight the possibility of molecular cellular features that are easily observable being conserved. Other significant benefits of this animal model are also present.

Zebrafish embryos serve as evidence that mobility is a complex habit, as they continue to posses a foundational capability to swim long after the hatching. The neocortex is deficient in the zebrafish, yet the synapse structures are as yet unblemished and are believed to be engaged with the transmission cycle as well as learning and memory. Even if there is a significant association between the various brain regions' functioning in zebrafish, this is nonetheless the case.

It is generally known that cholinergic signaling contributes to both memory and learning consolidation. Scopolamine (Sco), a nonselective muscarinic acetylcholine receptor antagonist, reduces learning and short-time memory and causes amnesia. In the object identification test, Sco reduces the ability of rodents to establish memories and weakens their cognitive abilities. Zebrafish, who are likewise entirely powerless against Sco, show amnesic impacts in the clever item acknowledgment (NOR) and Y-labyrinth tests. Sco-instigated amnesia models are thusly ordinarily used to assess the potential for neuroprotection of related substances and regular items.

The bulk of the Origanum species are indigenous to the Mediterranean, North Africa and Eurasia and are used in classical medications to cure gastrointestinal, respiratory, and cold-related conditions. The Origanum genus is a part of the Lamiaceae family. O. syriacum L. has been demonstrated to have neuroprotective properties and to be advantageous in the treatment of a range of ailments that impact numerous body systems, including the respiratory, cardiovascular, and neurological systems. Additionally, it improves memory and learning in mouse models of AD. The antioxidant, antibacterial, and anti-inflammatory capabilities of origanum's phenolic parts were traditionally thought to be responsible for the therapeutic actions of the herb in conventional medicine. O. vulgare's anti-inflammatory features have been investigated in numerous studies utilizing two of them cell and animal-based modelling. Avola et al. represented the anti-inflammatory and wound-healing properties of O. vulgare L. essential oil in a person keratinocyte cell culture. Han and Parker have provided affirmation of the anti-inflammatory, tissues re-modelling. Studies into the antioxidant benefits of O. vulgare, especially those of its essential oil derivatives, have been extensive. The chemical combination of O. vulgare essential oil contains carvacrol (35.00%) and thymol (35.19%), which is primarily to blame for Kakhri's demonstration of the oil's highest antioxidant activity.

Materials and Methods

Using and Extracting Plant Material

In the Touloum area, fresh Guiera senegalensis leaves were picked in June 2021. (Far North,Cameroon). The plant was identified

botanically by comparison to a botanically sample of Sabatié B. No. 698, which was discovered and got registered under No. 49838/HNC, at the National Herbarium of Cameroon.

The extraction process was followed exactly as Imam et al. [40] had previously reported.

Fresh G. senegalensis leaves were reduced to a fine powder after being dried in the shade for 1 week. The powder in result, 300 g, was macerated for 74 hours in a solvent made up of 80% ethanol and 25% distilled water. Whatman paper was used to filter the mixture. The final product was evaporated at 80°C in a rotating vapor to eliminate the ethanol and at 50°C for 45hours in an oven to remove the water. 7.67% (v/v) of the hydroethanolic extraction yield was obtained.

High-Performance Liquid Chromatograph (HPLC-PDA)

The extracts was initially diluted in a 3:7 methanol to H2O solution to create a stock solution with an initial concentration of 5 mg/mL. Clarifying the solutions required the use of a 0.2 m Millipore filter

The quaternary pump produced the mobile phase throughout the analysis by combining The sample analysis was done at a rate of flow 0.9 mL/min. Utilizing aliquots, the diluted extract was delivered in linearity gradients. For alignment bends with connection coefficients more noteworthy than 0, standard stock . Identification was done by comparing UV spectra and retention times, taking into account a match index of at least 950/1000. Sigma Aldrich provided all of the HPLC solvents and quality standards (Darmstadt, Germany).

Treatment and Animal

Adult fish received commercial flake fish feed twice daily (Gadstrup, Norwin Noryitall, Norwin, Denmark). Prior to the start of the study, fish was acclimated for 1 week.

Before the commencement of the behavioural tests, a 500 mL glass containing GS (1, 4, and 8 g/L) and Sco (100 M) was immersed once daily for an hour All strategies and conventions including the consideration and utilization of lab creatures have been supported by the Workforce of Science's Creature Exploration Morals Board of trustees at Alexandru Ioan Cuza College of Iaşi, Romania (No. 15308/30.07.2019), which found that they completely conformed to Order 2010/64/EU of the European Parliament and Gathering of September 22, 2010 on the insurance of creatures. The utilization of creatures was diminished to a base, and endeavors to improve

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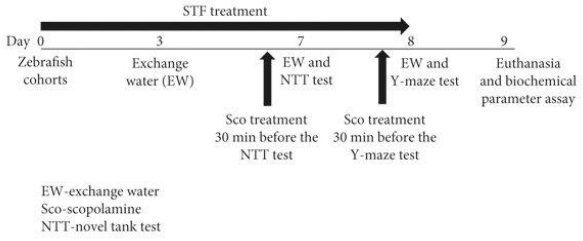


Figure 1: Design Procedure of experiment

Novel Tank Diving Test (NTT)

According to Cachat, the NTT is a specialized test utilized to measure zebrafish locomotor activity and anxiety reaction. The NTT was a 1.4 L trapezoidal tank (14.1 x 25.8 x 5.1 cm) with top and bottom parts separated by a virtually horizontal line. Zebrafish were evaluated one at a time for 6 minutes. Distance walked and average speed were the locomotor activity endpoints (measured in m and m/s, respectively). The quantity of passages to the top, time spent at the top (s), normal passage length (s), absolute distance went in the top (m), and freeze term (s) endpoints were evaluated to recognize tension like way of behaving.

Y-Maze Test

To find out how the zebrafish would react in an unknown setting, the Y-maze test was utilised. Zebrafish were put in the novel arm of a Y-shaped glass tank to test their recall (27 8 15 cm for each arm, 4 L). The "start" arm, "other" arm (which was consistently open), and "new" arm were the names of the Y-labyrinth arms (obstructed in instructional meeting and opened in test meeting). The fish were each independently positioned in the start arm, and the new arm was shut, for the term of the preparation (5 minutes). The fish were set in the beginning arm once more, yet this time the new arm was opened, and testing for five minutes began following an hour. Outright distance traveled (m) and turn point () were used to evaluate locomotor activity, while time spent in

the sharp arm (% of hard and fast arm time) was utilized to survey the reaction to interest.

Novel Object Detection Test

The NOR, a behavioral experiment, is often used to study the memory function of zebrafish. Zebrafish were introduced to the novel tank for a total of 5 minutes over the course of 3 days without the objects (30 cm filled with 6 cm of water). Zebrafish were exposed for ten minutes on the fourth day to two similar objects (training phase).

Results and Discussion

Extract from Guiera senegalensis: Chemical Makeup

The study was done to determine the chemical composition of the GS extract. According to the findings, several flavonoids and polyphenolic acids were identified. The following quantities were discovered: rutoside (204.81 g/g of dry extract), quercetin (305.6 g/g of dry extract), quercetin3-O-arabinoside (312.40 g/g of dry extracts), catechin (108.54 g/g of dry extracts), and luteolin-7-Oglucoside (55.35 g/g of dry extracts).

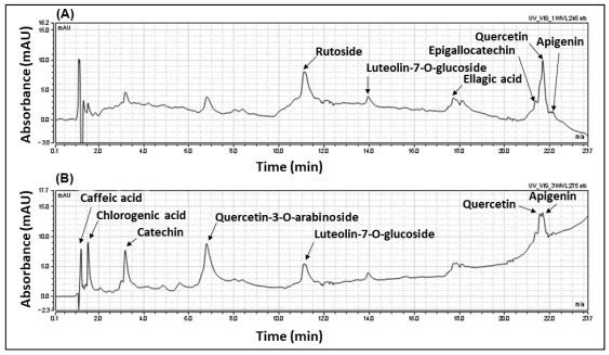
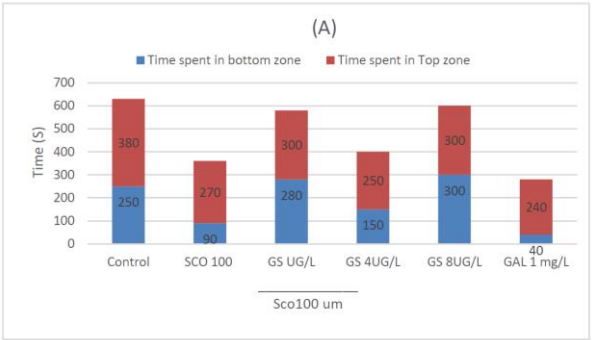


Figure 2: LC-PAD chromatography profile

Various distributions have exhibited that the vegetal material from G. senegalensis utilized in traditional medication contains in excess of 46 mixtures. According to earlier published data, chemicals that are similar to those in our results have been discovered in the leaves of G. senegalensis. In any case, most of the ongoing information just gives a conventional distinguishing proof without the measurements of these mixtures. Extractability is connected with the plant material, the solvent, and the preparation process. The most common combinations described included different polyphenolic acids, flavonoids, and gallic acid derivatives. As per the synthetic arrangement, the examined remove gives off an impression of being rich in polyphenols (flavonoids, catechins, and polyphenolic acids), which are referred to work as normal cell reinforcements. The free hydroxyl moieties likewise enact a safeguard component against free radicals in addition to connecting with cell layers. Additionally, quercetin, apigenin, and catechin derivatives inhibit oxidative enzymes like lipoxygenases to stop the denaturation of cells.

Impact of the NOR, Y-Maze and NTT Tests on Zebrafish Anxious Behavior and Spatial Memory

Zebrafish are in many cases utilized in the new tank plunging test (NTT) to analyze the impacts of uneasiness. The GS treatment brought about huge changes in the top/base proportion, complete distance went in the top zone, number of sections to the top, and time spent in the highest point of the tank, as per the one-way of ANOVA. Following Sco treatment, there was a sensational decline (p 0.0001) in how much time spent in the top zone of the tank, the top/base proportion, the distance went in the top zone, and the quantity of doorways to the top contrasted with the benchmark group (p 0.0001). Furthermore, the Sco-organization diminished the locomotory design in contrast with the benchmark group. For contrast, GS treatment had anxiolytic-like effects in Sco zebrafish at all dosages (1 g/L, 4 g/L, and 8 g/L). Iripramine (IMP), a favourable reference drug, showed anxiolytic effects.



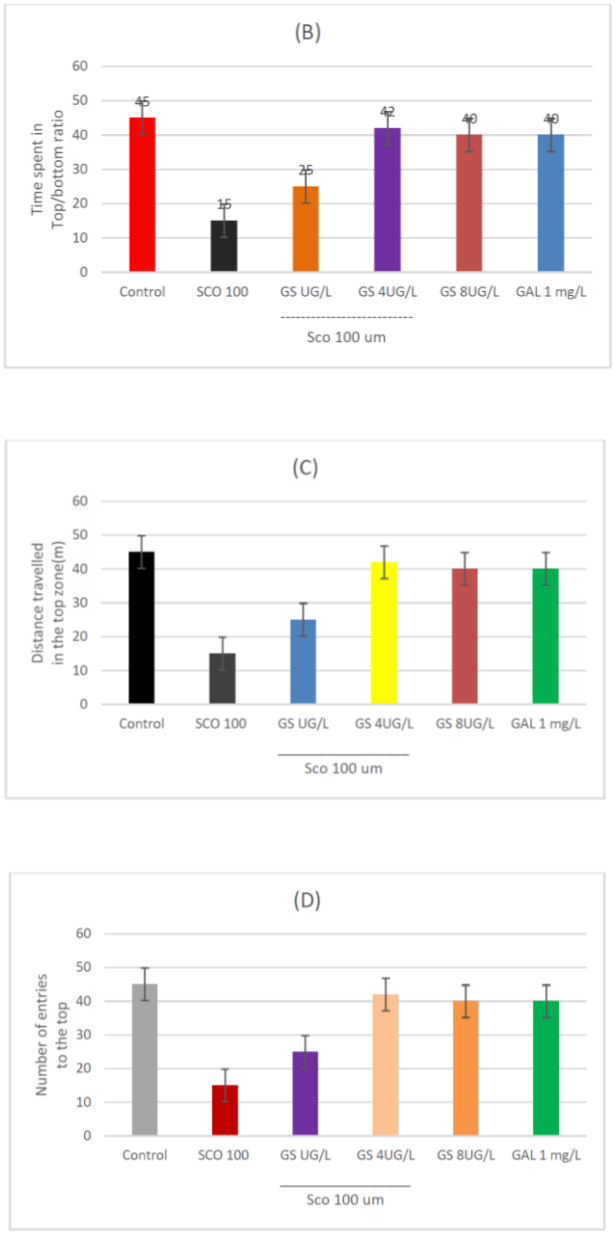


Figure 3: Guiera senegalensis hydroethanolic extract

Our findings confirm past research suggesting GS may have a neuroprotective effect. Sombie suggests a new possible source of naturally occurring neuroprotective and antioxidant components: the galls from G. senegalensis.

Additionally, Somewhat's exploration uncovered that G. senegalensis is as a treatment mental and neuropsychiatric sicknesses in the Hauts-Bassins locale of Burkina Faso. As per the creators, the advantages were achieved by the presence of the neuroprotective mixtures.

These results suggest that GS may be considered a therapeutic medication with a high potential for enhancing cognitive impairments. In fact, we demonstrated that GS has cognitive-improving and anxiolytic properties using the Sco zebrafish model.

Impact on AChE Activity in the Brain

AChE activity was investigated to learn more about the GS mechanism in the Sco zebrafish model. In mild cognitive impairment, AChE activity represented cholinergic status. When processing memories, the cholinergic system is crucial, and cholinergic neurons are destroyed. Acetylcholine levels fall as a result of AChE, which causes the learning and memory impairments associated with AD. Additionally, AChE inhibitors are implied in AD therapy, which improves cholinergic neurotransmission.

The AChE activity in the zebrafish treated with Sco was extremely higher than in the control group (p 0.0002). Additionally, the GS treatment significantly decreased the Sco-mediated AChE aggravation at all dosages (p 0.0001). The GS has cholinesterase inhibitory potential, which is connected to zebrafish with superior memory capacity, according to the results of the NTT, Y-maze, and NOR tests. Inhibiting AChE can be used to treat dementia caused by Alzheimer's disease, Parkinson's disease, senile dementia.

Particularly at low doses, GS dramatically reduced the In the zebrafish brain, Sco causes a rise in AChE. Since it modifies mental capability, hippocampal Hurt is a significant supporter of dementia-related cognitive declines and the fundamental component of mental problems..

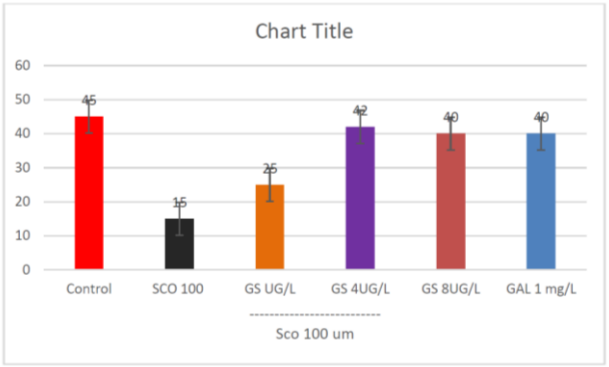


Figure 4: Impact of Guiera senegalensis hydroethanolic extract

Brain antioxidant capacity effects

The oxidative stress indicators were assessed to see if GS boosts antioxidant capability in vivo. Following exposure to Sco, Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), total reduced glutathione (GSH), as well as protein carbonyl (p 0.0001) and lipid peroxidation (malondialdehyde—MDA) activities, all extremely decreased (p 0.0003).

Additionally, GS treatment (1 g/L, 4 g/L, and 8 g/L) significantly reversed the fall in CAT,SOD specific activity, GPX, and the overall amount of reduced GSH (p 0.001, 0.0001, and 0.0004, respectively) (p 0.0001). In addition, animals treated with Sco had extremely lower levels of protein carbonyl (p 0.0001) and MDA (p 0.001) after receiving GS therapy, which in turn decreased protein and lipid peroxidation.

Conclusions

In this work, the chemical composition of OmEO was first determined, then the proteomes of AD model rats were analysed to look for any molecular alterations. This approach indicated four biological processes as potential OmEO side effects that might affect rat brain (hippocampi) performance: apoptosis, oxidative stress, neuriinflammation and cognitive processes mediated by neurotrophins.

Testing corresponding targets—recognized representatives of the biological processes of interest—led to the determination of the relevance of the biological processes in question. In this work, we discovered that OmEO improved cognition in rats treated with A1-42 and decreased brain oxidative stress. In order to demonstrate that OmEO improves memory performance in rats given A1-42 treatment, we finally connected the cellular and molecular findings with behavioural data.

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NOVEL COMPUTATIONAL APPROACHES FOR INVESTIGATING BACTERIAL INHIBITORS FROM MALABAR NUT PLANT

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Abstract: The issue of bacterial diseases is spreading throughout the globe. It is now recognised that bacterial diseases kill more people each year than malaria. Ayurvedic medicine was among the most effective treatments for bacterial infections in antiquity. It was discovered that the plant Malabar nut has a variety of pharmacological qualities after studying the most recent survey on the plant with antibacterial activity. The plant, which is an Acanthaceae member and was endemic to India, was known by the common name Adusa. Through a review of the literature, 102 phytochemical components from the Malabar nut plant were found for the present study project. Only 84 substances have their two-dimensional structure determined using PubChem databases. These compounds were subjected to virtual screening, the results of which indicated that only 28 compounds would be found to contain active drug molecules. Most of the bacterial illnesses that affected humans were brought on by the bacterial protein Cytochrome P450 14-alpha-sterol demethylases (CYP51). The three-dimensional protein structure was acquired from PDB databases and then utilised for the docking procedure. Using docking tools, only 1 chemical, out of 28, predicted the optimal binding relationship with the protein.

Keywords: *Malabar nut, Antibacterial activity, Phytochemical, Protein Cytochrome P450 14-alpha-serol demethylases, Docking*

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1. Introduction

Bacterial pathogens have a significant impact on public health. Any part of the body can develop disease, and it can be brought on by the organism itself or by the body's reaction to its existence[1]. Humans can contract bacteria via the air, water, food, or living things. The main means of bacterial infection transmission are contact, aerosol, droplet, vectors, and moving vehicles[2]. Morbidity and mortality are significantly impacted by preventive strategies. As we encroach into new environments, new species and novel variations of well-known species continue to be discovered[3]. Disease-causing pathogenic microorganisms have properties that enable them to sidestep the body's defenses and utilize its resources4. Last but not least, virulence refers to an organism's potential to spread disease through traits including invasiveness and toxin production[5]. In determining whether a disease will spread after a bacterial agent has been transmitted, host factors are crucial[6]. These variables include genetic make-up, nutritional status, age, length of organism exposure, and co-occurring diseases. Antibiotic resistance among bacteria is a rising issue that necessitates careful usage of antibiotics[7,8].

Adhatoda vasica Nees (Acanthaceae), sometimes referred to as Adusa or the Malabar nut tree[9]. The Assam, Bangladesh, India, Nepal, and Sri Lankan subcontinents, Laos, and Myanmar make up the natural range of the Malabar nut[10]. Many alkaloids, phenolics, flavonoids, sterols, and their glycoside derivatives have been identified from the well-known medicinal herb Malabar nut. Its wide range of therapeutic effects include hepatoprotective, antimicrobial, antitussive, abortifacient, antitubercular, antimutagenic, antiulcer, antiasthmatic and cardiovascular protection[11,12]. It is commonly used in South-East Asian traditional and folk medical systems, and it is also a well-known plant medication in Ayurvedic and Unani medicine[13]. The protein that was found to cause of the bacterial infections in human was Cytochrome P450 14-alpha-sterol demethylases (CYP51) are important enzymes in sterol in plants biosynthesis and cholesterol biosynthesis in humans[14]. In the pathways leading to the synthesis of ergosterol in fungus, various forms of sterols in plants, and cholesterol in humans, the demethylated products of the CYP51 reaction are essential intermediates[15]. These sterols are found in cells plasma membranes, where they serve a crucial structural function in the control of membrane permeability and fluidity[16]. They also have an impact on the activity of enzymes, ion channels, and other embedded cell components. In an effort to find novel ways to cure bacterial infections, drug researchers have started focusing on the 14-demethylase enzyme in bacteria[17]. By inhibiting the bacteria's production of ergosterol, the pathogen is killed since the breakdown of the plasma membrane causes cellular leakage[18]. The CYP51 family is an intriguing subject for fundamental P450 structure/function studies and is also an important clinical drug target[19]. *In silico* screening, large libraries of drug-like compounds that are commercially accessible are computationally screened against targets of known structure, and those that are predicted to bind well are experimentally tested[20]. Virtual Screening of the compound was excluded by Molinspiration software[21]. The software predicts the calculations of leading molecular properties as well as calculation of bioactivity score for the most important drug targets[22]. Docking method is one of the most important and frequently used methods in structural-based drug designing, which predict the

binding affinity of small molecules to their applicable target binding sites there by inhibiting the target functions[23].

2. Materials and Methods

Ligand selection

Through literature study, 102 phytochemicals were identified from the plant Malabar nut for computational analysis. From the Pubchem database, 2D structures were retrieved for 84 compounds and for remaining 18 compounds, 2D structures was not available in the database.

Prediction of oral drug activity using Molinspiration server

By using Molinspiration server, the drug likeness filter test was conducted for above 84 phytochemicals. Molinspiration expressed 6 rules (logP, Topological Polar Surface Area (TPSA), Molecular weight (MW), number of hydrogen bond acceptor (No.HA), number of hydrogen bond acceptor No.HD, and volume (Vol)) to determine whether a compound could be orally consummate. The compounds which clear the oral drug properties were further, analyzed for its biological properties using Molinspiration bioactivity server. Bioactivity predicts the 6 properties including GPCR ligand, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor. The compound which satisfies all the biological properties could be taken for further docking process.

Accession of target protein

The protein cytochrome P450 14-alpha-sterol demethylases (CYP51) were found to be most important bacterial protein. The three dimensional structure (1EA1) of that protein was retrieved using Protein Databank database which was resolute by experimental studies through X-Ray Diffraction with the Resolution of 2.21 Å.

Analysis of target active binding sites

The Binding sites of small molecules present in the protein structure were recognized by SCFBio - Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp). SCFBioActive Site prediction server which will predict the active site in the 3 dimensional structure of the protein for the binding of the ligand.

Molecular Docking Interactions using Argus Lab

The docking studies were performed to analyze the structural relationship between receptor and ligand using Argus Lab 4.0.1 docking software program28. The following factors were set for docking process: Population size, Grid resolution, Binding site box size, Maximum generation, Crossover rate, Mutation rate, Elitism, Dock engine used Lamarckian Genetic Algorithm. For the docking calculations, docking mode should be was set as “Dock” and “Flexible” ligand for each docking runs.

Visualization of docking interaction using PyMol

The best docking result were analyzed using PyMol which is an open source molecular visualization tool to view the hydrogen bond interactions between the protein and ligand and also predict the distance of hydrogen formation between them.

3. Results and Discussions

Preparation of small molecules:

Through literature survey, totally 102 phytoconstituents were identified from plant Malabar nut. The virtual screening was carried out for 84 phytoconstituents using Molinspiration server. From the result of Drug likeness properties revealed that only 38 compounds pass the Drug likeness screening test (Table 1). Further these compound were screened for its bioactivity test, the result proved that out of 38, only 28 compounds satisfies the oral drug properties (Table 2).

Table 1: The phytochemical compounds which satisfy the drug likeness properties

Compound	Pubchem ID	LogP	TPSA	MW	No. HA	No. HD	Vol
2-Glycylaminobutyric acid	86878	2.62	92	160	5	4	149
Anisotine	442884	2.99	73	349	6	1	312
Apigenin	5280443	2.46	90	270	5	3	224
Betaine	247	5.41	40	117	3	0	119
42752-07-8	206	2.64	110	180	6	5	51
Deoxyvasicine Hydrochloride	3046290	1.95	15	172	2	0	165
Deoxyvasicinone	68261	1.47	34	186	3	0	167
2-Aminoacetic acid	750	2.55	63	75	3	3	67
4-Heptanone	31246	2.36	17	114	1	0	131
Hydroxychalcone	97765	3.57	37	24	2	1	209
Kaempferol	5280863	2.17	111	86	6	4	232
178740-33-5	21769547	0.49	64	32	5	1	201
77500-13-1	110349	3.76	66	319	5	2	344
147-85-3	145742	1.72	49	115	3	2	108
Quercetin	5280343	1.68	131	02	7	5	240
2-Amino-3-hydroxypropanoic acid	617	1.67	83	105	4	4	92
2-amino-3-methylbutanoic acid	1182	1.91	63	17	3	3	117
Peganine	72610	1.04	35	88	3	1	173
Vasicinone	442935	0.48	55	02	4	1	175
Benzenamine	626005	3.33	18	291	3	0	282
Vasicolinon	627712	2.91	38	305	4	0	285
2-(3'-hydroxy-2'-oxopyrrolidinomethyl) aniline	14285793	0.02	66	06	4	3	91
Vasicinolone	13970119	0.03	75	218	5	2	183
Vasicinol	4486241	0.53	56	204	4	2	181
Vasnetine	9997066	3.03	73	335	6	1	296
Erythorbic acid	54675810	1.40	107	176	6	4	139
2,4-Dimethyl-2,4-Heptadiene	572949	3.93	10	24	0	0	150
Methyl 4-hydroxycinnamate	5319562	2.05	46	178	3	1	164

5,5-Dimethyl-1,3-Dioxane-2-Ethanol, Tert-Butyldimethylsilyl Ether	91714410	4.24	27	274	3	0	91
5-Isopropyl-1,3-Cyclohexanedione	566106	1.57	34	154	2	0	156
16688-21-4	595376	1.72	67	351	6	1	318
STK766913	5292275	4.53	72	370	6	1	288
Syringic Acid	10742	1.20	76	198	5	2	170
Methyl 4-hydroxy-3-methoxy-cinnamate	16830	1.86	55	208	4	1	189
1151-93-5	2825054	3.55	29	255	3	0	245
1-Cyclohexylsilatrane	208266	1.87	30	257	4	0	251
Hydroquinone	785	0.98	40	110	2	2	100
Dibutyl Phthalate	3026	4.43	52	278	4	0	273

Table 2: The phytochemical compounds which satisfy the bioactivity properties

S.No	***Pubchem ID***	***GPCR Ligand***	***Ion Channel Modulator***	***Kinase Inhibitor***	***Nuclear Receptor Ligand***	***Protease Inhibitor***	***Enzyme Inhibitor***
1.	86878	-0.30	0.05	-1.25	-1.16	0.04	-0.02
2.	442884	-0.14	-0.31	-0.19	-0.37	-0.41	-0.08
3.	247	-2.53	-1.79	-3.50	-3.75	-3.47	-2.12
4.	3046290	-0.26	0.09	-0.83	-1.22	-0.91	0.03
5.	68261	-0.98	-0.92	-0.75	-1.39	-1.22	-0.09
6.	750	-3.45	-3.11	-3.71	-3.55	-2.95	-3.29
7	31246	-3.17	-2.75	-3.66	-2.91	-3.06	-2.63
8.	97765	-0.34	-0.17	-0.54	-0.30	-0.51	-0.05
9.	21769547	-0.24	-0.43	-0.44	-0.67	-0.86	0.07
10.	145742	-2.15	-1.54	-3.08	-3.07	-1.72	-1.85
11.	617	-2.66	-2.54	-3.34	3.34	-2.36	-2.38
12.	1182	-2.66	-2.11	-3.40	-3.04	-2.12	-2.18
13.	627712	-0.40	-0.35	-0.53	-0.85	-0.97	0.03
14.	442935	-0.04	-0.26	-0.21	-0.35	-0.48	-0.01
15.	14285793	-0.13	0.04	-0.29	-0.52	0.14	0.16
16.	13970119	-0.26	-0.25	-0.33	-0.51	-0.87	0.15
17.	9997066	-0.24	-0.40	-0.26	-0.43	-0.50	-0.08
18.	54675810	-0.53	-0.24	-1.09	-1.01	-0.81	0.20
19.	572949	-2.13	-1.14	-2.53	-1.39	-2.35	-1.03
20.	5319562	-0.71	-0.35	-0.92	-0.30	-0.87	-0.28
21.	566106	-0.91	-0.38	-1.74	-0.67	-0.73	-0.32
22.	595376	0.01	-0.29	-0.32	-0.12	-0.36	0.12

23.	5292275	-0.58	-1.01	-0.37	-0.99	-0.66	-0.46
24.	10742	-0.65	-0.28	-0.69	-0.44	-0.82	-0.15
25.	16830	-0.58	-0.35	-0.72	-0.26	-0.78	-0.21
26.	2825054	-0.14	-0.13	-0.06	0.00	-0.39	-0.04
27.	785	-3.02	-2.48	-3.07	-2.84	-3.20	-2.66
28.	3026	-0.16	-0.09	-0.27	-0.12	-0.25	-0.07

3.1 Preparation of protein

The 3D structure of protein (1EA1) was retrieved using Protein Data Bank database. The possible binding site sites of the protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) were predicted using SCFBio. The secondary structure, name, position and of the amino acid present in the protein are as follows: Alpha Helix - SER260, THR261 and PHE399: Coil- GLY94, PRO319, ILE322, GLY388, ARG391, CYS394 and ALA397.

3.2 Docking analysis

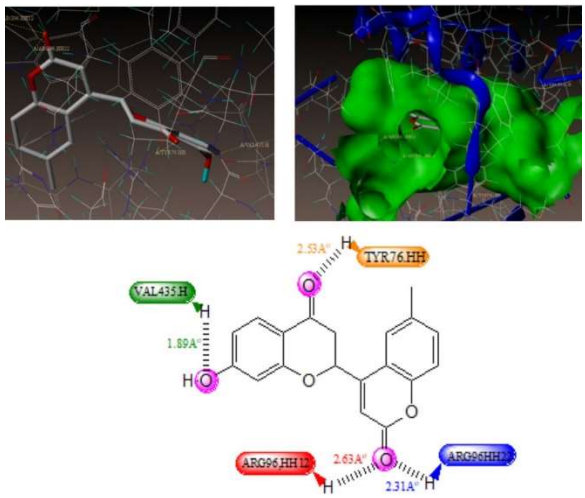
The predicted 10 active residues were used as the binding sites for 28 natural compounds from the plant Malabar nut for docking studies. The results of the binding interaction between the active site residues of target protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and 28 compounds were shown in the Table 3.

Table 3: Molecular Docking between Phytochemical Compounds and the Protein (1EA1)

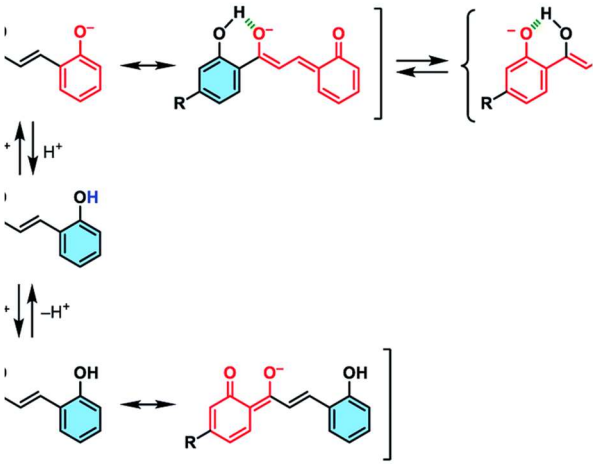
Compound	***Binding Affinity***	***Compound***	***Binding Affinity***
Hydroxychalcone	-8.9 Kcal/Mol	Dibutyl Phthalate	-7.4 Kcal/Mol
Methanone [4-(Dimethylamino)Phenyl] (4-Methoxyphenyl)	-8.7 Kcal/Mol	Methyl 3-(4-Hydroxy-3-Methoxyphenyl)Prop-2-Enoate	-7 Kcal/Mol
5-Isopropyl-1,3-Cyclohexanedione	-7 Kcal/Mol	2,4-Dimethyl-2,4-Heptadiene	-5.5 Kcal/Mol
Syringic Acid	-6.5 Kcal/Mol	Hydroquinone	-5.3 Kcal/Mol
4-Heptanone	-5.2 Kcal/Mol	Amino-N Butyric Acid	-5.1 Kcal/Mol
D-Erythro-Hex-2-Enonic Acid, Gamma-Lactone	-4.7 Kcal/Mol	Glycine	-4 Kcal/Mol

By analyzing the docking result, 2 compounds: Hydroxychalcone and Methanone [4-(Dimethylamino) Phenyl] (4-Methoxyphenyl) were found to have the highest inhibitory activity of -8.9 Kcal/Mol and -8.7 Kcal/Mol when compared with 10 other compounds which showed the inhibitory activity of < -8 Kcal/mol. The remaining 16 compounds do not exhibit any inhibitory activity between protein Cytochrome P450 14-alpha-sterol demethylases (CYP51).

Table 4: Docking interactions between hydroxychalcone – 1EA1



Best docking interactions between Hydroxychalcone - 1EA1



Hydrogen distance between Hydroxychalcone - 1EA1

The latest docking study on 1EA1 protein revealed that same binding inhibitory activity towards compounds Emodin when compared with the existing drugs[24]. From the current docking results, it was clearly conveyed that among the 28 plant compounds, Hydroxychalcone exhibits the best binding interaction with the Protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and further this study could be useful in designing of new preventive and therapeutic drug against bacterial infection.

Conclusion

The development of novel natural compounds with pharmacological activity is an urgent need in designing effective drug towards bacterial infections. In the present study, docking results revealed the binding interactions between protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and 28 natural flavonoid compounds. Among those compounds, Hydroxychalcone were found to have the best binding energy of -8.9 Kcal/mol and also satisfies the pharmacological properties which provide the strong recommendation for the compound to be considered for an oral drug. On the whole, this study could be concluded that compound Hydroxychalcone could be potent anti-bacterial drugs activity against the protein Cytochrome P450 14-alpha-sterol demethylases (CYP51). Further In vivo investigations are needed to enrich the bacterial activity of the Hydroxychalcone compound from Malabar nut to determine the dosage for the safety levels.

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Effect of a Suspension of *Carica papaya* Leaf Extract on the Thrombocytopenia Induced by Carboplatin in Mice

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Abstract: A low platelet count characterises thrombocytopenia. Thrombocytopenic disorders (dengue, chikungunya, malaria, etc.) are on the rise in cities. This is now present in over 125 countries. Low platelet counts are caused primarily by decreased platelet generation and increased platelet degradation. It has been shown in several research that papaya leaf extract may increase platelet count. The major objective of the proposed research was to examine the haematology and histopathology of different organs for any adverse effects of *Carica papaya* leaf extract on raising platelet count in induced thrombocytopenic experimental animals. To test whether a rise in platelet count was present, papaya leaves were macerated in a mixture of n-hexane, acetone, ethanol, and methanol in distilled water for 8 h using a Soxhlet device. Toxicologists may benefit from experimental animal haematological and histopathological studies.

Keywords: *Carica papaya* extracts, Organic solvents, Platelet count, Thrombocytopenia, Carboplatin

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Introduction

Platelet counts that are lower than the 2.5th lower percentile of the normal platelet count distribution are used to characterise the condition known as thrombocytopenia. U.S. data from the National Health and Nutrition Examination Survey III (NHANES III) back the long-held estimate of

150 x 109/L as the bare minimum of health (Chen *et al*., 2004). However, platelet counts between 100 and 150 x 109/L may not always indicate illness if they remain steady for more than 6 months (Stasi *et al*., 2012), suggesting that a cutoff value of 100 x 109/L may be more appropriate for detecting a pathologic condition (Rodeghiero *et al*., 2009). When platelet counts are above 200 x 109/L, clinical symptoms are typically limited to simple bruising; however, when platelet counts fall below 100 x 109/L, the chance of spontaneous mucocutaneous bleeding increases significantly (Sheikh *et al*., 2014). The major causes of thrombocytopenia are insufficient platelet production and excessive breakdown. Platelet production (PNH) is decreased in BM failure, megaloblastic anaemia, leukaemia, myeloma, myelofibrosis, solid tumour infiltration, aplastic anaemia, and paroxysmal nocturnal hemoglobinuria. In autoimmune ITP, SLE, and viral illnesses such as EBV, dengue, HIV, DIC, TTP, and hypersplenism, there is excessive damage. Thrombocytopenia is the most common cause of poor primary hemostasis. This might result in significant bleeding. Significant quantitative or qualitative platelet dysfunction causes mucocutaneous haemorrhage (Tahir *et al*., 2014). The most common causes of thrombocytopenia include bone marrow failure, reduced platelet survival and splenic platelet sequestration, leptospirosis, malaria, and dengue, all of which are associated with platelet destruction and bone marrow suppression, and sepsis. A combination of the aforementioned factors might result in thrombocytopenia. Multiple causes, including primary immune thrombocytopenia (ITP) and hepatitis C virus infection, may contribute to the development of thrombocytopenia in diverse kinds of thrombocytopenia (Chakravarty *et al*., 2013).

Carica papaya L. belongs to the plant family Caricaceae. The chemical components found in papaya leaf have been demonstrated to have antioxidant, anti-hypertensive, wound healing, hepatoprotective, anti-inflammatory, antibacterial, anti-amoebic, and anti-helminthic activities. Recent phytochemical analyses of plants show that they have a long folkloric history of use in the treatment of thrombocytopenia. Disease phyto-therapy research is a contemporary trend in the treatment of tropical illnesses and hereditary abnormalities like sickle cell anaemia, with the objective of developing cheaper, alternative medications that the general people may use right away (Imaga *et al*., 2009). It was studied how quickly platelet counts recovered in dengue-infected children. The boys were given a teaspoon of powdered papaya leaf paste every four hours. Platelet counts increased considerably after 12 hours of initiating medication, reaching 100,000 in one case and 250,000 in the other within two days. Patients were given fresh juice produced from *C. papaya* leaves 15 min after breakfast for three days. The study revealed a significant increase in platelet counts after 40 h when compared to values 8 h after the intervention began (Soobitha *et al*., 2013). When an experimental animal was given a suspension of powdered crude *Carica papaya* leaves in palm oil, the treatment group's thrombocyte counts rose regularly. The impact of papaya leaf formulations was larger and statistically significant, suggesting that they might be used to treat thrombocytopenic purpura (Kathiresan *et al*., 2009). Several clinical and pre-clinical research have shown that raising platelets has a favourable benefit trend. There was a large rise on the fourth day of admission. However, large-scale randomized clinical trials have been recommended to further demonstrate its importance in dengue management. According to Zunjar *et al*. (2016), *Carica papaya* leaves decoction possesses exceptionally potent anti-thrombocytopenic properties. According to the findings, the anti-thrombocytopenic effect of *Carica papaya* leaves is due to alkaloids. Furthermore, no clear toxicity or negative effect was seen in animals. Gamulle *et al*. (2012) discovered that mature leaf of *Carica papaya* concentration (MLCC) might be used to produce a plant-based treatment for thrombocytopenia. It was also discovered that MLCC made from mature *Carica papaya* leaves can be taken orally, is non-

toxic, and effectively increases platelet, WBC, and RBC counts in both normal (non-thrombocytopenic) and thrombocytopenic rats. The thrombocytopenic parameter of papaya leaf extract is studied using crude water extract, followed to some extent by methanolic and ethanolic extracts. The current study investigated at how different papaya leaf extracts performed in polar and non-polar solvents.

Materials and Methods

Sample preparation:

The authenticated, disease-free sapling of papaya plant of breed "Taiwan 786" was purchased from Ram Biotech Limited, Nashirabad, Maharashtra, India. These saplings were given organic manure at fifteen days intervals. Fifteen-day-old leaves were shade-dried, powdered and used in an extraction process. Powdered plant parts (leaves) weighing 25 g were soaked in 250 ml of ethanol, methanol, n-hexane, and acetone, respectively, for 6 h at the boiling point in a Soxhlet device. To minimize the volume, extracts were concentrated at room temperature. Same was macerated in batches of 250 ml of distilled water for 6 h at intervals. After 6 h, we filtered the extracts using Whatman No. 1 filter paper and concentrated the filtrates at room temperature to decrease the volume.

Experimental Design:

Phase-I:

Standardization of carboplatin dosage:

The carboplatin dose was standardised to assess the drug's toxicity and lethality. A single intraperitoneal dose of carboplatin produced thrombocytopenia in experimental Sprague Dawley rats (Fresenius Kabi Oncology Ltd). 12 Sprague Dawley rats of either sex were administered carboplatin doses of 50 mg/kg body weight, 100 mg/kg body weight, and 150 mg/kg body weight. The dosage of carboplatin of 50 mg/kg body weight was determined since it was shown to be acceptable for inducing thrombocytopenia with no or very little adverse effects and was non-fatal, while the other two doses were found to be deadly.

Phase-II:

Standardization of extract doze: A group of 6 animals (Sprague Dawley rats) of either sex was tested to determine the doze of extract for 5 different solvents in 2 different concentrations of 100 mg/kg body weight and 200 mg/kg body weight, as well as a set of standard drugs prednisolone. These findings were reached despite no increase in platelet count. As a result, the new preclinical experiment was designed with a double dose of 400 mg/kg body weight. This doze produced results consistent with the hypothesis, indicating an increase in platelet count.

Phase-III:

Animal trials:

A total of 48 Sprague Dawley rats were divided into eight groups of six (three males and three females) each. After establishing thrombocytopenia in all animals, on the sixth day, the control group (group 0) was given 0.3 ml distilled water, whereas the experimental groups were given 400 mg/kg body weight of extracts orally (groups 1 to 6) (Group 1: hexane extract, Group 2: acetone extract, Group 3: ethanol extract, Group 4: methanol extract, Group 5: distilled water extract, Group 6: standard). From day 6 through day 27, this was repeated every other day. On days 6, 15, and 25, blood was obtained retro-orbitally to assess platelet count increase.

Platelet Counting:

The induction of thrombocytopenia was confirmed by assessing platelet counts using the conventional techniques.

Histological studies: At the conclusion of the trial, animals were slaughtered for histopathological examinations of the liver, lungs, kidney, spleen, heart, and intestine to assess the extract's impact on organs and toxicity using conventional techniques.

Results and Discussion

Animal studies were performed on Sprague Dawley rats to assess the increase in platelet count as a consequence of extract therapy after inducing thrombocytopenia with carboplatin. Retero-orbital blood samples were taken from rats in each group on day 0, the sixth, fifteenth, and twenty-fifth days of the experiment. The platelet count in control, n-hexane, acetone, ethanol, methanol, distilled water extract and standard were 8.83±1.03, 8.99± 0.97, 8.90±1.04, 9.18±1.39, 9.40±1.29, 8.90±1.04, 9.68±1.45 x 10^5/cmm, respectively, before induction of thrombo-cytopenia in rats (day 0). The drug carboplatin has been used to cause thrombocytopenia. It is a Cis-platin derivative. It is a second-generation platinum medication that has shown clinical efficacy in the treatment of malignant tumours. It was selected because it has less adverse effects such as toxicity, nausea, and vomiting (Rang *et al*., 2007). Carboplatin induces a fast drop in circulating platelet number (Ulich *et al*., 1995), and it affects mature multi-lineage hemopoietic cells but not stem cells (Chabner *et al*., 2011).

On day 6, after carboplatin-induced thrombo-cytopenia, the platelet count was reduced by 71.46%, 74.22%, 73.53%, 77.89%, 79.03%, 75.51%, and 75.10% in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard, respectively. From day 6 onwards, on every other day until the completion of the trial, extract and standard were administered according to the predetermined doze and schedule. The platelet count increased by 18.19%, 42.49%, 42.26%, 49.92%, 60.10%, 70.97%, and 139.94% on the 15th day in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard groups, respectively. Platelet count increased by 126.52%, 166.36%, 178.94%, 181.20%, 190.36%, 216.96%, and 303.66% on the 25th day in the control, n-hexane, acetone, ethanol, methanol, and distilled water extract, and standard groups, respectively. The percentage increase in platelet count and the amount of platelets detected are shown in Table 1 and Figure 1.

The present study revealed that difference between male and female animal platelet count was not significant.

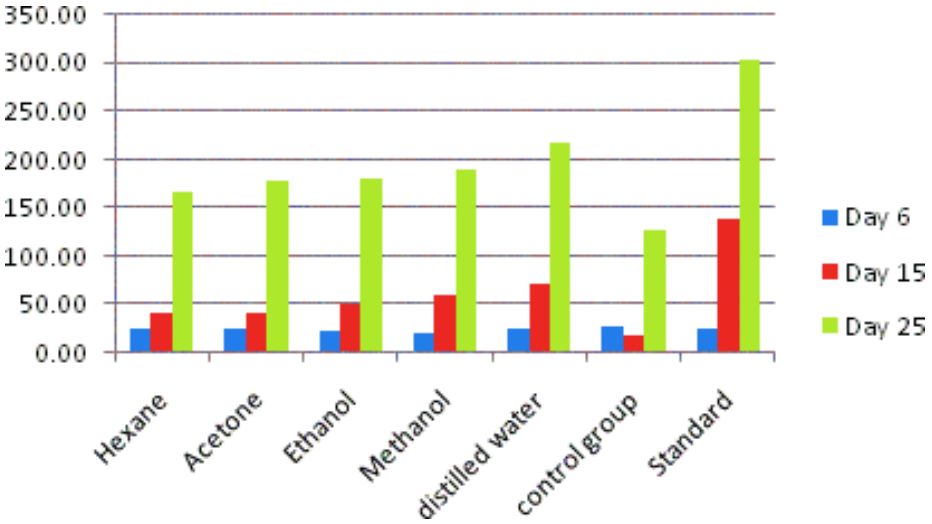
Even after 27 days of treatment, histopathological examination of the liver, lungs, kidney, intestine, heart, and spleen of the control and treated groups revealed no morphological differences or toxicity. This suggests that *Carica papaya* leaf extracts are safe and have no toxic or side effects. The representative histopathological images of the various organs are shown in Figure 2.

Following a carboplatin injection (50 mg/kg body weight), platelet count was decreased. On the seventh day, carboplatin had the highest effect, followed by an increase in platelet count. *Carica papaya* leaf extracts did not cause a decline in platelet count throughout the experiment period. Platelet count was higher on days 6, 15, and 25 in all experimental groups compared to day 1 in the same group (Fig. 1). According to the data, the platelet count significantly increased above baseline. When platelet count in experimental groups was compared to the standard (Prednisolone) and control (Natural recovery), it was observed that the standard group's platelet count increased quicker and at a greater level than the experimental groups, while the control group's platelet count was lower. The gender difference in platelet count was not significant in any of the groups. One animal study indicated that drinking papaya leaf juice increased platelet count in 5 healthy mice within hours, suggesting an increase in platelets from bone marrow. Gammulle *et al*. (2012) reported at how papaya leaf juice affected hydroxyl urea-induced thrombocytopenia in mice. When 7.2 ml/kg of juice was given for three days in a row, it increased platelet count by 76.5% above the control.

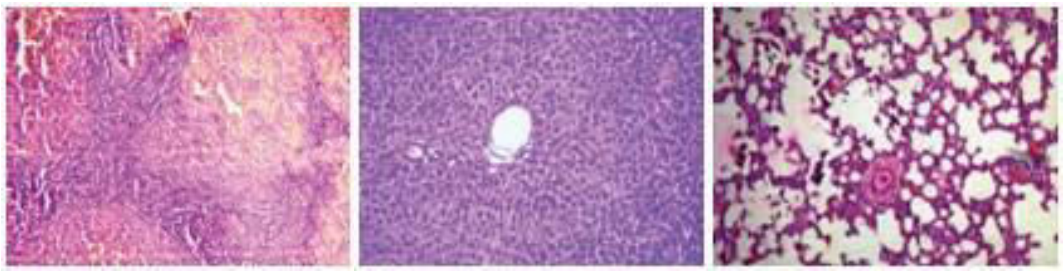
Tahir *et al*. (2014) reported at the platelet count differences between male and female Carica papaya leaf juice. The difference between male

Table 1: Platelet Count × 10^5/ cmm Mean ± SD observed in various extracts treated rat groups

Group name	Day 0	Day 6	Day 15	Day 25
Hexane	8.99 ±0.97	2.31±0.63	3.30±0.54	6.17.5±0.73
Acetone	8.90±1.04	2.35±0.84	3.35±0.78	6.57±0.64
Ethanol	9.18±1.39	2.03±1.12	3.04±0.95	5.70±0.52
Methanol	9.40±1.29	1.97±0.86	3.15±0.96	6.00±0.68
Distilled water	8.90±1.04	2.18±0.89	3.73±0.84	6.91±0.85
Control	8.83±1.03	2.52±0.75	2.97±0.84	5.70±0.28
Standard	9.68±1.45	2.41±0.62	5.78±1.12	9.73±1.30



Kidney **Heart** **Intestine**



Spleen **Liver** **Lung**

Fig. 2: Histopathological images of the variousorgans.

and female plants was not statistically significant, but a higher dose (10 ml/kg) evoked a much greater response than a low dose (5 ml/kg). This is consistent with our findings. Many active ingredients in papaya leaf juice may be responsible for increasing blood cell counts in a variety of ways. Papain, chymopapain, alkaloids, flavonoids, flavonols, tannins, and benzyl glucosinolate are all active chemicals found in papaya leaves. These substances improve and/or augment the ability of megakaryocytes to produce enough platelets to maintain an adequate platelet count in mammals, especially during chemotherapy. Leaf proteolytic enzymes, such as papain and chymopapain, are biologically active substances. Protein breakdown by these enzymes may increase platelet count because caspase (protease) activation regulates proplatelet production. Flavonols and flavonoids (including kaempferol, quercetin, myricetin, and fisetin) are commonly used in pharmaceutical formulations. They have been shown to have an anabolic effect (Songlin *et al*., 2009), which could explain why they stimulate blood cell production. Tannins are large polyphenolic compounds that contain sufficient hydroxyls and other groups (including carboxyl) to form strong complexes with proteins and other macromolecules. Tannins' complex-forming properties may contribute to their beneficial effects on platelet count in the blood because retraction events release individual pro-platelets (Patel *et al*., 2005). Using the protocols provided by Godkar and Godkar (2006), animals were sacrificed at the end of the experiment for histopathological examinations of the liver, lungs, kidney, spleen, heart, and intestine to assess the effect of the extract on organs and its toxicity. All extracts demonstrated no harmful effects on vital organs such as the liver, lungs, kidney, spleen, heart, and intestine, according to histopathological investigations. As a consequence, these extracts are safe to use orally in order to boost platelet count.

Conclusion

The initial dip in platelet count produced by carboplatin was averted by papaya leaf extract, and the platelet count eventually rose without any hazardous side effects. The effect of extracts on other haematological parameters was insignificant, and extracts had no effect on male or female rats. It is clear from this that *Carica papaya* extracts contain potential component(s) responsible for increasing platelet count; thus, additional research into the isolation, purification, and characterization of such component(s) is urgently required. However, this is a preliminary study, and more research is needed to isolate and identify the active bio-components of *Carica papaya* leaves in order to develop a suitable platelet-producing formulation.

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Biochemical and Behavioral Changes in Mulberry Silkworm Larvae Fed Micronutrient-Enhanced Mulberry Leaves

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Abstract: Larvae from a PMxCSR hybrid of the insect pest Bombyx mori L. are being fed mulberry leaves and micronutrients to see how they fare. We conducted a field experiment with twelve different treatments (T0-T11) including control (T0) group that received different amounts of each micronutrient. Twenty B. mori early instar larvae/replication stages were given mulberry leaves, twice a day (morning and evening) throughout their fourth and fifth instars. Compared to the control group (4th and 5th instars), treatment T9 (4 kg/ha of ZnSO4 + 3 kg/ha of FeSO4 + 3 kg/ha of CuSO4) increased food intake and absorption by up to 31.56 and 22.98%, respectively (4th and 5th instar, respectively). In comparison to T10 (4 kg/ha of CuSO4 + 3 kg/ha of FeSO4 + 3 kg/ha of ZnSO4), T11 (1:1:1 kg/ha of CuSO4, FeSO4 and ZnSO4) exhibited a growth of 94.95 and a tissue growth efficiency of 65.67. A longer larval period (7.93±1.16 days) was seen in the T1 (FeSO4 10 kg/ha alone) group compared to the control group (T0). In T9, the larvae grew by 50.0% in length and breadth and 18.0 and 18.51% in length and width, respectively. Maximum chawki larval weight increased by 16.78 and 29.44%, and T9 had the largest silk gland at 2.14±0.05 g.

Keywords: *Bombyx mori*, Micronutrients, Mulberry, Larval assimilation, Digestibility, Morphometrics

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Introduction

Fast development and transformation of *Bombyx mori*, made possible by the nutritional content of mulberry leaves, constitute the physiological basis for sericulture. *Bombyx mori* silkworms specifically feed only on mulberry leaves (*Morus alba* L). Ninety-seven per cent of the silkworm's total food intake and eighty-five per cent to eighty-five per cent of feed utilization in the fifth instar

larva came from the metabolically active leaves ingested during the last two instars (i.e., the fourth and fifth instars). Either improving the quality of the food or making the silkworm larvae instars hungrier might significantly boost silk production performance. Nutritional characteristics of food, biochemical state of nutrients in food, amount of hormones in body, and condition of environment all have a role in the growth, development, and eventually the physiology of insect bodies. When fed various mulberry leaves, silkworms efficiently metabolise their diet and transform it into silk (Yamamoto and Fujimaki, 1982). The quantity and quality of the leaves are directly related to the pace of growth and the weight of the larvae. Micronutrients, which function as cofactors in the activity of numerous enzymes, increase the silkworm's energy efficiency and, by extension, its silk production. Foliar vitamin supplementation has also been shown to improve mulberry leaf production and quality, which in turn promotes the healthy growth of silkworms and, in turn, boosts cocoon output and quality. Recently, it has been reported how supplementation with vitamins might alter the metabolic features of *B. mori* larvae (Marin *et al*., 2021). Analyzing the larvae's nutritional indicators in addition to its morphometrics and characteristics would help us better grasp the silkworm's larval parameters. As a result of the above, scientists investigated whether or not micronutrient addition to the soil influenced the development of *B. mori* in mulberry plants. We conducted a field experiment with twelve different treatments (T0-T11) including a control (T0) that received the optimal amount of each micronutrient.

Materials and Methods

The experiment was conducted at an altitude of 29 metres above sea level in Poovancode village, Kanyakumari district, Tamil Nadu, India (8.3031° N, 77.2881° E), where the mulberry garden was three years old. The test plot was planted in a completely bare, well-watered, and sun-drenched environment. A mulberry plant (*Morus alba*) of the MR2 (Mildew Resistant Variety -2) strain was provided by the Sericulture Department of the Government of Tamil Nadu's experimental station. A 90x60 cm gap was left between each plant. In June, before the trial began, the mulberry trees were felled. This was followed by ploughing, an annual application of 20 metric tonnes of farmyard manure, and a uniform single dose of 120 metric tonnes of nitrogen, phosphorous, and potassium (kg/ha). Every five days (or so, depending on the weather), water was applied, and twenty days after pruning, micronutrients were tilled into the soil. The experimental plants were protected from pests and any diseased or damaged sections were removed and discarded. The field experiment included 12 different treatments, and each treatment was replicated 3 times, for a grand total of 36 potential results. Every treatment received the appropriate quantity of each micronutrient, either alone or in different combinations. To clarify, Treatment 0 (No nutrients), Treatment 1 (10 kg/ha of FeSO4), Treatment 2 (10 kg/ha of ZnSO4), Treatment 3 (10 kg/ha of CuSO4), Treatment 4 (5 kg/ha of CuSO4 + 5 kg/ha of ZnSO4), Treatment 5 (5 kg/ha of CuSO4 + 5 kg/ha of FeSO4), Treatment 6 (5 kg/ha of ZnSO4 + 5 kg/ha of FeSO4), Treatment 7 (5 kg/ha of ZnSO4 + 5 kg/ha of CuSO4), Treatment 8 (4 kg/ha of FeSO4 + 3 kg/ha of ZnSO4 + 3 kg/ha of CuSO4), Treatment 9 (4 kg/ha of ZnSO4 + 3 kg/ha of FeSO4 + 3 kg/ha of CuSO4), Treatment 10 (4 kg/ha of CuSO4 + 3 kg/ha of FeSO4 + 3 kg/ha of ZnSO4) and Treatment 11 (1:1:1 kg/ha of CuSO4, FeSO4 and ZnSO4). Each set of 20 B. mori fourth- and fifth-instar larvae was fed a meal of 5-6 completely grown mulberry leaves twice a day (morning and evening) to all the 12 groups (T0-T11). In order to conduct this research, we acquired B. mori PMxCSR2 hybrids from the Government Sericulture Training Centre in Konam, Nagercoil, Kanyakumari, Tamil Nadu, India. The procedure of rearing silkworms began when the mulberry trees were 45 days old. Because of the constant attention they required, the silkworms used in the trials were reared in a rearing chamber according to Krishnaswami's (1978) technique. Each day, the weight of the *B.*

mori larvae, the discarded mulberry leaves, and the litter was recorded. We dried the faeces and the leftover leaves in a hot air oven at 70°C until they were both the same weight. As suggested by Waldbauer (1968) we recorded the larvae's initial and final weights, as well as their food intake, assimilation, total growth, approximate digestibility, tissue development efficiency, and ecological growth efficiency. To check for statistical significance between the test and control groups, the student's t-test was used. Standard deviation and mean were confirmed for all data (S.D.).

Results and Discussion

The fourth and fifth instar *B. mori* larvae were subjected to several treatments, and the results were mixed. On both the fourth and fifth stages, T9 was the most beneficial treatment. Fourth and fifth instar T9 had the highest food consumption (31.56% and 22.98% higher, respectively; 1032.8 48.3 and 2080.4 164.6 mg/larva/day). In comparison to the control group (T0), T1 had the lowest food consumption throughout the fourth and fifth instars (848.3 26.5 and 1819.2 113.3 mg/ larva/day, respectively). The largest increase in absorption was seen in T9 (730.3 21.4 and 1694.7 110.7 mg/ larva/day; 51.38% and 34.56% above control, respectively). The worst absorption was found in the T11 strain (504.326.9 mg/larva/day, an increase of 4.50% over the control; the best assimilation was found in the T1 strain, an increase of 3.41% over the control). As a whole, the fourth instar grew by 522.1 mg/larva/day in T9, an increase of 94.95% over control, and by 296.3 mg/larva/day in T1, an increase of 10.64% over control. We also observed significant increases in the frequency of the fifth instar in the T9 population (954.8 14.7 mg/larva/day) and the T1 population (612.7 8.5 mg/larva/day), both compared to the control group by a factor of 65.67% and 6.31%, respectively. The quantity and composition of the food eaten have a significant bearing on the size of the silkworm larvae (Shivakumar, 1995). Because of their enhanced flavour, increased nutritional content, and ability to keep water in the leaves for longer periods of time, T9 showed significantly higher rates of food intake in the fourth and fifth instars (31.56% and 22.98%) as compared to controls. In addition, the insect's physiological state and the meal's physical and chemical makeup would have affected its rate of digestion and digestion.

All treatments, as compared to the control, greatly increased assimilation in the fourth and fifth instars. According to Thrived *et al*. (2003), a higher absorption rate is the result of better digestion and assimilation. Researchers Lalfelpuii *et al*. (2014 a, b) claim that larval performance may be explained by the larvae's ability to digest and absorb the nutritional components contained in mulberry leaves. The amount of food eaten and the humidity of the leaves are two elements that affect digestion. It is possible that silkworms' digestion and enzyme production improve as they consume more food. The larvae's growth depends on the quality and quantity of the food they ingest. The majority of food is eaten towards the end of its life cycle. As previously established, micronutrients have been proven to speed up larval development, and the present research verified this by showing that overall larval growth was higher in T9. Growth-promoting hormonal processes and an uptick in food intake and the larvae's capacity to turn nutrients into biomass were also implicated.

In comparison to the control, T9 exhibited an increased estimated digestibility of 15.14% in the fourth instar (70.74±4.9%) and 9.33% in the fifth instar (81.44± 6.7%). The lowest approximative digestibility was found in T11 (55.56x5.8%) and T1 (70.97±7.9%), both of which were decreased by -9.60% and -4.70%, respectively, from the control. Tissue growth efficiency was best in the fourth and fifth instars at T11, with values of 91.77± 4.2% and 68.45± 4.6%, respectively; these values were increased by 65.35% and 49.61% above the control. Their smallest T1 values, in comparison, were 54.18±4.6% and 45.45x6.4%, decreases of -2.37% and increases of 3.71% from the control. Maximum and minimum ecological

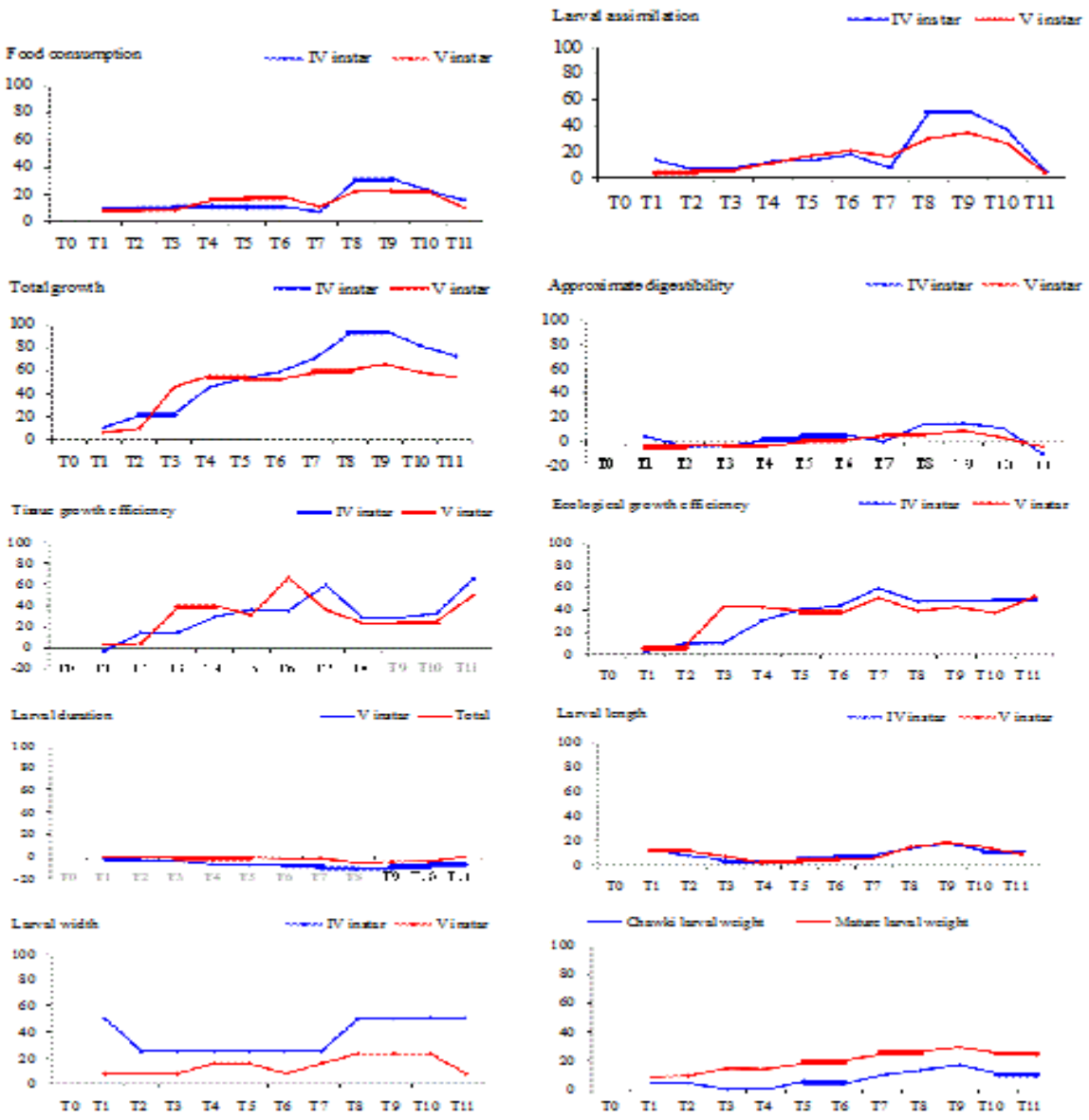

Fig. 1: *B. mori* larval parameters-effects of treatments.

growth efficiency values were recorded by the fourth instar larvae in T11 (51.07± 3.3%) and T1 (34.93± 5.2%), representing increases of 49.58% and 2.31%, respectively, over the control, while the fifth instar recorded 48.9±1.5% and 33.67±2.6%, representing increases of 52.0% and 4.66%, respectively, over the control (Table 1; Fig. 1). Food's approximative digestibility is measured as the percentage of food that is absorbed in comparison to how much is consumed. In this research, differences in leaf quality were the primary source of variance across treatments. Food's estimated digestibility increases as it moves through the stomach and is digested and assimilated more thoroughly. It was previously believed that larvae's variable reactions to the physical and chemical composition of food were to blame for the age-related decline in tissue development efficiency and ecological growth efficiency.

Both the adult and larval stages of T1 lived for a very long time (7.93 ± 1.16 days). When

Table 1: Effect of treatments on the nutritional characteristics of *B. mori* larvae

Treatment	Food consumption (mg/ larva/ day)		Larval assimilation (mg/ larva/ day)		Total growth (mg/ larva/ day)		Approximate digestibility (%)		Tissue growth efficiency (%)		Ecological growth efficiency (%)	
	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar	Fourth instar	Fifth instar
T0	784.4± 31.4	1691.6± 123.5	482.4± 25.3	1259.4± 93.8	267.8± 3.6	576.3± 4.7	61.49± 6.2	74.45± 8.6	55.5± 6.8	45.75± 7.9	34.14± 4.1	32.17± 4.3
T1	848.3± 26.5	1819.2± 113.3	546.8± 18.4*	1291.8± 104.3	296.3± 3.4*	612.7± 8.5	64.45± 3.7	70.97± 7.9	54.18± 4.6	47.45± 6.4	34.93± 5.2	33.67± 2.6
T2	862.4± 49.2*	1830.4± 122.7	510.9± 19.4	1320.6± 95.3	323.7± 7.5*	631.0± 6.4*	59.24± 4.5	72.13± 6.4	63.35± 5.2*	47.80± 4.2	37.53± 3.7*	34.47± 2.3
T3	868.5± 21.6*	1834.6± 142.9	516.3± 22.4	1334.1± 104.8	327.8± 9.8*	842.3± 7.5*	59.44± 3.1	72.74± 5.7	63.49± 4.4*	63.14± 3.7*	37.74± 3.6*	45.91± 3.2*
T4	875.6± 14.3*	1954.7± 124.7*	543.2± 38.6*	1402.7± 129.5*	390.4± 5.9*	897.2± 8.2*	62.03± 3.6	71.75± 8.3	71.87± 5.1*	63.96± 3.2*	44.58± 3.4*	45.89± 5.2*
T5	858.7± 48.8*	1974.3± 134.7*	546.2± 24.3*	1474.8± 113.4*	411.8± 3.4*	884.1± 10.4*	63.60± 4.1	74.71± 8.9	75.42± 5.7*	59.94± 3.6*	47.95± 3.2*	44.78± 2.2*
T6	870.3± 32.4*	1998.5± 152.6*	568.1± 31.2*	1518.2± 108.5*	426.9± 6.4*	879.4± 7.2*	65.28± 4.7	75.97± 5.6	75.14± 5.6*	57.92± 3.8*	49.05± 3.6*	44.0± 2.3*
T7	840.4± 24.5	1875.4± 117.8*	518.7± 21.6	1465.1± 104.6*	457.8± 4.1*	911.3± 12.4*	61.72± 5.8	78.13± 7.8	88.25± 3.4*	62.20± 3.1*	54.47± 3.3*	48.59± 2.7*
T8	1023.3± 31.6*	2067.9± 103.7*	721.9± 33.7*	1637.6± 112.5*	515.5± 9.5*	926.7± 13.4*	70.48± 2.9*	79.19± 6.3	71.40± 3.2*	56.58± 5.7*	50.37± 4.1*	44.81± 3.4*
T9	1032.8± 48.3*	2080.4± 164.6*	730.3± 21.4*	1694.7± 110.7*	522.1± 4.7*	954.8± 14.7*	70.74± 4.9*	81.44± 6.7*	71.49± 4.1*	56.34± 4.4*	50.55± 4.2*	45.89± 3.8*
T10	964.2± 36.1*	2064.7± 142.5*	662.5± 25.2*	1594.9± 97.9*	487.2± 3.6*	912.4± 10.3*	68.65± 6.4*	77.24± 7.8	73.53± 5.3*	57.20± 5.1*	50.52± 3.7*	44.19± 4.1*
T11	906.1± 46.8*	1862.5± 136.3*	504.3± 26.9	1302.4± 97.2	462.8± 4.6*	891.3± 12.6*	55.56± 5.8	71.48± 6.5	91.77± 4.2*	68.45± 4.6*	51.07± 4.2*	48.90± 1.5*

Values mean± S.D. *Significant P≤0.05 (t-test)

Table 2: Effect of treatments on the morphometrics of *B. mori* larvae

Treatment	Larval duration (days)		Larval length (cm)		Larval width (cm)		Chawki larval weight (g/ 10)	Mature larval weight (g/ 10)	Silk gland weight (g)
	Fifth instar	Total	Fifth instar	Total	Fifth instar	Total			
T0	6.79± 1.12	19.22± 5.11	5.0± 0.12	5.4± 0.17	0.4± 0.09	1.0± 0.22	1.43± 0.59	33.89± 4.8	1.44± 0.02
T1	7.93± 1.16	25.08± 5.44	5.6± 0.15*	6.1± 0.26*	0.6± 0.3*	1.4± 0.3	1.50± 0.87	36.86± 5.2	1.58± 0.05
T2	7.51± 2.22	25.06± 5.14	5.4± 0.17	6.0± 0.19*	0.5± 0.1*	1.4± 0.14	1.49± 0.51	37.07± 3.9*	1.53± 0.1
T3	7.46± 3.45	25.06± 5.17	5.2± 0.14	5.8± 0.19	0.5± 0.2*	1.4± 0.3	1.43± 0.47	38.83± 1.6*	1.67± 0.03
T4	7.22± 3.23	24.46± 6.23	5.1± 0.15	5.5± 0.18	0.5± 0.1*	1.5± 0.23*	1.43± 0.83	38.67± 2.9*	1.62± 0.01
T5	7.20± 2.45	24.87± 6.23	5.2± 0.11	5.6± 0.11	0.5± 0.2*	1.5± 0.21	1.51± 0.20	40.05± 4.22*	1.83± 0.02
T6	7.20± 3.21	24.73± 6.34	5.3± 0.19	5.7± 0.08	0.5± 0.2*	1.4± 0.26	1.48± 0.14	40.72± 3.54*	1.87± 0.04
T7	7.01± 3.26	24.68± 6.56	5.4± 0.21	5.7± 0.23	0.5± 0.15*	1.5± 0.11*	1.56± 0.13*	42.11± 1.11*	1.71± 0.07
T8	6.86± 3.35	23.81± 6.12	5.7± 0.10*	6.2± 0.08*	0.6± 0.18*	1.6± 0.10*	1.62± 0.14*	42.87± 5.30*	2.00± 0.04
T9	6.94± 2.21	23.96± 7.13	5.9± 0.13*	6.4± 0.10*	0.6± 0.1*	1.6± 0.43*	1.67± 0.15*	43.87± 3.29*	2.14± 0.05
T10	7.14± 3.45	23.03± 7.16	5.6± 0.16*	6.2± 0.15*	0.6± 0.1*	1.6± 0.13*	1.58± 0.94*	42.45± 3.69*	1.98± 0.02
T11	7.29± 4.32	24.04± 7.18	5.5± 0.14*	5.9± 0.12*	0.6± 0.16*	1.4± 0.19	1.56± 0.39*	41.97± 4.62*	1.84± 0.01

Values mean± S.D. *Significant P≤0.05 (t-test)

compared to the control group (T0), its duration was notably high. As a whole, T8 (6.86± 3.35) lasted only 23.81± 6.12. Compared to the control group, T9 showed an increase of 18.0% in the maximum and 2.0% in the minimum length of the fourth instar (5.9± 0.13 cm and 5.1± 0.15 cm, respectively). The fifth instar grew by 18.51% in T9, or 6.4 ± 0.10 cm, and by 1.85%, or 5.5 ± 0.18 cm, over the control period. Maximum values for T8 (0.6 ± 0.18 cm) were significantly higher than those for T11 (0.6 ± 0.1 cm), T1 (0.6 ± 0.3 cm), T10 (0.6 ± 0.1 cm), and T9 (0.6 ± 0.1 cm), all of which increased by 50.0% relative to the control, while minimum values for the other treatments (i.e. T2, T3, T4, T5, T6, T7, and T8) were significantly lower than 0.5 cm. The widest treatments were T9 (1.6± 0.43 cm), T10 (1.6± 0.13 cm), and T8 (1.6± 0.10 cm), all of which were increases of 23.07% over the control, while the narrowest were 1.0 cm or less (Table 2; Fig. 1). In T9, the chawki larval weight reached its maximum point (1.67± 0.15 g/ 10), a 16.78% increase over the control, and its lowest point (1.43± 0.47 g/ 10 and 1.43± 0.83 g/ 10), which was equivalent to the control (1.43± 0.59 g/ 10). Maximum and lowest mature larval weight values were 43.87 ± 3.29 g/ 10 in T9 and 36.86 ± 5.2 g/ 10 in T1, up 29.44% and 8.76% from control, respectively. T9 had the heaviest silk glands (2.14 ± 0.05 g), while T2 had the lightest (1.53 ± 0.1 g) (Table 2; Fig. 1).

This research significantly enhanced the morphometrics of late-stage larvae (stages four and five). The increased larval weight could be attributed to high-quality leaves with vitamin content.

Other characteristics of larvae include healthier larvae with improved micronutrient use and absorption (Bose *et al*., 1994; Sarker *et al.*,1995; Nirwani and Kaliwal, 1995, 1996; Etebari *et al.*, 2004; Ramarethinam and Chandra, 2007; Balasundaram *et al*., 2008). Higher larval weights result in higher silk gland weights. Silk glands develop at their fastest rate around the end of the fifth instar due to fibroin synthesis, and it is obvious that the weight of the silk glands is one of the most important factors to consider when evaluating the larvae's capacity to produce silk. According to studies on micronutrients and the development of the silk gland and larval body (Bhattacharya and Kaliwal, 2005a, b), the latter finding was also observed in the current investigation. When compared to other treatments, T9 had the highest value across all therapies. When zinc is administered through mulberry leaves, the weight of silkworm larvae increases significantly (Chamundeswari and Radhakrishnaiah, 1994; Hugar and Kaliwal, 1999, 2002; Ashfaq *et al*., 2010). Furthermore, zinc promotes the development of the silk gland and the larval body, shifting the balance toward a higher gland-body ratio during the 5th instar (Kavitha *et al*., 2012).

Conclusion

This study clearly showed that the supplementation of the different micronutrients on different ratio have notable impact on the nutritional and morphometric characteristic features of the larvae. The positive impacts of the micronutrients on the total growth, food consumption, larval assimilation, digestibility, tissue growth and ecological growth characteristics were clearly observed. The notable effect of micronutrients on the larval length, larval width, larval weight, larval duration and silk gland weights were also clearly understood.

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A Comprehensive Review of the effects of Toxicity and Prevailing conditions on the Thyroid Gland and its Herbal Medicament

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Abstract

The human body is among the most significant examples for understanding how many systems must operate in harmony and harmony in order for the body as a whole to function. This encompasses interconnected systems, including the cardiovascular, respiratory, renal, neurological, gastrointestinal, endocrine, and many more. Each system in the body contributes to the maintenance of life in several wonderful and distinctive ways. Many details regarding the endocrine system's duties in the human body, which include regulating key processes, may be learned through shedding light on it. Lack of stasis in the system may interfere with the body's capacity to adjust to various physical consequences. One of the most common disorders caused by the endocrine system's dysfunction is thyroid disorder. There are a variety of potential prefactors for these illnesses, including toxicity, dietary habits, drug use, comorbid disorders, and many more. Toxicity may occur for a number of causes, such as those that impact the body's numerous biological processes. The hormonal balance, which depends on enzymes and chemical compounds to support its normal cycle, interferes with the metals, chemicals, and other elements present in the environment and produces toxic agents that disrupt normal synthesis, resulting in a variety of disabilities, illnesses, and disorders, etc.

Keywords: Endocrine system, Hormonal imbalance, Health, Thyroid, Environmental effect, Herbal remedies, Sustainability.

1. Introduction

The human body is made up of several unique and diverse organs, each of which functions differently yet together sustain life. To keep the body stable, these systems must function in harmony. When a system in the body is not functioning in equilibrium, it may cause a number of issues that can eventually result in health problems. The dysfunction of a system like the endocrine system, which results in problems of this system and often results in a hormonal imbalance, is a good illustration of this. This imbalance can cause a variety of health issues, including PCOD, PCOS, diabetes and its variants, such as Type 1 diabetes, Type 2 diabetes, juvenile diabetes, gestational diabetes, and others, as well as the other prevalent condition of the endocrine system's imbalance, the thyroid disorder, which can include hyperthyroidism and hypothyroidism as well as an autoimmune condition. Such diseases may be caused by a variety of variables, including toxicity as well as other elements including lifestyle choices or underlying medical disorders. The environment contains various harmful substances in the form of metals, chemicals, pollutants, etc., which cause biological alterations, which do not occur in vivo. The quantity and degree of toxicity of the affected metals have a negative impact on the human body, resulting in hormonal and biological disorders. However, since these medicinal plants have so many important pharmacological and therapeutic properties, there are excellent methods for the treatment and cure of such problems via the use of herbal medicines.

2. Factors that Distress Hormonal Balance

The endocrine gland and system, one of the most important glands in the body, is governed by a variety of hormones found in

the human body. These hormones are often produced in the specialised system and gladly as said, the endocrine gland that really assists in managing as well as modulating distinct actions in the human body via the control of cells and other systems of the body. The human body tends to have many wonderful characteristics like these that support a variety of functions including regulating body temperature, mood, emotions, body metabolism, circadian rhythm, and fostering growth and development. However, the control of hormones does not always seem to be in a state of equilibrium stability. There are many hidden antecedents, and it is important to shed light on the many different factors that are connected to the hormonal imbalance in question.

The phrase "hormonal imbalance" refers to a condition where too many or too few hormones are generated by the human body. The endocrine system's natural signals are sent out by these hormones, which are also recognised for their functions as chemical messengers. When there is an imbalance in how these hormones signal in the human body, it is the cause of several problems that ultimately result in different health consequences. Numerous variables, particularly modern lifestyle choices, are to be cited that might contribute to this hormone imbalance, which has been seen in the majority of the population. These lifestyle practises have to do with a person's degree of physical activity, nutritional choices, circadian rhythm, and social practises. Such lifestyle factors that are compromised make it impossible for the body to function on its own, which makes it impossible for hormones to be created or communicated effectively enough to support physical functions. While a lifestyle component is one of the factors, medical issues or comorbidities are the other major element.

Diabetes, PCOD, PCOS, thyroid disorders, addison's disease, and many other medical conditions are among the most prevalent ones. The status of the thyroid gland, which may cause a variety of illnesses as was described above, is one of the key contributing elements and pharmacological conditions that results in hormonal imbalance. Thyroxine, also known as the T3 hormone, tri-iodothyronine, also known as the T4 hormone, and thyroid-stimulating hormone, often known as TSH, are all produced by the thyroid gland. Below the Adam's apple on the front of the neck is where the thyroid gland may be seen. One of the most common medical disorders is a disorder of this hormone. Numerous millions of individuals of all ages and genders are diagnosed with endocrine disorders, according to previous research (Anushka Agarwal et al., 2018). Similar to hyperthyroidism, which results from excessive thyroid hormone production, hypothyroidism results from inadequate thyroid hormone production or gland, thyroid, it is, which results in thyroid gland inflammation, autoimmune disease known as Hashimoto's thyroiditis, as well as thyroid cancer. In order to grasp hormonal imbalance and its contributing causes, it is necessary to comprehend how this gland might become dysfunctional owing to a number of circumstances.

3. RISK FACTORS THAT CAUSE THYROID

With the manifestation of a disease, condition is those with its correlating pointers that are said to trigger and become the causative factor in the prevalence of the condition, having said that it is to be shed light on that many factors are to be precursors that lead to causing hormonal balance and are such which is associated with factors leading to the conditions. It can be caused by a variety of factors, including age, gender, pregnancy period, genetic factors, an existing or prevailing medical condition, improper lifestyle, particularly sedentary factors, and improper nutrition, dietary factors, particularly concerning iodine levels in the diet, medications, and the presence of iodine in scans, as well as certain disease conditions that include the condition as a side effect, and one of the major factors that result in the thyroid is the thyroid. In terms of pregnancy and breastfeeding, the lifecycle has curved more towards the increase of hormonal swings, leading to illnesses such as gestational diabetes and thyroid where the levels have risen substantially. It should also be mentioned that illnesses of the brain, particularly the hypothalamus, may lead to hypothyroidism as well as decreased functioning of other glands such as the pituitary gland, which suppresses the generation of thyroid-stimulating hormone (TSH) (Michael, 2009). When the thyroid hormone is impaired, it usually causes a significant increase in thyroid-stimulating hormone, which may be detected in blood indicators of values such as TSH levels, T3 levels, and T4 levels to provide a comprehensive picture (Thea et al., 2003). However, in the condition of Autoimmune condition that is a very prevailing factor of the thyroid condition that would not be seen clearly just with the profile of markers mentioned above and the thyroid peroxidase auto antibodies (TPOAb), thyroglobulin autoantibodies (TgAb) values are ought to be tested upon and in such autoimmune conditions is seen a very high curve of values of the markers and however, the prevailing causative factors are seen to be diagnosed at the (Mounika et al., 2013). It may be deduced that studying the disease and its underlying variables, screening at an early stage, and following conventional procedures for treating and curing the sickness will help reduce the disorder's prevalence.

4. ENVIRONMENTAL TOXICITY:

The most important cause for polycystic ovarian syndrome (PCOS) and polycystic ovarian disease (PCOD) could not just be the in vivo conditions. Due to many external factors, surprisingly toxicity is present in everyday used products. Not just reproduction-related problems but also the future generation will have genetic defects for example Attention deficit hyperactive disorder (ADHD)etc, To the surprise, many day-to-day usage products are involved, the levels of the toxic chemical that invade and imbibe into the human physical body majorly the drastic effect is on gene system (Kelly, 2021). Many free metals are spread in the environment according to their density the metals vary as to their impact. Environmental

toxicity is an increasing problem for ecological, environmental, nutritional and evolutionary reasons. Endocrine-Disruptors (ED's) caused due to heavy chemicals that involve in reproductive, development, brain and immune-related disorders. People are exposed to multiple ED's hence it is difficult to assess what type of toxicity causes what type of an effect (Amaral, 2002).

a. Everyday usable plastics like storage and food containers etc.

b. Herbicide applied vegetables

c. Flexible plastic toys

d. Cosmetics like creams, moisturisers, soaps, Liquid body wash, Lotions etc

e. Tofu, Soya products

f. Furniture, Foam and carpets

Environmental toxicity simply means the body's sensitivity that leads to health problems which occur during diet, air, skin, and water etc.

5. CHEMICAL TOXICITY:

As the word says the toxic aspects that cause an imbalance in the hormonal activity and any type of biochemical cycle disruption due to various chemicals that are present in the environment and other modes of toxicity s from synthetic drugs that causes side effects etc. The below-mentioned chemicals are not just toxicity-causing agents but also Endocrine disruptor's too due to which the endocrine hormonal imbalance happens (Monisha, 2014).

A. Phytoestrogens: They mimic hormonal activity, Genistein and daidzein that are in soy products like Tofu and soya etc.

B. Triclosan: Personal care products like body wash and face wash, hand wash etc which ever has Anti-microbial activity is rich in Triclosan chemical.

C. Polychlorinated Biphenyls (PCB): PCBs are the first industrial compounds that were banned worldwide, PCB's bioaccumulation leads to Hypothyroidism, Behavioral Problems, Auto Immunity and Cognitive disorders etc. PCB's presence in electrical equipment like transformers, lubricants and plasticizers etc.

D. Polybrominated Di Phenyl Ethers (PBDE): Used in flame retardants especially in households like furniture, furniture foam (Polyurethane) and carpets etc. These chemicals cause Tumours, Neurodisorders, hormonal imbalance, especially thyroid.

6. METAL TOXICITY:

Metal toxicity or metal poisoning is the toxic and adverse effect of metals on the forms of life however the doses or the levels of the metal cause poisonous insoluble compounds. The general symptoms of chemical toxicity are diarrhoea, nausea, abdominal pain, vomiting, shortness of breath, chills and weakness etc. Toxic heavy metals are listed down and their effects on the various parts of the body (Mahadi, 2021).

i) Mercury (Hg): Mercury is found in air, water and soil. Mercury is mostly in liquid form at room temperature which can easily be evaporated. In the vapour state mercury is most hazardous. Mercury enters the food chain of aquatic animals and eventually via seafood consumption it enters the human body. Chronic mercury toxicity causes neurological damage, ataxia, muscle weakness, numb limbs, speech disturbances, chewing, brisk and increased tendon reflex, Infants who have severe developmental disorders were born from the affected pregnant mothers.

ii) Lead (Pb): Lead is equally a heavy toxic metal that is easily absorbed by the skin, respiratory system, and digestive tracts. Lead exposure can induce neurological, respiratory, urinary, and cardiovascular, exposure to lead can produce alteration in physiological functions of the body like neurological, biological and cognitive functions in the body.

iii) Chromium (Cr): Naturally occurring heavy metal that is found in the earth's crust and seawater. Chromium is a compound that is released by chemical industries that is absorbed in soil, groundwater and air. Chromium can cause a variety of diseases like lung cancer, larynx, bladder, kidneys, testicles, bone and thyroid etc.

iv) Cadmium (Cd): Cadmium is a very rare metal that occurs naturally in soil, air and water. High levels of Cd in water, air, and soil cause severe disorders. Substantial exposure to Cd occurring due to smoking elevates blood and urine Cd concentrations. Cd may also occur in alloys, glass, and battery production. When toxic levels increase it will affect the kidneys, heart and lungs (Abirami, 2007).

v) Arsenic (As): Arsenic is one of the harmful heavy metals, it is known as the King of poison as it is present as contamination in food, water, and the environment. Arsenic exits in the form of metalloid. DNA damage of airway cells was observed following, Intake of as leads to spontaneous abortion, stillbirth, preterm birth, and the male reproductive system are affected (Hopenhayn et al., 2012).

7. HERBAL TREATMENTS

Herbs are plants or parts of plants that have aroma, taste, and medicinal characteristics. Herbal medications have no negative effects and are not always toxic, contrary to popular opinion. Many potential adverse effects A specific ingredient or component must be isolated and utilised as a medication or decoction when frequent consumption of that specific secondary metabolite compound from any part of the plant may heal many ailments by regularising the biochemical production. Though herbal therapies or cures may be used as an alternative or supporting medication, they are not medically acceptable during crises. Herbal medications are taken in the form of tablets, powders, or tinctures. They are made as tea and used to treat allergies on the skin as gels, lotions, and creams. Some plants are used to make oils, pain reliever baths, saunas, and other products. Using herbal supplements as treatment or remedy agents has been used for thousands of years. However, as is now widely suggested across the world, it is always advisable to visit a doctor for symptoms and adverse effects. (Tasel, Linda, 2006).

Table 1: Medicinal plants and its therapeutic effects are listed below

Medicinal Plant Associated with The Endocrine System	**Common Name**	**Coherent Pharmacological Activities**
Nigella sativa	Black cumin	Being one of the medicinal plants, containing the components thymoquinone governing stasis of hormones in the body (Mahdieh et al., 2016).
Withania somnifera	Ashwagandha	The property of this herb being an Adaptogen helps in aid to cure of major stress in the human body due that occurs due to the dysfunction of the Endocrine system and is also known to help balance the hormonal levels due to various pharmacological properties there by curing conditions such as PCOD, thyroid Etc (Umadevi et al., 2012).
Vitex agnus-castus	Chaste tree	Yet another medicinal herb that works in order primarily to fix hormonal imbalances mainly to do with thyroid, PCOD working with coherence with the pituitary and the endocrine gland (Branka, 2016).
Capparis spinosa L	Caper bush	Providing various chemical components that go in relation with pharmacological activities with providing cure to hormonal fluctuations there by help stabilizing the balance of such functions due to its coherent work with the Endocrine system (Sharrif et al., 2012).
Melissa officinalis	Lemon Balm	Melissa officinalis, commonly called lemon balm plays many specific roles with equalizing Hormonal patterns in the body and thereby fix conditions such as thyroid, diabetes, PCOD and many more pharmacological conditions (Eric et al., 2006).
Nasturtium officinale	Watercress	Nasturtium officinale a very known medicinal herb Encompassing various features of the ability on working in par with different systems of the human body to cure and maintain stasis of the system. Due to its presence of

		alkaloids, flavonoid, and many other chemical constituents along with vitamins and minerals helps with an order to create activities that in order to cure fluctuations of impaired bodily functions (Ali Esmail et al., 2020)
Cimicifuga racemosa L	Black cohosh	Compound Remifemin present in Actaea racemosa has various medicinal properties like gastrointestinal upsets, rashes, Hepatic, Adrenal gands disorders, cardiovascular and circulatory disorders (Sakineh et al., 2013).
Origanum majorana	Majorana	This herb is an effective food preserving agent, It is an effective antimicrobial agent, compounds Terpenoic and phenolic compounds, such as carvacrol and thymol present in this herb is used as Antioxidant agent and adrenal gland balancing agent (Jana et al., 2017).
Ajugareptans	Bugleweed/Carpetweed	Bugleweed is used to lower high levels of thyroid hormones (hyperthyroidism).It is also used to treat premenstrual syndrome, breast pain, nervousness, (insomnia) and bleeding, especially nosebleeds and heavy bleeding during menstruation (Edward, 2019).
Lithospermum erythrorhizon	Gromwell	It is a soap root, effective for anal cancer, Arthrititis, Hyperthyroidism, Tumors, Bladder stones, Fatty acids, Antiviral, Grave's disease (Kelly et al., 2018).
Astragalus propinquus	Huang qi or Milkvetch/locoweed	Protects the immune system, prevent cold, and upper respiratory infections treats disbetes, protects liver and corrects adrenal glands (Mohammad et al., 2020).
Eleutherococcus senticosus	Siberian Ginseng	It is the best Adaptogen to prevent colds and flu, increase energy, longevity, and vitality. Hyperthyroidism (Mathias et al., 2014).
Palmari palmata	Dulse	The Astringent aspect of Palmaria palmate used to treat gum ailments, toothache, bleeding Hypothyroidism (Kaushik, 2018).
Eisenia bicyclis	Sea oak/ Arame	This herb is a package of vitamins, Minerals, Iodine, iron, magnesium, calcium and other forms of important metals that are useful for the body. The Iodine content present in the herb is helpful to cure Thyroid (Chapman, 2013).

NIGELLA SATIVA

Nigella sativa, often known as black cumin seeds and a member of the Ranunculaceae family, is one of the most prominent plants with several pharmacological properties. It has a variety of therapeutic characteristics and ingredients that aid in the

treatment and cure of human bodily illnesses. It is often employed in numerous forms of medicine, including Ayurveda, Unani, and Siddha, owing to its capacity to treat and cure a variety of medical disorders (Aftab et al., 2013). This excellent medicinal plant has been found to play an important part in thyroid therapy. The presence of the component quinine is the reason why this medicinal plant has such powerful medical properties. Previous research has shown that when Nigella sativa in powdered form was administered to subjects with Hashimoto's thyroiditis, a much more complicated stage and form of thyroid condition, it resulted in improved values of blood markers as well as anthropometric levels, as well as a change in vascular endothelial growth factor parameters (Mahdieh Abbasalizad et al., 2016). Another research found that when Nigella sativa was delivered to wistar albino rats in the form of oils by gastric gavage at a dose of 200 mg/kg body weight to 48 rats, the group treated with the oil had a progressive drop in triiodothyronine levels in the blood (Gulcan Avci et al., 2021).

WITHANIA SOMNIFERA

Withania somnifera, also known as Ashwagandha, Indian Ginseng, and Indian winter Cherry, is claimed to be one of the most important medicinal herbs used in numerous branches of medicine like as Ayurveda and Siddha for many centuries. There are several plant components that are used to cure sickness and have many medicinal properties. Because of the presence of several chemical and pharmacological ingredients, it may be assumed that both the leaves and roots of this medicinal plant have held varied therapeutic benefits (Umadevi et al., 2012). According to studies, the use of this medicinal plant resulted in the stability of values across multiple thyroid profiles such as serum TSH (thyroid stimulating hormone), serum triiodothyronine (T3), and thyroxine (T4) levels in hypothyroid diagnosed participants (Sharma et al., 2017). Evidence suggests that the root extract of this medicinal plant was supplied to mice every day for around 20 days by stomach incubation and was shown to directly boost blood triiodothyronine and tetraiodothyronine levels (Panda et al., 1998).

BACOPA MONNIERI

The Plantaginaceae family's Bacopa monnieri, often known as Brahmi or Indian pennywort, has been demonstrated to be deficient in numerous important therapeutic properties. It is often employed in the ayurveda medical system owing to its medicinal elements that are associated with ailment cure. It has been demonstrated to have anti-inflammatory, anti-convulsant, anti-depressant, analgesic, and anti-microbial effects, as well as therapeutic applications in treating many ailments of the human system (Sebastian Aguiar et al., 2013). According to data, using this herb may help enhance the thyroid panel and can be used as a prophylactic in the state of hypothyroidism. According to one research, using this herb increased the T4 content in the body as well as the TSH levels (Kar et al., 2002). According to the papers, the use of Bacopa monnieri revitalises the thyroid panel in the body and so aids in boosting the thyroid panel, particularly the T4, and that it is a well-known and important herb in the area of Ayurvedic medicine (Tarun et al., 2015).

FUCUS VESICULOSUS

Fucus vesiculosus also called Bladder wrack or the sea grapes has shown to increase levels of T1,T2,T3,T4,T5 hormone levels in the blood serum of rats that are treated with Fucus vesiculosus for 3 weeks with a control group noticed to inhibit thyroid peroxidase, high levels of TSH leads to decrease T3 and T4 in blood serum, and this result agrees that dietary intake of sea grapes targeted areas of free radicals as they contain anti thyroid which causes oxidative stress, after continuous 3 weeks of treatment it is found that the tissues of Thyroid gland there are clear changes in the thyroid glands for the treatment (Mary 2010). Bladder wrack is air filled bladders which keeps the upper regions of the sea as it has the high capacity of absorbing the iodine content from the sea, believed to have a remedy for Thyroid (Jill 2012).

EMBLICA OFFICINALIS

Emblica officinalis, commonly known as the Wonder berry/Amla,and the role of Emblica officinalis and extracts in regulating thyroid functions were studied in male mice. Oral administration of Emblica officinalis fruit extract for 20 days decreased serum T3 and T4 concentrations and hepatic oxygen (O2) consumption. Both the plant extracts exhibited hepato-protective effects as evidenced by decreased lipid peroxidation. In animals treated with Emblica officinalis, activities of super-oxide dismutase and catalase remained unaffected, indicating that extract of Emblica officinalis, may have a direct free radical scavenging role, there is a change in the size and activities of the thyroid gland (Bhavesh et al., 2016).

PHAEOPHYCEAE

Phaeophyceae commonly called Brown seaweed is characterized with usual deficiency of iodine that can yield harmful effects on the thyroid as well as in increased iodine intake. One source of iodine is seaweed which is used as a foodstuff in Western countries. Apart from its potential involvement in thyroidal health, gaseous iodine released from seaweed plays a significant role in treating thyroid conditions. The iodine content present in these sea plants has a natural remedy factor (Robert 2003). Iodine is an essential micronutrient that requires the necessary quantity for thyroid hormones, These Phaeophyceae has the efficiency to suffice the bodily need for iodine. As the quantity suffices the demand it is been used as edible property in many foods and beverages factories (Inger 2020).

8. Discussion

Many facts about current pharmaceutical circumstances emerge as a result of patterns in other fields that do not interact with the human body. As previously said, medical disease and its consequences may be caused not only by patterns of the body's

defunction, but also by characteristics of the domicile and its toxicity. It should be noted that the epidemiology and pathology of such illnesses differ in nature, as do their treatment and cure. Nonetheless, the use of pharmacological plants as restorative remedies not only leads in significant improvements in reversing the ailment or curing the symptoms, but it also has fewer side effects than other medical systems. Harmful elements that impact the human body are found in the environment, and medication to treat toxic levels is also available. When there is a lock, there is also a key, and when there is a problem, there is also a medication. There are several pollutants and poisons present; how they are exposed and the degree of exposure determine the bad situation. Exposure to certain chemicals and metals, for example, is affected by both physical and mental states. Herbal therapies, on the other hand, are a cure factor or a control agent for restoring the human system to health. Other Asian countries that believe in natural and herbal treatments include (Sargassum spp., Sargassaceae), Chinese yam (Dioscorea oppositifolia, Dioscoreaceae), fritillaria (Fritillaria spp., Liliaceae), Prunella (Prunella vulgaris, Lamiaceae), scrophularia (Scrophularia ningpoensis, Scrophularia These are not prevalent on the Indian subcontinent, although they are abundant throughout the Asian continent.

Conflict of Interest

The authors declare they have no competing interests.

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Smart Investigation on Nanocomposites Composed of Carbon Dioxide-Derived, Repeatable Biological Polymers – A review

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Abstract

In recent years, an increasing number of people have begun to focus their attention on the environmental impacts that are caused by the widespread use of therapeutic polymeric composites that are generated from fossil energy. Another factor that probably contributes to the short shelf life of biomedical polymer products is the fact that many of them are designed to be used just once before being discarded. When a biomedical polymer product goes over its sell-by date, it must often be burned before being discarded, increasing carbon dioxide emissions (CO_2). By ultimately replacing their unsustainable fossil-based equivalents, biomedical goods based on polymers produced from CO_2 fixation would improve CO_2 recycling in this industry and aid in the mitigation of the greenhouse effect. However, the bulk of presently available polymer materials manufactured from renewable raw materials do not satisfy these expectations due to a number of property deficiencies, and the superiority and stuff values for biomedical devices are constantly expanding. The materials don't have the essential characteristics to satisfy the requirements. Many people are trying to apply nanotechnology in this field due to these problems. In addition to discussing replicable CO_2-fixed polymer-based nanocomposites that may be used in biological applications, this work gives a number of intriguing suggestions for further research areas in this field.

Keywords: Biopolymers, gelatin, nanocomposites, emission, chitin

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INTRODUCTION

Due to rising populations and economies, there has been a rise in the need for energy and the production of greenhouse gases [1–4]. Since the start of the industrial age in the 17th century, greenhouse gas emissions have increased. The results were a worsening of the greenhouse effect and other problems. The majority of the greenhouse effect is due to carbon dioxide (CO_2), which is produced by burning fossil fuels and its byproducts. Multidisciplinary teams of scientists have worked together to cut down on carbon dioxide production. This action is necessary to forestall further contributions to global warming gases [5–7].

Many biomedical polymer products that we use regularly are blamed by healthcare specialists for contributing to global CO_2 emissions. Biomedical polymers have several applications in the medical field, including in tissue engineering, artificial

organs, medication production, diagnostic devices, MPE, consumables, and packaging (Figure 1). Primarily responsible are the advantages and low cost of biomedical polymers [8]. Most of these discarded medications emit carbon dioxide when burned. More than one hundred biomedical polymer polymers are used in more than 1800 pharmaceutical products [9]. From 2011 to 2019, biomedical polymer material production and distribution rose 5.6%, from 4.391 million metric tonnes to 6.771 million. Biomedical polymer waste generates 20 million tonnes of CO_2. The manufacturing of medical devices adds considerably to carbon dioxide emissions due to its stringent process limitations and high failure rate. Prioritizing CO_2 recycling of biomedical polymers is important since it lessens the material's effect on the environment.

Biomedical devices designed to prevent epidemics have seen greater usage after the spread of COVID-19. China's production of COVID-19 masks increased by about 100%. Numerous systems for the organisation of biological polymers have been proposed [10, 11]. It's possible to find both biodegradable and nonbiodegradable biomedical polymers. Biomedical polymers include thermoplastics, thermosets, and elastomers. Biomedical polymers may either be derived from living sources or from remains. This is supported by the studies. Bio-based polymers, also known as naturally generated polymers, have a lower global warming potential than polymers made from $_{fossil}$ fuels. Because the components of bio-based polymers are sustainable [12]. Biomedical polymers are more carbon-efficient than their petrochemical counterparts since they are often produced by plant photosynthesis or other natural CO_2 fixation processes.

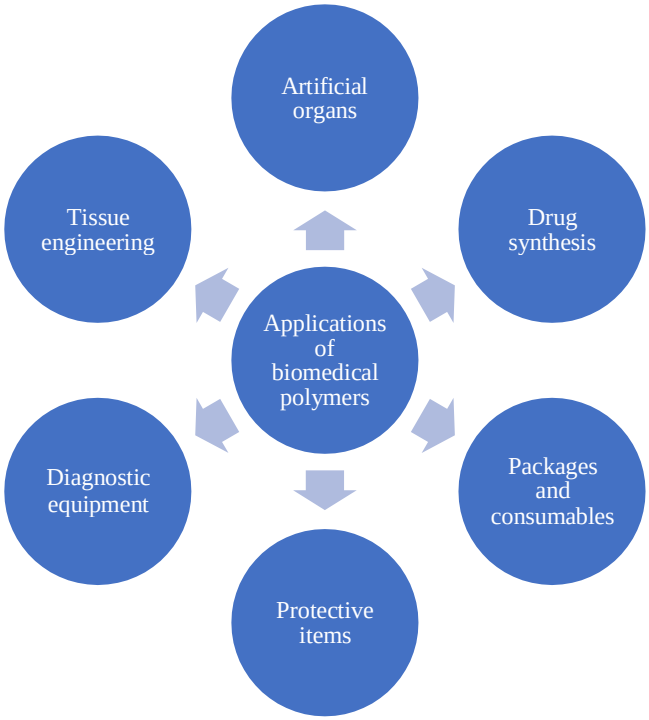

Figure 1. Applications of biomedical polymer products.

There are three repeating polymers that are able to repair CO_2. Examples include polymers generated from natural sources (Figure 2), polymers made from renewable resources, and polymers made from carbon dioxide. The term "synthetic renewable polymers" refers to polymers that are derived from renewable sources such as those that are created by the polymerization of natural subunits. The production of CO_2-based polymers requires polymerizing carbon dioxide gas with chemicals sourced either from biological sources or from fossil fuels [13]. Carbon dioxide (CO_2) fixation is used to create carbon-neutral repeating polymers. To encourage CO_2 recycling and reduce the carbon footprints of medical supplies, petrochemical alternatives must be phased out in favour of biomedical products

created from polymers from CO_2 fixation using repeatable sources. The vast majority of polymers produced by CO_2 fixation fall short of achieving the ever-increasing quality and performance demands for biomedical applications. This is true regardless of whether the polymers are based on CO_2 or on renewable resources.

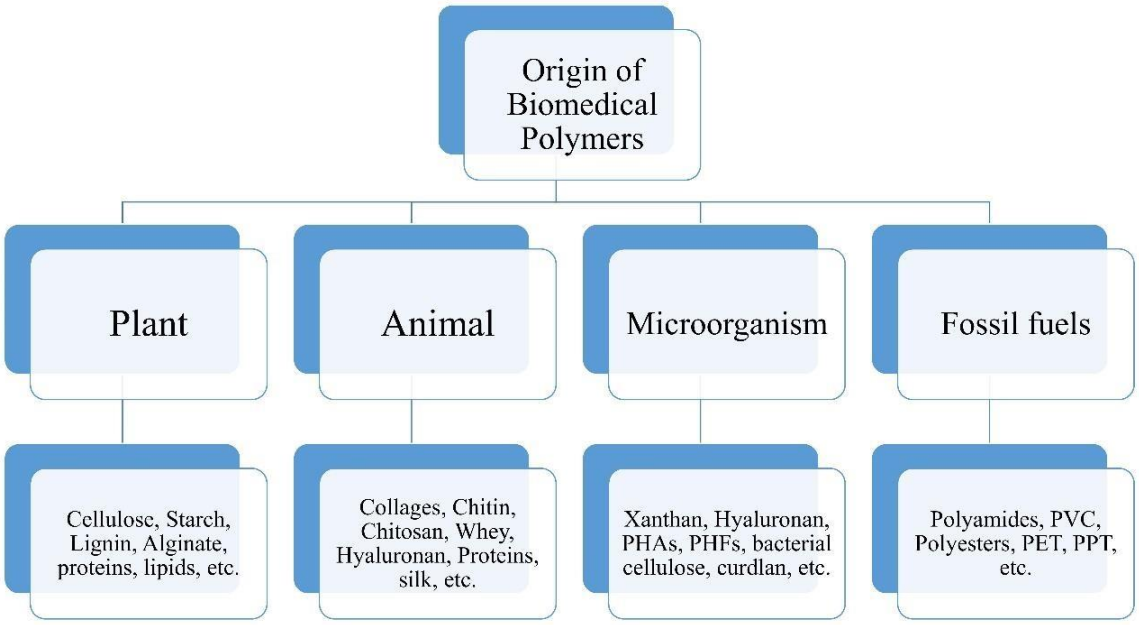

Figure 2. Biomedical polymer materials from different origins.

Over the last several decades, advancements in nanotechnology have allowed for significant upgrades to previously used materials. Many scientists have been modifying carbon dioxide polymers by mixing in nanoparticles in an effort to make environmentally acceptable medicinal polymers (CO_2). Both organic and synthetic polymers may be generated by the process of CO_2 fixation. These repeatable polymers derived from CO_2 fixation are increasingly being used as nanocomposites to enhance the performance of applications. There is a lot of hope for eco-friendly biomedical polymer goods when these nanocomposites are used. Carbon dioxide fixation may produce biological polymers with a high degree of reproducibility, however after their useful life or death, most of these nanocomposites would be incinerated. Combustion-derived CO_2 gas may be utilised by creating repeatable polymers [14].

NATURALLY-DERIVED POLYMERS

Natural polymers (Figure 3) have been used for a very long time in the field of biomedical research. Around the year 3500 BC, the Egyptians were the first known people to utilise cotton or horsehair to stitch up wounds. Skulls from the same time period that were found in Mexico may have had wood chips glued on them. Since the 1950s, synthetic polymers have gained a significant advantage over their naturally occurring counterparts. The fact that polymers generated from natural sources are both biocompatible and biodegradable ensures that they will continue to play a significant role in the area of biomedical applications. The development of biomedical products often involves the use of biomaterials that are based on polysaccharides [16–18]. These biomaterials may be represented by things like cellulose, chitin/chitosan, starch, alginate, and derivatives of hyaluronic acid, as few examples. In this part, we will talk about the biological uses of nanocomposites that are made from regular polymers that are derived from natural sources. These nanocomposites are created in a lab.

Collagen

Collagens are a kind of protein having molecular weights (Mw) below 300,000. Skin and skeletal muscle rely on it because of its large concentration in the body [19–21]. Although more than 20 forms of collagen exist in the body, types I through IV account for the vast majority. Collagen molecules are

biomedical nanoparticles because their chain lengths are on the order of microns. The amino acids in collagen may be broken down by enzymes. Collagen has been investigated for potential medicinal uses because to its interesting combination of physicochemical, mechanical, biological, enzymatic degradation, biocompatibility, and osteoconductivity features [22]. Collagen degradation might be stopped by cross-linking polymer chains or treating them with enzymes beforehand.

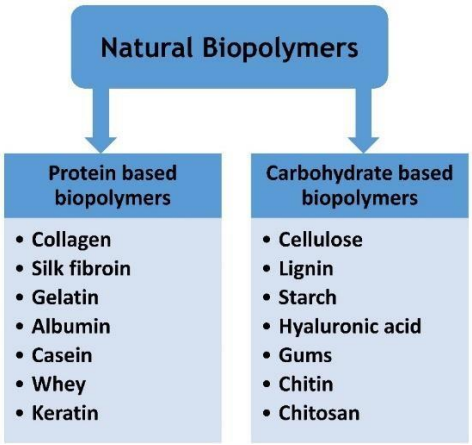

Figure 3. Naturally- derived polymer materials.

Nanocomposites made of collagen and calcium phosphate have been the focus of a significant amount of study over the last few years [23, 24]. This is due to the considerable potential that these nanocomposites offer for usage as bionic materials for the replacement and regeneration of bone. Bone transplants made of collagen-hydroxyapatite nanocomposites are becoming more popular owing to the compositional and structural similarity of these nanocomposites to native bone, as well as their innovative functional properties and increased mechanical strength. Nanocomposites containing collagen may improve cell-recognition sites, which in turn accelerates bone regeneration [25]. Collagen-hydroxyapatite nanocomposites rapidly stimulate cell mineralization even in the absence of an osteogenic additive in the culture medium.

There is a wide range of potential uses for commercially available collagen-hydroxyapatite nanocomposites. An FDA-approved synthetic bone transplant, Collagraft® is constructed of fibrous collagen, hydroxyapatite, and tricalcium phosphate. The thrombogenic characteristics of collagen are used in the hemostatic product Floseal®, a high-viscosity gel. For repairs to the dura, or inner lining of the eye, doctors recommend using Duragen® suture-free sutures. Collagen's clinical research use is shown by products like the bandages Biobrane®, Sulmycin®-Implan, and the three-dimensional collagen matrix grafts.

Fibrin

The clotting enzyme thrombin converts the protein fibrinogen into the biopolymers fibrin. The paring off of two polypeptide chains causes fibrin to have a molecular weight of about 360 kDa. Its four structural domains consist of the core fibrin peptide E, two pairs of fibrin peptides A and B, and two domains of fibrin peptide D. Fibrin was one of the first biopolymers used in medicine because of its desirable properties such as bioactivity, renewability, injectability, and the ability to promote cell growth and adhesion. Fibrin and its involvement in wound healing have come under considerable scrutiny in recent years [26–28]. This is likely due to the fibrous network properties that are inherent in blood production. Bioseed®, a commercial product, combines keratin-forming cells with fibrin to speed up the healing of chronic wounds. Using the patient's own plasma, Cryoseal® may be utilised to manufacture hemostatic tissue sealants for use in the treatment of burn injuries (Thermogenesis, United States). Cell adhesion, proliferation, and encapsulation are all facilitated by fibrin networks, which are used as a sealer on the surface of fibrin scaffolds or gels. Although fibrin is mechanically unstable and

is rapidly hydrolyzed by hydrolases, it may be improved by complexing it with other polymers, hence increasing its resistance to hydrolysis [29].

Gelatin

When collagen in animal tissues is hydrolyzed, gelatin is the byproduct. Therefore, the kind of collagen employed in the transformation and the method used impact the machine-driven, enlargement, and other physicochemical characteristics of gelatin. Gelatin, like many other naturally derived polymers, is non-toxic, biodegradable, and has a low immunogenicity. Natural and affordable polymer gelatin has several uses in medicine and pharmaceuticals [30, 31]. Many vaccinations and capsules taken orally are stabilised with gelatin. Gelatin may be shaped into a variety of drug delivery vehicles, including hydrogels, microspheres, nanoparticles, and nanofibers. For administration into the brain, nothing beats gelatin nanoparticles. Gelatin nanocomposites including various compounds existence are explored due to their prospective in tissue engineering. Middle-membrane vascular grafts constructed from synthetic tissue [24].

Chitin/chitosan

Biodegradable chitin and chitosan may be produced indefinitely. Due to their cell attachment and growth characteristics, they are utilized in the production of biomedical scaffolds, gels, particles, films, and more [5, 18, 32]. A greater number of negatively charged cells were attracted to positively charged chitosan. Chitosan dressings prevent bleeding and fight infection. Skeletal substitutes made from chitosan are used in tissue engineering regenerative scaffolding. By fusing with neighbouring muscle and maintaining automatic strength on par with innate tissue, filamentous protein/chitosan scaffolds were able to address myofascial anomalies in the abdominal wall. It has been proposed that chitosan colloidal particles might encapsulate a wide range of biological molecules, providing new avenues for medication administration. Rabbit eyes were injected with vancomycin by Khangtragool, who used chitosan as a delivery vehicle. Chitosan is not bone tissue-friendly on its own [33]. Membrane swelling, protein adsorption, and roughness were all enhanced in diatomaceous earth-doped chitosan composite membranes. Saos-2 cell proliferation and alkaline phosphatase activity were both boosted by the use of biocompatible composite membranes.

Starch

Starch is found in the endosperm and tubers of several plants [15, 34]. Starch nanocrystals are frequently employed in biomedicine because they are cheap, biocompatible, and biodegradable. Osteointegration, thermal-mechanical characteristics, and cell adhesion might all be enhanced with the use of starch-hydroxyapatite in tissue engineering. A combination of nanoscale magnesium olivine, PVA, thermoplastic starch, and vitamin E. Nanocomposite showed superior biological and mechanical characteristics, breakdown, and secondary osteoblast development compared to starch-PVA. It is also possible to use starch that has been chemically modified to deliver drugs. Due to its nonionic nature, starch is an effective co-blendant for polymers, allowing them to better absorb water and expand their pore structure [35]. Hydrogels degraded when treated with or without ketoprofen, and gellan gum and starch were involved in both instances. Starch hydrogel characteristics were modulated by the concentrations of polymer and cross-linker. Hydrogels' malleability made them excellent candidates as medicine delivery vehicles for a wide range of medical conditions. The shear and heat resistance of SPCL, which is made from starch and biodegradable polycaprolactone, is higher than that of starch alone (PCL). PCL lowered the stiffness and water sensitivity of the starch, which enhanced the SPCL's capacity to be processed, degraded, have better mechanical characteristics, and promote cell proliferation [36]. Starch has the potential to reduce manufacturing costs and boost PCL's biodegradability.

Cellulose

Cellulose is the most valuable renewable biological resource [37, 9, 11] due to its high economic value and abundance as the most common natural polymer. Cellulose is a straight-chain polymer

composed of -D-glucopyranose disaccharides linked together through glycosidic-(1, 4). The cellulose in plants forms nanofibrillar networks between 20 and 100 nm in size. Nanofibrils of bacterial cellulose have a size of 20-50 nm. Bacterial cellulose may be able to hold more water than plant cellulose because it is less crystallin. The glycan polymers found in plant cellulose are resistant to biotransformation, limiting its use [7, 26, 38]. Efforts are being made to study bacterial cellulose. Bacterial cellulose might be used in drug delivery methods. Silver bacillus coagulans, often known as Ag-BC, is a powerful antibacterial agent that can kill off any kind of bacterium, including Gram-positive and -negative strains. Specialties such as orthopaedics, ophthalmology, urology, neurology, and otolaryngology are included. Scaffolds made from bacterial cellulose are often used for tissue repair [39].

Hyaluronic Acid

1934 saw Meyer and Palmer achieve their goal of successfully isolating HA from vitreous fluid. The backbone of HA, an abundant anionic linear polymer, is formed by the combination of D-glucuronic acid and N-acetyl-D-glucosamine. In the field of tissue engineering, natural polymers such as HA are often used because of the biocompatibility and biodegradability of these materials. Scientists just recently realised that HA might be used in tissue engineering scaffolds to regulate cell mobility and adhesion, speed up the healing process, and distribute medications. This discovery was made over twenty years ago. Motor receptors such as CD44 bind HA as part of a pathway that is mediated by HA (RHAMM). When cancer cells take up CD44-HA, the expression of CD44 is lowered, which makes it possible for anticancer drugs to penetrate the cells with greater efficiency [40]. Esterified HA derivatives, such as ethyl/benzyl esters (HYAFF®), and cross-linked HA gels, both of which have been extensively researched as wound dressings, are just two examples of the many biomedical products that are based on HA derivatives. HA derivatives are also used in a wide variety of other applications. Mucoadhesive HA solutions, such as SYNVISC® and ORTHOVISC®, may reduce pain and restore mobility in arthritic joints by acting as synovial fluid replacement therapies for osteoarthritis patients. AMVISC® vitreous fluid replacement made from HA assures the safety of delicate eye tissue when it is put to use during ocular surgery. Using Hyalomatrix® (Anika treatment) as a dermal replacement for major surgical wounds has been hypothesised to have the potential to promote capillary development, fibroblast attraction, and wound healing [13, 41].

Alginate

Alginate is a kind of anionic polymer that may be found naturally occurring in the cellular walls and intercellular gaps of some types of algae. It is also known by the name sodium alginate. Non-Newtonian aqueous solutions are characteristic of glycosaminoglycans and also of alginate, a block copolymer of two glycuronates. It has low cost, low toxicity, biocompatibility, lack of immunogenicity, ease of gelation, and regulated biodegradability, alginate has several potential applications in the biomedical industry. Soft alginate gels may encapsulate chemicals or even living beings without damaging them. The implantation of alginate capsules containing pancreatic cells into people first occurred in the 1980s. Two examples of alginate derivatives that are used in the therapy of infected wounds, allograft wound dressings, and pressure injuries are called Kaltostat® and AlgiSite® respectively [41–43]. Bioactive substances and cells may be transported through alginate hydrogels, making them a promising scaffold for tissue regeneration. Proteins and bioactive chemicals are more easily targeted by alginate gels, making them a promising therapeutic option.

Synthetic Renewable Polymers

It is possible to create renewable synthetic polymers using biological or chemical processes [44]. Natural chemicals or macromolecules are used to create the monomers for this polymer (such as sugar, starch, cellulose, proteins, etc.). Their carbon footprints are less than those of polymers made from fossil fuels since they are produced via photosynthesis or microbial CO_2 fixing. In order for a substance to significantly add to the greenhouse effect, it must see widespread use. The possibility for decarbonization has attracted the greatest attention and research into synthetic renewable polymers like polyhydroxyalkanoates and polylactic acid -related polymers.

Poly Hydroxylalkanoates

Bacterial PHAs, a kind of intracellular polyester (Fig. 4), have evolved into a biosynthetic renewable polymer in recent decades. PHAs are the biggest class of biopolymers, and they are biosynthesized by genetically engineered bacteria. PHAs are capable of being biosynthesized by live cells from a wide range of various nutrients, such as sucrose generated from sugar cane or sugar beets, glucose derived from maize starch, vegetable fats (such as bean, palms, grain, etc.), and bovine fats. It has been shown that the presence of PHAs in surgical sutures, drug administration systems, substrates, and transplants encourages the growth of stem cells [1, 22, 45] due to their biocompatibility, biodegradability, and availability.

Polylactic Acid

Biomedical applications of PLA, a synthetic renewable polymer (Figure 5), are many. Carbohydrates like maltose, sucrose, lactose, etc. are fermented to produce PLA. The structure of stereoisomeric PLA has an effect on its performance. Different from the amorphous PDLLA and meso-PLA, the semi-crystalline PDLA and PLLA are a useful material [46].

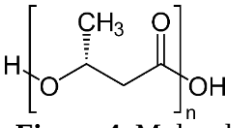

Figure 4. Molecular form of PHAs.

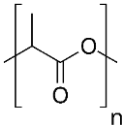

Figure 5. Molecular form of PLAs.

Despite its intricate molecular stereo structure, polylactic acid (PLA) has excellent chemical, mechanical, biocompatibility, and biodegradability properties. Surgical clips, sutures, adhesives for wound closure, bone staples and plates, regenerative scaffolds, drug delivery systems, medical equipment, packaging, and more are just few examples of the wide range of biomedical items that may be manufactured from PLA. PDLLA is great for coatings in orthopaedic biomedical applications since it is both biocompatible and mechanically stable. Antibiotics and other medications with a low molecular weight may be localised using PDLLA. PLLA's safety, biocompatibility, and biodegradability make it an attractive candidate for use in cardiac tissue [16, 25, 47, 48].

CO_2-based Polymers

Polymers that can repair CO_2 are quite promising. Using catalysts for ring-opening copolymerization (ROCP), polymers may be synthesised from carbon dioxide (CO^2) [33]. This procedure generates mostly aliphatic polycarbonates, which have the potential to replace petroleum plastics in applications such as medical and food packaging, agricultural films, trash bags, etc. Poly (ether carbonate) polyols might be made from CO2-based polymers with a low molecular weight. Using Bio based monomers from poly (ether carbonate) polyols in polyurethane manufacture would reduce emissions by 20%. Every year, 3–4 Mt of polyols are required for polyurethane manufacture. Poly (ether carbonate) polyols of CO_2-based polymers are a potential replacement for polyether polyols in the context of decarbonisation [49].

Poly Propylene Carbonate

Alternating carbon dioxide and propylene oxide copolymerization produces PPC (Figure 6). Cheap propylene oxide and CO_2 make PPC one of the most promising eco-friendly synthetic polymers. Biomedical product-friendly PPC is non-toxic, non-polluting, transparent, renewable, degradable, barrier, flexible, and electrically conductive. PPC's dimensional instability and low glass transition temperature restrict its employment in high-temperature applications (Tg). Water and CO_2, PPC breakdown products in vivo, decrease inflammation and protect PLA and PLGA. PPC scaffold tissue engineering advantages. Another experiment developed PLA-TCP-PPC polymer scaffold PPTE. PPTE composite-inoculated mouse osteoblasts adhered, proliferated, and calcified. Bone healing was regulated by PPTE composites [46, 47]. PPC delays and transports medicines [22, 34]. PPC's strong

water barrier and low gas permeability boost antibacterial action. PPC helps medical packaging, dressings, and devices. Bone fixation, surgical sutures, and polymer "additives" may use PPC [48]. PPC and its nanocomposites—the best CO_2-based polymers for biological applications—need additional study.

Poly Cyclohexene Carbonate

Another CO_2-based polymer that has received a lot of attention is PCHC (Figure 7). The six-member ring structure of PCHC makes it more efficient at retaining heat than PPC. It has a Tg that is higher than 100 degrees Celsius. A novel epoxide/CO_2 copolymer that can withstand extreme heat. The biodegradability of PCHC molecules is diminished due to the presence of hydrophobic cyclohexane groups throughout the molecular chain. Incorporating PCHC becomes more challenging due to these characteristics. PCHC composites have been the subject of substantial research for their potential use in drug-loaded nanoparticles, hydrogels, pore-forming chemicals, biodegradable surfactants, and regenerative scaffolds [29, 48].

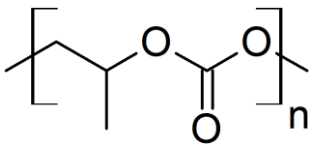

Figure 6. Molecular form of Poly propylene carbonates.

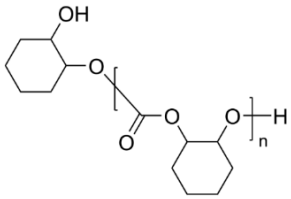

Figure 7. Molecular form of Poly cyclohexene carbonate.

Poly Ethylene Carbonate

The well-studied fatty acid polycarbonate poly ethylene carbonate (Figure 8) is produced via reductive organic carbonation polymerization (ROCP) of ethylene oxide and carbon dioxide, and its surface erodes in vitro and in vivo. Thus, PEC-based delivery systems' drug-release behaviour is intimately linked with polymer mass loss, enhancing predictability. PEC is easily degraded by cells and enzymes. If successful, this strategy might enhance medicine delivery in the body. Microorganism growth was suppressed by rifampicin released by PLA microparticles. In vitro, macrophages caused degradation of PEC surfaces. Surface degradation caused by macrophages reduced the breakdown of 41-kDa PEC. Methods based on PECs disperse medicines that stimulate macrophages [12]. Long-term medication administration is not feasible since PEC degrades in two to four weeks [50]. Degradation of PECs led to the development of controlled-release, extended-release medication delivery devices.

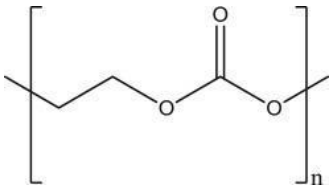

Figure 8. Molecular form of Poly ethylene carbonate.

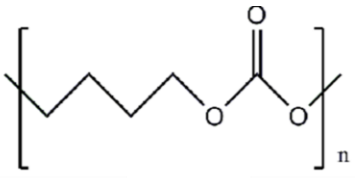

Figure 9. Molecular form of Poly butyl carbonate.

Poly Butyl Carbonate

The combination of epoxy butane with CO_2 monomers gives PBC remarkable elasticity, tensile strength, elongation at break of more than 400%, and a glass transition temperature (Tg) of 32°C. Despite being biocompatible, PBC (Figure 9) is very unstable and melts at about 55 degrees Celsius. Potentially higher grade compounds may be made from PBC by blending polymers [51–53]. Breakdown of PBC nanofiber membranes. Hydrophilic, antibacterial, and mechanical PBC/PLA/chitosan nanofiber membranes produced by electrostatic spinning. Axial electrostatic

spinning was used to create core-shell PVA/PBC nanofibers for doxorubicin administration (DOX). The results that DOX-loaded PVA/PBC core-shell nanofibers suppressed SKOV3 ovarian cell adhesion and proliferation have implications for tissue engineering and chemotherapeutic applications. PBC has the potential to affect drug development. By combining PBC, PLA, and GO, Gu et al. (2018) created nanofibers with anti-tumor properties. The use of a PBC/PLA/GO nanofiber matrix for cell imaging and drug transfer was successful, demonstrating the matrix's biological potential. Incorporating PBC into biodegradable polymers improves their mechanical and chemical properties [54–56]. Biodegradable polycarbonate PBC needs further research and implementation.

CONCLUSION

This investigation explores state-of-the-art CO_2-fixing polymer nanocomposites, which might be used to minimise the environmental effect of biomedical polymers. CO_2 fixing results in the creation of polymers, which may be found in nature or engineered in a lab. Biomaterial, non-cytotoxic, recyclable, and environmentally beneficial, nanomaterials of renewable polymers created by CO_2 fixation are used by numerous researchers to build novel biomedical materials. Research in this area focuses mostly on tissue engineering, medication delivery, cancer treatment, and organ transplantation. These businesses have a smaller carbon impact since they seldom employ biological resources. Researchers have overlooked the fact that many disposable biomedical items are constructed from petroleum-based polymers. The single-use items pollute biopolymers. Therefore, it is critical to develop CO_2-fixation-based repeating polymers for usage in short-term medical devices.

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Does Egg Clutch Size Determine the Abundance of Indian Golden Gecko *Calodactylodes aureus* (Beddome 1870)? An Example from SPSR Nellore District, Andhra Pradesh, India

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Abstract: The Indian Golden Gecko *Calodactylodes aureus* is an endangered, Scheduled I geckonid lizard. It is endemic to Peninsular India. It is rediscovered by many scientists in the recent past, redefining its distribution and raising lot of concerns of conservation issues. In this backdrop, we undertook a survey to record its presence in SPSR Nellore district, Andhra Pradesh, India. Following line transect method, the distribution is recorded, and we tried to relate the abundance of the animal to communal egg clutch size. 15 habitats were identified. It is observed that population size has a positive correlation to egg clutch size. Since egg clutches are prone to various threats, it is strongly suggested to initiate conservation measures.

Keywords: Indian Golden Gecko, Endangered animal, Line Transect Method, Calodactylodes

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Introduction

Many of the primitive and nocturnal Geckonid lizards are mainly confined to tropical countries (Daniel, 1983). The Indian Golden Gecko, *Calodactylodes aureus,* is notified as endangered animal as per the Indian Wildlife Act, 1972. It is one of the least appreciated primitive lizards among the South Asian Herpetofauna (Deraniyagala, 1953). It has been consistently

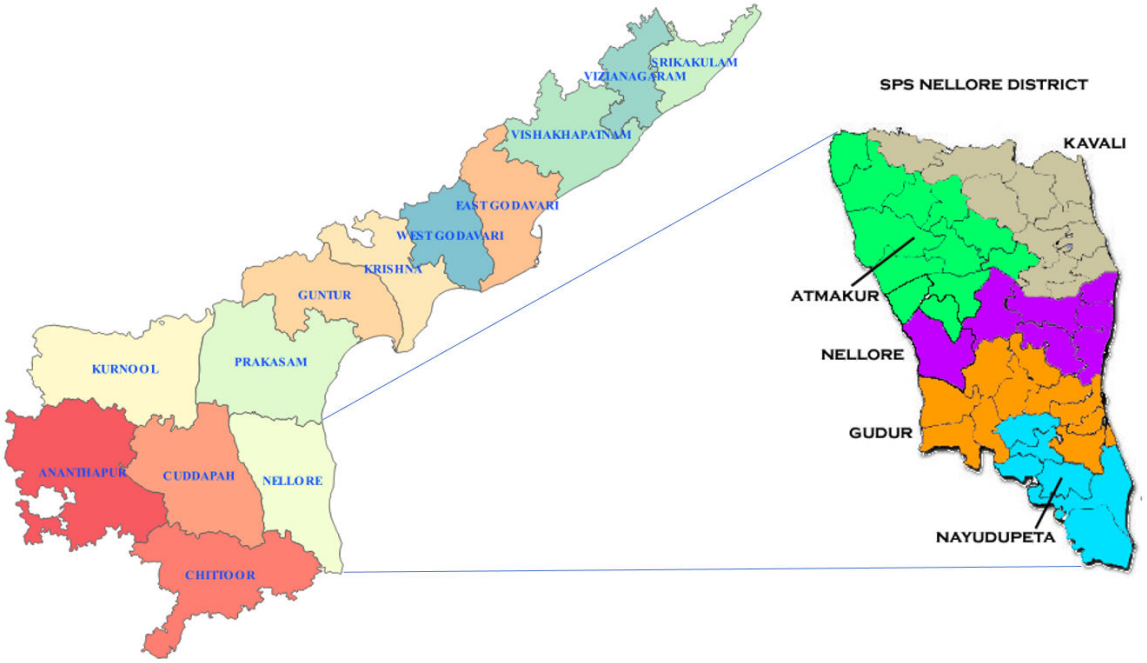

Fig. 1: Map showing SPSR Nellore District.

enlisted in various checklists (Boulinger, 1885; Wermuth, 1965; Kluge, 1993; Dilip Venugopal, 2010). Its biology is largely unknown (Aaron and Das, 2000). First reported by Beddome in 1870, it was thought to have been extinct, but later on observed in 1986 (Daniel *et al*., 1986). Nandakumar *et al*. (2000) reported this animal from Seshachalm Biosphere Reserve. It is being cited along the East coast of India (Dutta *et al*., 2005; Rajaskhar and Nandakumar, 2007; Javed *et al*., 2007; Basundhara Chettri and Bhupathy, 2010; Sreekar *et al*., 2010; Rajasekahar and Reddy, 2010; Amaranath Reddy *et al*., 2012, 2013) and many new locations of its habitat were reported by Rajasekhar and Chakrapani (2012). Being a Gondwanian relic (Boulinger, 1885), Golden Gecko is of much interest. The present study is taken up in SPSR Nellore dist, Andhra Pradesh, India. It aims at correlating the animals abundance to its communal egg clutch size.

Materials and Methods

Study area:

Study area comprised parts of SPSR Nellore district of Andhra Pradesh (Fig. 1), surved between July 2011 and June 2013. Nellore District is located at the Southern most end of Andhra Pradesh, India. It lies between13^{0}30' and 15^{0}6' of the Northern latitude and 70^{0}5' and 80^{0}15' of the Eastern Longitude. Veligonda hills form the western border of the district. Along the coast, fertile lands are present and on the western side of the district has low westward elevation and has shrub jungles and rocky plains. Maximum temperature ranges between 40-45 ℃ during May and minimum temperature ranges from 17-30 ℃ during December.

Direct counting method was followed and conventional distance sampling method was adopted for this survey. The expanded phalanges (Rajasekhar and Chakrapani, 2012) with large trapezoidal penultimate and distal expansions are the key characters to identify this Indian Golden Gecko (Smith, 1935). Male and female can be identified by colour. Opportunistic searches were conducted among rock boulders. These were carried out over a vast area. Vertical and horizontal crevices were intensely searched. During day light searches, presence of communal egg patch and morphological correlates of the animal were recorded. Where the density of golden geckos is assumed to be high enough,

nocturnal search was conducted using line transects. Before attempting, one transect line for every 5 meters was drawn in the day time. Survey was conducted after dusk, with the help of 6V spotlights and hand torches. Morphometric details were collected as per the method given by Daniel *et al.*, (1986). Habitat analysis was done as per Kotwal (1982). Sympatric reptiles were identified according to the keys provided by Smith (1935) and Aaron and Das (2000).

Animal Description:

By noosing, a male golden gecko was captured (Fig. 2). It was observed for morphological and morphometric characters and released safely into the habitat, Sri Penusila Lakshmi Narasimha Wildlife Sanctuary (Rajasekhar and Chakrapani, 2012). Characterized by flat body, proportionately big head and oval neck, Golden gecko has a dorsal surface covered by small granules and tubercles. Squarish scales are present on the ventral surface (Smith, 1935). Long slender tail, round supra orbital, presence of canthal ridge, vertical pupil, long snout are observed. Canthal ridge has slightly larger granules (Chakrapani *et al*., 2014). Digits have large trapezoidal penultimate and distal expansions. Each expansion is grooved and has a retractile claw (Daniel *et al*., 1986). Golden gecko efficiently camouflages its surroundings. Dorso-ventral side of males is golden yellow (De Silva, 1995) and females have mixed colours. It has a peculiar habit of depositing eggs in communal patches, sticking the eggs to the roof of rock boulders (Fig. 3).

Results and Discussion

Unlike the Western Ghats, the Eastern Ghats are a chain of discontinuous row of hills and hillocks, running from North to South between coastal plains and plateau. This may be the reason for the discontinuous distribution of Golden Geckos in these rocky terrains. Normally these locations are known for semi-arid to arid climate. But, SPSR Nellore being a coastal district, has humid to sub-humid climate. As a result, SPSR Nellore district, experiences different temperatures, humidity and rainfall (Hoe *et al*., 1986). The study locations differ geologically also, as the geological formations range from Dharwar schists to the recent alluvium. These contain rich minerals.

Geologically and meteorologically Nellore district differs considerably and hence we focussed our attention on the habitat and the population of Golden geckos in the study locations. As a result, work has been carried out to see the correlation between abundance of Golden geckos and selected climatic parameters of rock boulder habitat.

In SPSR Nellore district covering 5.68 km^2, golden geckos were found in 15 new locations. Humidity range was between 76% and 100%. Temperature varied between 22°C and 41°C in those habitats where golden geckos were observed. Rock Boulder height varied between 1.2 m and 2.8 m. Egg clutch were found in all the 15 of the study locations. Number of eggs in one egg patch ranged from 38 in the smallest to 130 in the largest. Excreta were found in 10 locations surveyed. A total of 104 were found in the present study. The number of animals in each location ranged from 4 to 16. Consolidated survey results are given in Table 1.

Golden gecko population is more where the observed eggclutch is of larger size. In all the 15 locations surveyed in Nellore district, egg clutch was found. The number of eggs in each egg patch ranged from 38 to 130. When the egg patch size was more, naturally more animals were found in locations like Konamalleswaram. Egg patch composition in each of the study locations in SPSR Nellore district is presented in Table 2 and Figure 4.

As in the case of study in Chittoor district, a positive correlation between egg patch size and animal abundance and vice-versa could be found (Fig. 5). Karl Pearson coefficient of correlation indicates a highly positive relation between egg patch size and animal abundance ($r = 0.8475$).

Results of the t-test showed that egg clutch size is a significant factor in determining the

Fig. 2: *Calodactylodes aureus*, displaying golden yellow color, nostril, canthal ridge, vertical pupil, labials, dorsal tubercles and ear opening prominent. Photograph at Chakrateerdham of Tirumala Hills.

Fig. 3: Communal egg clutch of Calodactylodes – (a) freshly laid eggs and (b) an old scar of the egg clutch.

population size in the study locations.

As per the formula, $\left|t_c\right| = \left|\frac{r-\rho_0}{\sqrt{n-2}}\right|$

Where r = 0.8475

t tab at 5%= t n-2 =t 15-2

5%=1.771

$$\left|t_c\right| = \left|\frac{r-\rho_0}{\sqrt{n-2}}\right| = \left|\frac{0.8475-0}{\sqrt{15-2}}\right| = 0.2351$$

Since t_c is less than $t_{tab,}$ the assumption that the egg clutch size is a significant factor in determining population size holds good.

To draw definite conclusion from this survey in SPSR Nellore district, the mean values of selected parameters were taken into consideration. We predicted Golden gecko populations to exist in considerable numbers.

In conclusion, we tried to estimate Golden gecko population in the entire rocky terrain of Nellore district, based on our field observations. The details of population density estimates in the study locations are given in Table 3.

Population density of Golden geckos in SPSR Nellore district was 18.29/km^2. It is noteworthy that, though the absolute number of Golden geckos

Table 1: Consolidated Survey results showing abundance of Golden geckos in relation to climatic variables SPSR Nellore District

S. No.	Location	Temperature (℃)	Humidity %	Egg patch size	Height of the Rock Boulder (m)	Number of animals
1	Vempalli toka	28	100	50	1.5	4
2	Udayagiri	25	100	64	1.5	6
3	Somasila	30	100	42	1.4	4
4	Panchalingalakona	30	100	44	1.5	5
5	Mallemkonda	32	98	68	1.4	14
6	Penchalakona	33	98	100	2.3	12
7	Bhairavakona	28	100	80	2.6	13
8	Akilavalasa	41	87	42	1.6	4
9	Sidduleswarakona	23	92	40	1.8	4
10	Dattanagaram	40	82	45	1.6	6
11	Attalasiddavaram	28	78	60	1.3	4
12	Stambhalakona	24	83	48	1.2	3
13	Konamalleswaram	22	100	130	2.8	16
14	Varadanapalli	27	88	38	1.5	5
15	Paravolu	24	76	60	1.8	4

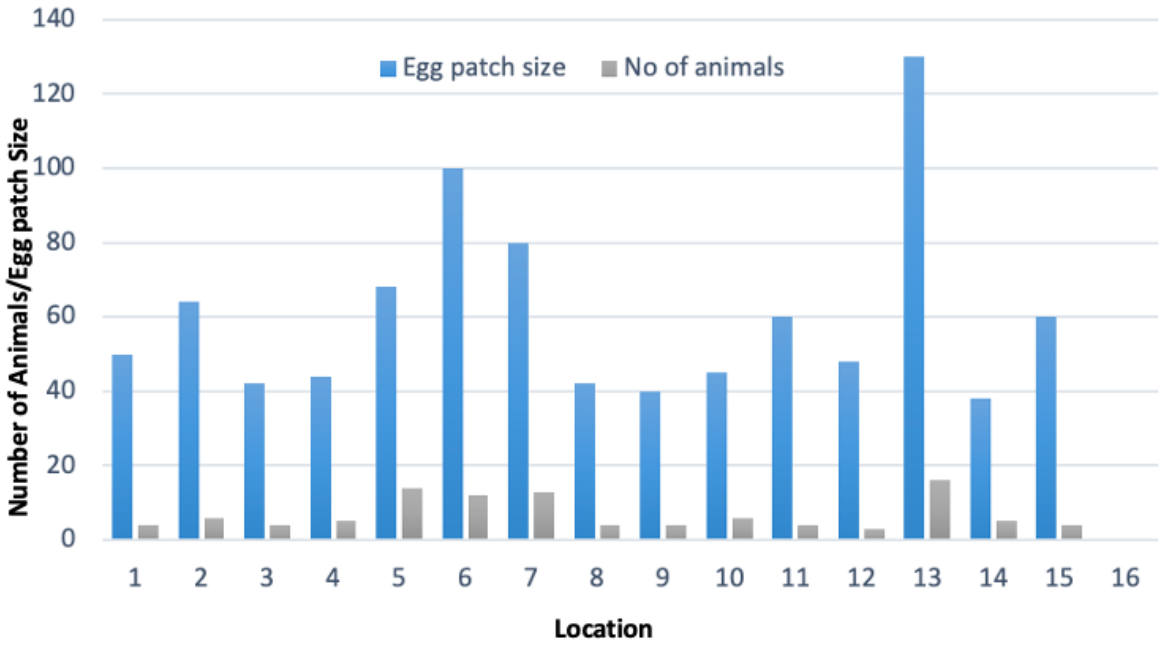

Fig. 4: Animal abundance in relation to egg clutch size in SPSR Nellore District.

Table 2: Communal egg clutch composition and animal abundance in SPSR Nellore district

S. No.	Location	No. of egg scars observed	Number of eggs observed				Number of animals
			Hatched	Unhatched	Spoiled	Total	
1	Vempallitoka	2	20	18	12	50	4
2	Udayagiri	1	15	30	19	64	6
3	Somasila	4	6	16	20	42	4
4	Panchalingalakona	3	12	8	24	44	5
5	Mallemkonda	2	22	20	26	68	14
6	Penchalakona	6	42	38	20	100	12
7	Bhairavakona	4	34	30	16	80	13
8	Akilavalasa	2	12	10	20	42	4
9	Sidduleswarakona	3	8	16	16	40	4
10	Dattanagaram	2	20	5	20	45	6
11	Attalasiddavaram	1	15	20	25	60	4
12	Stambhalakona	6	20	4	24	48	3
13	Konamalleswaram	8	54	36	40	130	16
14	Varadanapalli	1	16	10	12	38	5
15	Paravolu	0	20	14	26	60	4

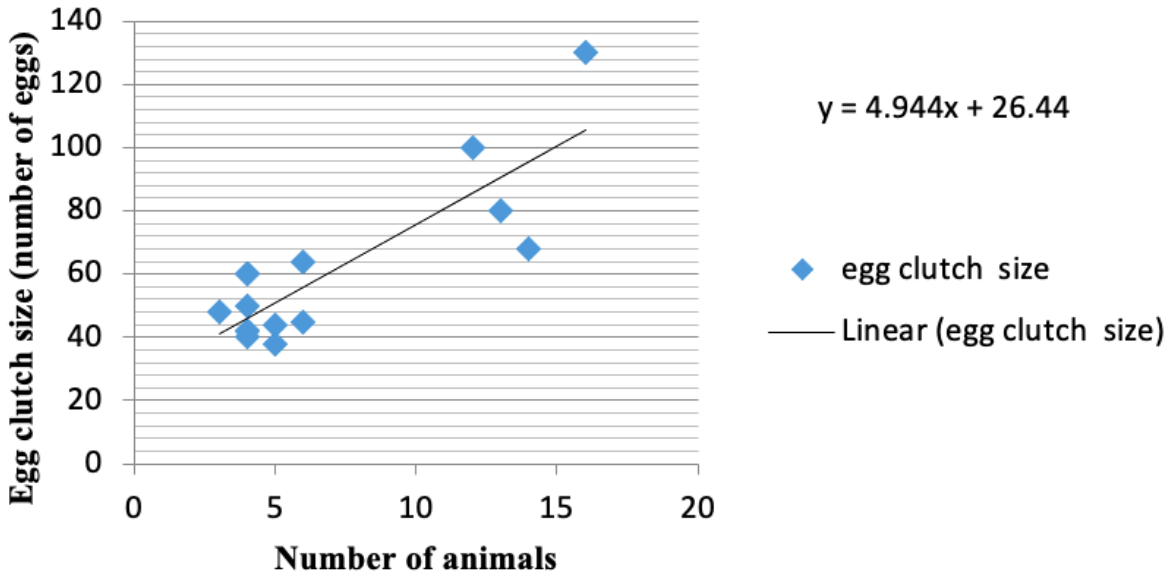

Fig. 5: Egg clutch size in relation to number of animals present in SPSR Nellore district.

Table 3: Population Density of the Indian Golden Gecko *Calodactyldoes aureus* in the study locations of SPSR Nellore District

S. No.	Location	Area (m^2)	Number of Golden Geckos Present	Population Density (number of animals/km^2)	Standard Deviation
1	Vempallitoka	105.55	4	0.1429	0.378
2	Udayagiri	563.11	6	0.142	0.377
3	Somasila	211.1	4	0.1262	0.3552
4	Panchalingalakona	492.87	5	0.0892	0.2986
5	Mallemkonda	422.39	14	0.25	0.5
6	Penchalakona	1320.61	12	1.1713	1.0822
7	Bhairavakona	880.3	13	0.1711	0.4163
8	Akilavalasa	211.1	4	0.0759	0.2754
9	Sidduleswarakona	105.64	4	0.1428	0.3778
10	Dattanagaram	105.64	6	0.142	0.3768
11	Attalasiddavaram	140.89	4	0.1428	0.3776
12	Stambhalakona	105.64	3	0.1423	0.3772
13	Konamalleswaram	563.11	16	0.3788	0.6154
14	Varadanapalli	351.91	5	0.0892	0.2986
15	Paravolu	105.64	4	0.1138	0.3373

is less, expected population density is high in SPSR Nellore district in the observed study locations. Given the general observation that SPSR Nellore district experiences heavy down pour of rain due to frequent cyclones, average humidity and mean temperatures of the district is more. Due to this reason, forest cover may be thick, which in turn would affect population of insects, which is the main prey for Golden geckos. This would have an indirect positive effect on the reproductive capacity of individuals. Hence, the limited rocky terrain of SPSR Nellore district might support rich populations.

We calculated the estimated population density of Golden geckos in the entire district. The expected population density in SPSR Nellore district is 151.84/km^2. In the estimation of any population density, the parameter follows negative binomial distribution where the mean is always less than the variance (Table 3). This implies that the actual population density may be less, because entire rocky terrain cannot be expected to be suitable habitat. As mentioned previously, many deeper pockets of forest areas are not accessible due to limitations of administrative restrictions imposed in view of red sanders smuggling and other illicit trade in forests. Keeping these findings in view, we suggest an intensive survey to be conducted in the rocky terrains of SPSR Nellore district, with the active help of all the stake holders to elucidate, protect and conserve Golden gecko habitat.

As the existence of Golden gecko has been largely unknown until its discovery by Beddome (1870), the Indian government has declared this animal as endangered and included it in Schedule I of the Wildlife Act, 1972. Issues like anthropogenic interference, loss and fragmentation of habitat, global warming etc., are threatening Golden geckos' existence (Chakrapani *et al*., 2014). The reduction of gecko population would likely to have repercussions on the integrity of food chains, thereby affecting the food web. Golden geckos are prey animals for many reptiles and birds, while

Table 4: Seasonal variation in Golden gecko population at specified locations

S. No.	Location	No. of Golden Geckos observed		
		Rainy season	Winter	Summer
1	Mallemkonda	22	16	14
2	Penchalakona	18	16	12
3	Konamalleswaram	24	18	16

simultaneously being important predators of insects (Knowlton George, 1938). Many scientists have reported drastic decline and extinction of amphibian and reptiles around the globe. These extinctions are attributed mostly to the spread of fungal diseases, environmental change and climate change, especially the global warming (Sinervo *et al*., 2010). The present study also emphasizes the importance of the temperature and humidity on the Golden gecko population. Many studies are emphasizing that the climate warming is the major cause of extinction of lizards (Sinervo *et al*., 2010; Bharati *et al*., 2017). We also observed greater number of golden geckos in rainy and winter seasons and less number during summer at three specified locations in SPSR Nellore district. We visited Golden gecko habitats three times successively every week in August, December and May. The mean number of animals noticed was observed, and we find less number of animals during summer (Table 4).

This finding corroborates with the results of many researchers. If temperature continues to raise as in the present scale, most of the geckos, existing right now, will become extinct by 2080 (Sinervo *et al*., 2010). Hence, we suggest that there is an urgent need to initiate measures for conservation of this precious animal.

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New record of *Dictyota bartayresiana*, a marine brown algal species revealed from Rich Seaweed Diversity Area of South India

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Keywords

Macroalgal species, Phylogenetic analysis, Indian coastal region, *D.bartayresiana* , rbcl gene sequencing.

Abstract

In the current paper, *Dictyota bartayresianais* account for the earliest time, a novel record for the Indian macroalgal flora. A present species of Dictyota, previously recognized as *D. bartayresiana*, is illustrated from southeast coast region of India. Morphological observation and phylogenetic analysis, as well to molecular sequence data of specimens collected from Mandabam and species were evaluated and facilitated us to authenticate that *D. bartayresiana*. There were 38 species of Dictyota species used for the comparison.

1. Introduction

Seaweed is found as an inhabitant in submerged oceanic waters. Based on their chemical composition and structure, they are classified as Chlorophytes, Rhodophytes, and Phaeophytes. Phaeophytes are mostly submerged in seawater, resulting in unicellular and multicellular architecture. Chlorophyll a and c, Beta carotene, Lutein, Fucoxanthin, and Dioanthin are some of the respiratory and phytochemical pigments.

Brown algae can tolerate adverse environmental conditions due to the presence of bioactive compounds and oxidizing capacity [1]. Secondary metabolites have contained excellent biological

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activity besides providing resistance against other organisms. As far as, more than 2400 seaweed bioactive compounds are identified that acting as blueprint for developing novel drugs. The bioagenic compounds be grouped as aldehydes, alcohols, halogenates and terpenoids that synthesized from algal species and acting as antibacterial, antifungal, antibiofouling agents [2, 3].

Seaweeds are definitely the most promising bioactive molecules and still paved the way of research and exploitation to be fully implemented in human health benefits. However, further studies are needed to evaluate the efficacy of the seaweed on medicinal purpose in pharmaceutical applications [4, 5].

Dictyota bartayresiana is rare seaweed that is multicellular and belongs to the Phylum Phaeophytes. *Dictyota bartayresiana* extracts contain essential phenolic compounds such as alkaloids, steroids, phenols, flavonoids, saponins, tannins, glycosides, and sugars, and it also aids in the scavenging of free radicals, as evidenced by in vitro studies, and it also acts as an antioxidant. The crude extract of *D. bartayresiana* has shown cytotoxicity and antibacterial activity against the HT29 cancer cell line as well as urinary pathogens [6]. Because of external morphological similarities, species limitations, and the need for analytical natures, the Dictyota family has a long and difficult taxonomic history.

Our indulgent of species range has been progressed, and the biogeographical variety of individual species has been purified, thanks to current research combining DNA-support species restricted techniques and characteristic morphological scrutiny. The molecular systematic aids to Dictyota organization may be useful in allocating taxonomists to improve recognizing of species diversity at the genus level [7, 8].

Species identification is important for some industries to invest in technology depends on the final product of algal extraction. The following industries are utilizing algal products such as Pharmaceuticals, food and agro industries. There have been various techniques employed for identification of algal species [9]. The rbcl gene sequencing is one among the method which targets chloroplast gene and facilitates the further phylogenetical studies. Amplification of rbcl gene can be easy than other techniques and that uniquely present in photosynthetic organisms [10].

This research uncovers new evidence of the rare Mandabam species *Dictyota bartayresiana*. A molecular phylogeny demonstrates the spot of *D. bartayresiana* presented the genus and in similarity to a closely relay genus, and the morphology, locality, and environments of the species are illustrated. They are the first rbcl gene sequences discovered in this careful species. These species were discovered over a century ago, but due to their rarity, they have yet to be investigated.

Dictyota bartayresiana are widely distributed in southeast coast area of India exclusively pudumadam, seeniappa darga, krusadai island, pamban and thonithurai and other areas including goa, okha, Lakshadweep, bhatkal, malvan. This species is distributed in the tropical western central Pacific Ocean and the tropical western Atlantic Ocean, as well as the Gulf of Mexico which occurs at depths of 108 m.

2. Methodology

Brown algae Dictyota bartayresiana prefers the submerged portion of the ocean column. It was collected for the experiment from Mandabam in Kanyakumari district (Figure 1) and washed in running tap water for 15 min before soaking to remove unwanted soil residues. Live algae are preserved in order to better understand their morphological structure and compare them to other species for future research.

Finally, distilled water is used to obtain purified seaweeds. The authentication code of our sample is BSI/SRC/5/23/2019/Tech/3070, provided by Botanical Survey of India, Govt of India.

3. Structure Analysis

External structure of *Dictyota bartayresiana* clearly indicates that internodal segments are less than 4 breadth long; upper sinuses broad, but sinuses usually narrower in other Dictyota family. The lamoroux are usually variable, intermingled masses form the bushy and dense structure. Stipes are not present, instead of stipes, holdfast helps the attachment to substratum by their irregularly

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shaped structure. The holdfast usually binds with rhizoids.

4. Polymerase Chain Reaction

DNA extraction was done by using Xpress DNA plant kit as per manufacturing protocol with minor modifications. Amplification of isolated genome carried out by PCR techniques. The 25 µl PCR reaction mixture were contains following components such as 1.5 units of Taq polymerase, 0.2µm of each dNTP and primer, 2mM $MgCl_2$ with reaction buffer (Applied Biosystems), 1µl of temFig DNA, final volume made with double distilled water. The universal primers of rbcl-68F [11] and rbcl-708R [12] were used and the PCR band size approximated to be 520bp.

PCR amplification was carried out through Applied Biosystem to accomplish 40 cycles following an early denaturation cycle at 95°C for 10 min. Every cycle comprised of a denaturation step for 30sec at 95°C subsequent to an annealing step for 20 sec at 50°C further extension step for 30 sec at 72°C. The final extension was continued for 15 mins at 72°C. The resulting product was resolved by electrophoresis technique in 1.5% agarose gel and purified with purification kit (Roche diagnostics). Sequencing was performed using ABI BigDye terminator (Applied Biosystems).

The evolutionary distances were calculated by means of the Kimura 2-parameter method. The sequences were analyzed by MEGA X package and submitted to gen bank [13]. The rbcL PCR primers were doing well to intensify and fabricate PCR product with anticipated band size (≈ 520 bp).

5. Results

Dictyota bartayresiana extended up to the height of 9 to 14 cm and thallus widens around 6 to 10 mm. In young stage of growth, thallus is branched but the further development which become bent and twisted for attaining typical structure of dichotomous in Indian coastal water. The dichotomus structure are equally angled the ranges 45^0-90^0, mostly that exhibits in 80^0. The thallus of *Dictyota bartayresiana* lamouroux have seems very soft, membranous and brittle nature which does not contain any midribs, 2-7mm broad [11].

The collected raw seaweed materials were washed thoroughly with seawater for removing sand particles and unwanted debris. Double distilled water used for washing the seaweeds to make germ free material which avoid bias during sequencing process. The resulting material was chopped into thin pieces and immersed with preservatives for further studies until which kept by vials and stored at -40^0C. Approximately 1050 bp of rbcl genes sequence was recovered for *Dictyota bartayresiana* seaweed. First fifteen sequences were selected from blast tool based on maximum score of identity and aligned using multiple alignment software program ClustalW [11].

The rbcl sequences of dictyota species shared similarity score higher than 93% with sequences of *Dictyota bartayresiana* in genbank database which is previously sequenced from Canary Islands (GQ425107.1). Hence, it has been concluded that the seaweeds collected in present study is *Dictyota bartayresiana*. The sequence were uploaded in NCBI and got accession number: MT015586 for further reference (Table 1).

Figure 1: A map published on Aquamaps, showing the Distribution range colours indicate degree of suitability of habitat which can be interpreted as probabilities of occurrence.
(Source:https://www.aquamaps.org/imagethumb/cached_maps/pointmap/pic_SLB-131834.jpg)

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Figure 2: Habit of *Dictyota bartayresiana*

Figure 3: Base pair of rbcl gene sequence alignment through software program

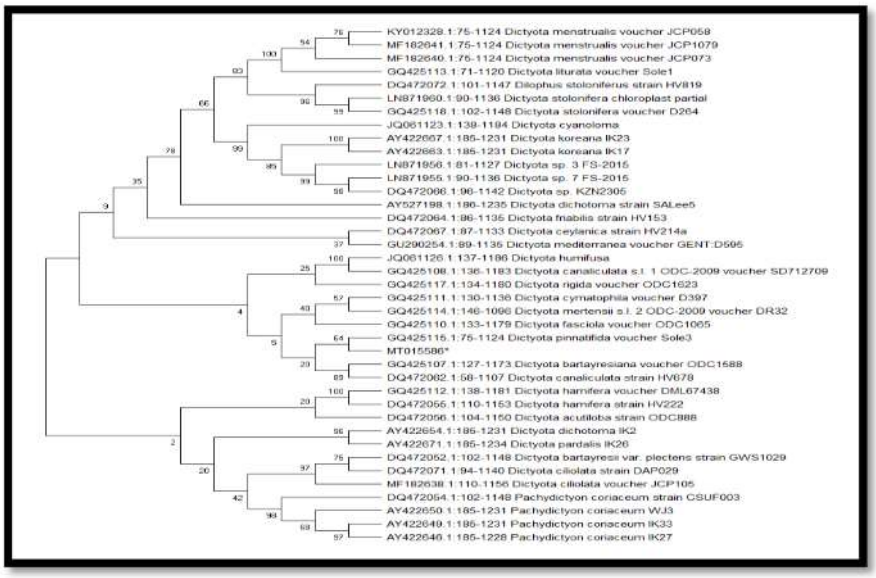

Figure 4: Bayesian consensus tree for the alignment of rbcL sequences of brown algal species with bootstrap values for Maximum Parsimony (1000), Neighbour Joining (1000) analyses overlaid on the branches.

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Table 1: The sequences used in the concatenated alignment are listed with their Gen Bank accession numbers

Species	Rbcl gene sequence
Dictyota menstrualis	KY012328
Dictyota menstrualis	MF182641
Dictyota menstrualis	MF182640
Dictyota liturata	GQ425113
Dictyota stolonifera	DQ472072
Dictyota stolonifera	LN871960
Dictyota stolonifera	GQ425118
Dictyota cyanoloma	JQ061123
Dictyota koreana	AY422667
Dictyota koreana	AY422663
Dictyota sp.	LN871956
Dictyota sp.	LN871955
Dictyota sp.	DQ472066
Dictyota dichotoma	AY527198
Dictyota friabilis	DQ472064
Dictyota ceylanica	DQ472067
Dictyota mediterranea	GU290254
Dictyota humifusa	JQ061126
Dictyota canaliculata	GQ425108
Dictyota rigida	GQ425117
Dictyota cymatophila	GQ425111
Dictyota mertensii	GQ425114
Dictyota fasciola	GQ425110
Dictyota pinnatifida	GQ425115
Dictyota bartayresiana	GQ425107
Dictyota canaliculata	DQ472062
Dictyota hamifera	GQ425112
Dictyota hamifera	DQ472055
Dictyota hamifera	GQ425112
Dictyota hamifera	DQ472055
Dictyota acutiloba	DQ472056
Dictyota dichotoma	AY422654
Dictyota pardalis	AY422671
Dictyota bartayresiana	DQ472052
Dictyota ciliolata	DQ472071
Dictyota ciliolata	MF182638
Pachydictyon conaceum	DQ472054
Pachydictyon conaceum	AY422650
Pachydictyon conaceum	AY422649
Pachydictyon conaceum	AY422646

6. Discussion

In order to strengthen the results, phylogenetic analysis was included in the present study. The evolutionary history was inferred using the Neighbor-Joining method [14]. The bootstrap consensus tree inferred from 100 replicates is taken to signify the evolutionary account of the taxa analyzed. Branches corresponding to dividers reproduced in fewer than 50% bootstrap imitates are collapsed. The percentage of replicate trees in which the associated taxa gathered together in the bootstrap test (100 replicates) are revealed after that to the branches. The evolutionary remoteness was figured by the Kimura 2-parameter method and in the components of the numeral of base replacements per site. This study occupied 39 nucleotide sequences [15]. Codon spots comprised were 1st+2nd+3rd+Noncoding sequence. All indefinite spots were eliminated for every sequence pair (pairwise deletion option). There were a whole of 1508 spots in the concluding dataset. Evolutionary analyses were carried out in MEGA X which shows in Figure 2, 3 and 4 [13].

7. Conclusion

The present study concluded that rbcl gene sequences useful to understand the family and genus level relationship with evolutionary lineage of seaweed. The molecular identification of brown algal species was performed by using rbcl as marker gene. Phylogenetic analysis with aligned rbcl sequences were done by neighbor joining method. Our investigations give you an idea about the rare species of *Dictyota bartayresiana* (Indian Ocean), its evolutionary significance, and the locality was not yet completely recognized by previous researcher. There is need to explore the species of *Dictyota bartayresiana* occurring in Indian sea coastal region using combined morphological and molecular approaches.

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Conflict Of Interest

Authors declare no conflict of interests.

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Biological and antimicrobial activity

EXPLORING THE BIOACTIVE POTENTIAL, ANTIMICROBIAL PROPERTIES AND TOXICITY OF PEPTIDES AND PROTEINS FROM *Nigella sativa* FOR DRUG DISCOVERY

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ABSTRACT

Nigella sativa, a medicinal plant with a long history of traditional use, is renowned for its wide range of pharmacological properties. Peptides and proteins extracted from *Nigella sativa* have shown to possess various bioactive potentials including antimicrobial activity, anticancer and anti-inflammatory, properties. This study aims to explore the bioactive potential, antimicrobial properties, and toxicity of peptides and proteins extracted from *Nigella sativa* for drug discovery. The peptides and proteins are extracted using various techniques including solvent extraction, enzymatic hydrolysis, and size exclusion chromatography. The extracted peptides and proteins are screened for their bioactive potential using *in vitro* assays such as cell viability assays, enzyme

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inhibition assays, and cytokine release assays. The antimicrobial properties of the peptides and proteins are evaluated using minimum inhibitory concentration (MIC) assays against various bacteria and fungi. The toxicity of the peptides and proteins is assessed using *in vitro* and *in vivo* assays. The results potentially contribute to the development of novel drugs from *Nigella sativa* peptides and proteins.

Keywords: *Nigella sativa*, peptides and proteins, antimicrobial activity, anti-inflammatory properties, toxicity of peptides.

AIMS AND BACKGROUND

The bioactive potential, antimicrobial properties, and toxicity of peptides and proteins are important for drug discovery because they can provide a source of novel compounds that may have therapeutic potential. Peptides and proteins are naturally occurring molecules that can be isolated from plants, and have diverse biological activities. They interact with specific receptors and enzymes, and modulate various biological processes, including immune responses, cell signaling, and metabolic pathways. *Nigella sativa* is a plant that has been traditionally used for its medicinal properties in many cultures. *Nigella sativa* seeds contain various bioactive compounds, including proteins, peptides, and essential oils, with wide range of pharmacological properties. Studies have shown that peptides and proteins from *Nigella sativa* possess antimicrobial activities in contradiction of various microorganisms. The major active ingredient of the medicinal plant *Nigella sativa* L. was identified as thymoquinone (TQ), and it was discovered that its use in nanoscaffold delivery systems increased therapeutic potential[1]. It was discovered, enriched, and tested against pathogens and spoilage bacteria that the wheat gene TdLTP4 encodes an antifungal protein[2]. Pro10-1D, a new powerful peptide antibiotic, was discovered to have strong antibacterial action against gram-negative bacteria[3]. Bioactive peptides from *Acacia catechu* extract were discovered to have strong anti-DENV action. These peptides were obtained from Thai medicinal herbs[4]. Researchers have discovered novel antimicrobial peptides (AMPs) derived from pine needles that exhibit potent activity against bacteria responsible for causing foodborne illnesses[5]. High antioxidant and free radical-scavenging activity were discovered in the extracts from *Lantana camara*'s leaves and flowers[6]. Enzymatic hydrolysis was discovered to dramatically cleave proteins in crab leg muscle hydrolysates and create peptides that may have health effects[7]. Using in-silico experiments, the research determined the toxicity of bioactive compounds extracted from two species of the genus Morus of glucosamine-6-phosphate synthase and its relations in binding with enzyme. It also evaluated the antibacterial activities of these compounds against certain pathogens[8]. Volatile oils of Fennel and cumin were shown to dramatically reduce the activation of human neutrophils after being evaluated for several biological activities, in a study of six herbs used often in the Mediterranean diet[9]. The volatile and aromatic chemicals of sweet orange, cumin, and allspice essential oils discovered as possible bioactive molecules for treating chronic disorders with underlying inflammation[10]. From laba

garlic, three antimicrobial peptide fractions were extracted, identified, and their possible antibacterial mechanisms were examined[11]. An anti-inflammatory peptide with strong denaturation inhibition and membrane protection action was isolated, purified, and identified using marine crab body muscle[12]. Antifungal peptide of MCh-AMP1 was isolated, sequenced, and discovered to show antifungal efficacy against Candida and Aspergillus species[13]. At the cellular and molecular levels of synthetic action, the mechanism against *Candida albicans* by MCh-AMP1 was examined using methods such as electron microscopy, potassium ion leakage, ROS generation, and plasma membrane permeabilisation experiments[14]. Increased plasma membrane permeabilisation, ROS generation, and potassium ion leakage were all signs that MCh-AMP1 had dose-dependent antifungal action against *Candida albicans*. At greater doses of MCh-AMP1, ultrastructural examinations employing electron microscopy indicated severe damage to the fungal cells[15].

EXPERIMENTAL

Plant material collection and authentication. The *Nigella sativa* seeds were collected from a trusted source and authenticated using standard botanical procedures to ensure their identity.

Seed preparation. The seeds were thoroughly cleaned and dried at 40°C in oven until the moisture content was reduced to less than 10%. Then, the seeds were crushed using a grinder to form a fine powder.

Solvent extraction. Three solvents, ethanol, methanol, and water, were used for the extraction of peptides and proteins. For each solvent, 50 g of the seed powder were mixed with 100 ml of the solvent in a flask at room temperature and stirred for 24 h. The mixture was then centrifuged at 10 000 rpm for 15 min to isolate the clear solution from the residue. The clear solution was collected, and the extraction was repeated three times for each solvent.

Protein purification. The extracted proteins were purified using size exclusion chromatography. The supernatant obtained from the solvent extraction step was followed by prepacked Sephadex G-25 column (10 × 300 mm) equalised with 50 ml Tris-HCl buffer of pH 7 of 0.5 ml/min of flow rate. The column was then washed with an inert buffer that did not interact with the protein. This helped to remove any impurities or unwanted proteins that was bounded to the resin. After washing, the protein was eluted from the column by running a buffer that specifically interacts with the protein. In this case, the same buffer that was used to load the protein sample onto the column was used to elute the protein fractions. As the eluting buffer flowed through the column, the protein was released from the stationary phase and flows out of the column. The protein fractions were collected in individual tubes or containers as they elute from the column. These fractions were then analysed for protein content using the Bradford assay. The protein binds with the dye and causes a shift in colour that can be measured

using a spectrophotometer. By measuring the absorbance of each protein fraction at a specific wavelength, the protein content of each fraction was determined. This information is important in determining the purity of the protein and the amount of protein recovered from the column.

Enzymatic hydrolysis. The purified proteins were subjected to enzymatic hydrolysis using trypsin. The purified proteins were liquified in Tris-HCl buffer of pH 7 of 75 ml and incubated with ratio 75:1 with trypsin for 5 h at 38°C. The reaction was stopped by adding 10% trifluoroacetic acid (TFA) to the mixture. The resulting peptides were extracted using 50% acetonitrile containing 0.1% TFA and centrifuged at 10 000 rpm for 15 min. The collected clear solution was dried under vacuum, and kept at –70°C for further examination.

Analysis of peptides and proteins. The extracted peptides and proteins were analysed by using liquid chromatography gel electrophoresis, and amino acid sequencing to identify and characterise the different proteins and peptides extracted from *Nigella sativa* seeds.

SCREENING FOR BIOACTIVE POTENTIAL

Cell culture. Appropriate cell lines were cultured in complete growth medium in sterile conditions with 10% CO_2 at 38°C.

Cell viability assay. A colorimetric MTT assay was used to analyse cell viability. The cells were planted in plates of 96-well and different concentrations of the extracted peptides and proteins were added. After incubation for 48 h, for each well MTT reagent was added and incubated for another 3 h. The crystals of formazan melted in DMSO, and absorbance was measured at 570 nm.

ENZYME INHIBITION ASSAY

The inhibitory activity of the extracted peptides and proteins against various enzymes such as α-amylase, α-glucosidase, lipase, and protease was evaluated using appropriate enzyme assays. The preparation of substrate solutions for α-amylase, α-glucosidase, lipase, and protease assays was by dissolving the starch in the Tris-HCl buffer (pH 7.0). The solution was mixed well and heated for 10 min at 37°C to activate the enzyme. After incubation, the identification of enzyme activity was measured by absorbance at 400–450 nm. The percentage enzyme activity inhibition was calculated using the following equation (1):

$$\text{inhibition \%} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100. \quad (1)$$

DETERMINATION OF IC_{50} VALUES

IC_{50} values were determined from an enzyme inhibition assay. The IC_{50} value is a measure of the inhibitory potency of a compound and represents compound concentration required to inhibit 50% of the enzyme activity. Thus, IC_{50} value provides a quantitative measure of the effectiveness of a compound in inhibiting the activity of an enzyme.

ANTIMICROBIAL PROPERTIES

The evaluation of antimicrobial properties of extracted peptides and proteins was carried out by using minimum inhibitory concentration (MIC) assays against bacteria and fungi.

Bacterial *Staphylococcus aureus* and fungal *Trichophyton mentagrophytes* strains were obtained from authenticated sources and cultured on appropriate media. The extracted peptides and proteins to obtain a stock solution, were dissolved in sterile distilled water. The serially diluted stock solutions were obtained by range of concentrations from 10–50 µg/ml. The MIC assays was performed by adding 100 µl (10^5–10^6) of bacterial cells to each well of a 96-well microplate, along with varying concentrations of 10–50 µg/ml of the extracted peptides and proteins. Control wells contained only the cells and media without any extract. *Staphylococcus aureus* was incubated for 24–48 h at 35–37°C, while *Trichophyton mentagrophytes* was incubated for 7 days at 25–30°C. The wells were visually inspected for visible growth and the lowest concentration of the extract that completely inhibited the visible growth of microbes was determined as the MIC. The experiment was performed in triplicate, and the mean MIC value was calculated. The data were analysed statistically to determine the significance of the results.

TOXICITY ASSESSMENT

Toxicity assessment of extracted peptides and proteins was performed using a combination of *in vivo* and *in vitro* assays.

In vitro assays. The cell viability assay using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], MTT assay was performed. The MTT assay is a cell viability assay that measures the activity of mitochondrial enzymes to assess cell metabolic activity and viability. The 96-well plates were cultured with cells at of 1×10^4 cells/well density. After 8–10 h incubation, cells were treated with the working solutions with 5% CO_2 at 37°C for 24 h. For each cell wall, MTT was added and incubated at 37°C for 4 h. DMSO was added to media to dissolve the formazan crystals. The microplate reader was used to record absorbance at 570 nm. The cell viability percentage was determined by relative to the untreated control.

In vivo assays. The *in vivo* acute toxicity study was performed in rodents. The study was conducted according to the OECD (Organisation for Economic Cooperation and Development) guidelines for acute oral toxicity testing. The test substance was admin-

istered to the rodents at a single dose of 1000 mg/kg weight of body via forced feed orally. The animals were monitored 14 days for any toxicity signs, such as changes in body weight, behaviour, clinical signs, or mortality. The blood samples were collected at 24 h time interval.

The data obtained from the cell viability assay and the acute toxicity study were analysed using appropriate statistical methods. The one-way ANOVA test was used to determine cell viability data. The acute toxicity data were analysed using the Probit method to determine the LD_{50} value.

RESULTS AND DISCUSSION

The study aimed to extract and characterise proteins and peptides from *Nigella sativa* seeds and assess their bioactive potential. Figure 1 shows the relative abundance of different peptides and proteins in different samples. The plant material was collected from a trusted source and authenticated using standard botanical procedures to ensure its identity. The seeds were cleaned, dried, and ground into a fine powder before solvent extraction with three solvents, ethanol, methanol, and water. The extracted proteins were purified using size exclusion chromatography and analysed for protein content using the Bradford assay. The purified proteins were subjected to enzymatic hydrolysis using trypsin to obtain peptides that were analysed using liquid chromatography gel electrophoresis and amino acid sequencing.

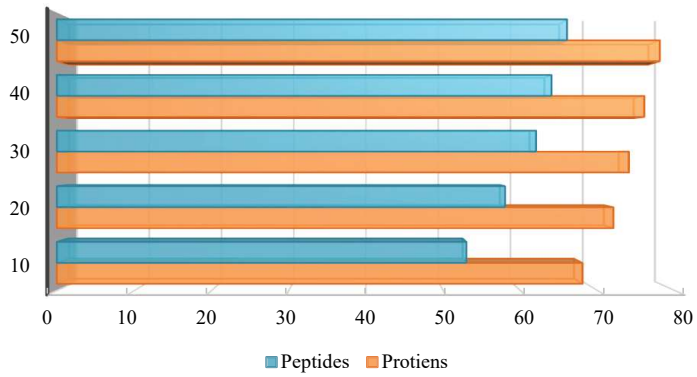

Fig. 1. Relative abundance of different peptides and proteins in different samples

Different peptides identified by amino acid sequencing are short chains of derived amino acids from the proteins using trypsin by enzymatic hydrolysis. The bioactive potential of the extracted peptides and proteins, appropriate cell lines were cultured, and colorimetric MTT assay is used to evaluate cell viability. Figure 2 shows the absorbance of various enzymes.

The inhibitory activity of the extracted peptides and proteins against various enzymes, including α-amylase, α-glucosidase, lipase, and protease, was evaluated using enzyme assays. The IC_{50} values were determined from the enzyme inhibition assay which is shown in Table 1. The antimicrobial properties of the extracted peptides and

proteins were also evaluated using minimum inhibitory concentration assays against bacteria and fungi.

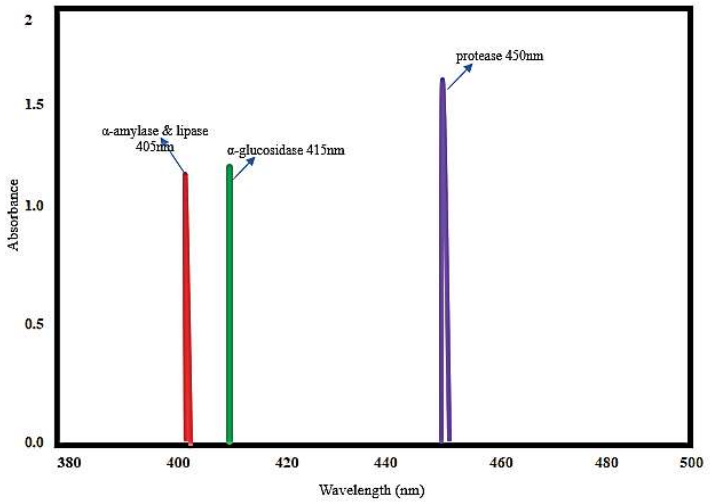

Fig. 2. Absorbance of various enzymes

Table 1. IC_{50} values of various enzymes

S. No	Enzymes	IC_{50} values (mg/ml)
1	α-amylase	0.15
2	α-glucosidase	0.18
3	lipase	0.24
4	protease	0.23

The study found that *Nigella sativa* seeds contain proteins and peptides that have bioactive potential. The extracted peptides and proteins showed inhibitory activity against α-amylase, α-glucosidase, lipase, and protease enzymes. Figure 3 shows the antimicrobial activity against *Staphylococcus aureus*.

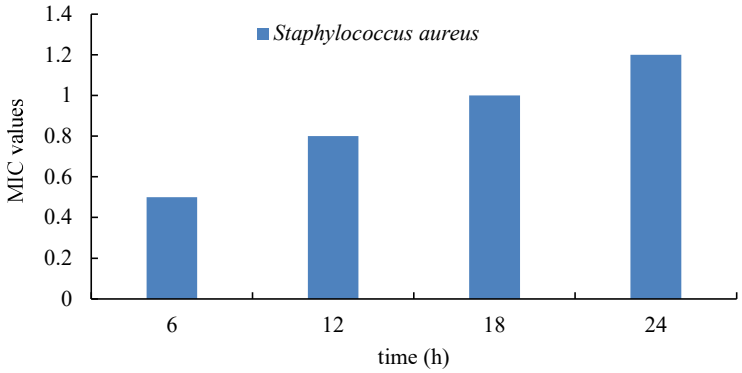

Fig. 3. Antimicrobial activity against *Staphylococcus aureus*

The antimicrobial activity of extracted peptides and proteins against *Staphylococcus aureus* and *Trichophyton mentagrophytes*, with MIC values range from 0.5–1.2 and 0.8–1.4 mg/ml, correspondingly is shown in Fig. 4.

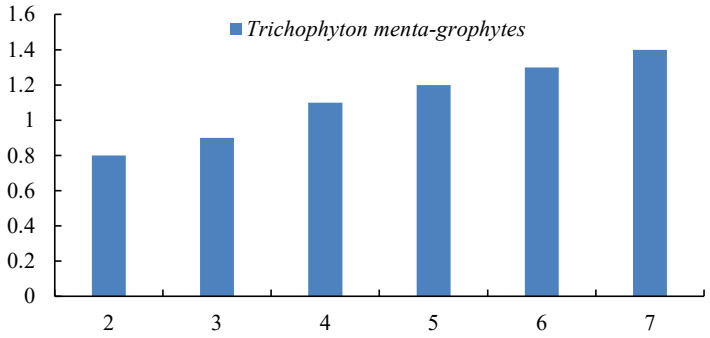

Fig. 4. Antimicrobial activity against fungi

The toxicity assessment of extracted peptides and proteins involves using a combination of *in vitro* and *in vivo* assays to evaluate the safety of these compounds. The acute toxicity data is analysed using the Probit method to determine the LD_{50} value and no morality was found for 14 days. These methods help to provide an objective evaluation of the toxicity of extracted peptides and proteins, which can be used to guide the development of safe and effective therapeutics.

After this dose, there is no change in any of the investigated toxicity measures, including behavioural changes, relative organ and body weight, white blood cell count differences, and abnormalities examination. The extracted peptides and proteins from *Nigella sativa* seeds could not be determined because no deaths of rodents were observed for 14 days at the highest tested dose of 1000 mg/kg. This indicates that the LD_{50} value is greater than 1000 mg/kg, which is a positive indication of the safety of these compounds. The Cell viability data are presented in Table 2.

Table 2. Cell viability data

Dose	Days	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Basophil (%)	Eosinophil (%)
1000 mg/kg	1	0	1	0	0	1.2
	3	12	4	3	0	1.0
	5	15	5	4	0	1.0
	7	18	6	6	0	0.8
	9	20	7	8	0	1.0
	11	21	8	9	0	0.8
	14	23	9	12	0	0.8

A rich source of proteins and peptides with bioactive potential is found in *Nigella sativa*, including inhibitory activity against key enzymes and antimicrobial properties.

These findings suggest the potential use of *N. sativa* in the development of nutraceuticals, and pharmaceuticals.

CONCLUSIONS

The study provides a comprehensive evaluation of the bioactive potential, antimicrobial properties, and toxicity of proteins and peptides extracted from *Nigella sativa* seeds. The study results suggest that *Nigella sativa* seeds contain proteins and peptides that can inhibit key enzymes and show antimicrobial activity against bacterial and fungal strains. Moreover, the toxicity assessment showed that the extracted peptides and proteins are safe for use, as there were no adverse effects observed in *in vitro* and *in vivo* assays. Additionally, no deaths of rodents were observed for 14 days at the highest tested dose of 1000 mg/kg. These findings provide valuable insights into the potential use of *Nigella sativa* as bioactive compounds for drug discovery. The study highlights the importance of exploring natural sources for discovering potential drugs that are safe and effective.

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ORIGINAL ARTICLE

Development of thermally reduced corn stover biochar and its satin weaved sisal-reinforced vinyl ester composites

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Abstract
The aim of this research is to develop thermally reduced corn stover biochar and combined it with the satin weaved sisal-reinforced vinyl ester composites. This investigation focus on mechanical, wear, DMA, fatigue, and hydrophobic properties of this composite, characterized according to ASTM standards. Composites are made after silane surface treatment on reinforcements and fabricated by hand layup process. Results show that the composite with the 5.0 vol.% biochar had the highest values of mechanical properties, which were 134 MPa, 4372 MPa, and 4.74 J for tensile strength, flexural strength, and Izod impact, respectively. But further increased in biochar up to 5.0 vol.% shows the reduction in mechanical properties. On the other hand, the composite designation VS3 was discovered to have the maximum storage modulus and lowest loss factor with inclusion of 5.0 vol.% of biochar particles as well as it shows the better wear characteristics of about 0.28 coefficient of friction and 0.009mm^3/Nm sp. wear rate. However, maximum fatigue life counts of about 28,813 were observed by addition of 3.0 vol.% of biochar. The composite material designated VS3 exhibits the highest recorded contact angle, which is around 71°, indicating that it is hydrophobic in nature. SEM fractography demonstrates better fiber-to-matrix adhesion as a result of surface treatment on reinforcing materials. Furthermore, such composites could be used in industrial and domestic applications.

Keywords Satin weaved sisal fiber · DMA · Biochar · Fatigue life counts · Mechanical properties

1 Introduction

Composites are made up among different materials blended together while still retaining the unique characteristics of each material. Together, these parts work to provide the necessary toughness and mechanical qualities to composite materials [1]. It is made up of two or more separate phases (the matrix part and the dispersed section), each of which has large volume characteristics that are noticeably different from the others. The major phase with a continuous property is the matrix section, which is typically more ductile [2]. Fiber or reinforcements distribute the load equally and hold the secondary phase, also known as the scattered phase. The matrix contains a discontinuous form of the

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phase separation. This phase is also known as the reinforcement agent because it is typically stronger than the matrix [3]. Vinyl ester is one of the most durable alternatives for regions subjected to such problems due to its great chemical, thermal, and mechanical resistance. For increased endurance, the majority of vinyl ester systems have several layers. However, over the last few hundreds of years, natural fibers such as cotton, bamboo, jute, hemp, flax, and sisal have been utilized as substitute reinforcing fibers in wide applications of polymer composites due to several advantages over synthetic fibers, e.g., minimal cost, minimal wear rate throughout processing, light weight, ecologically friendly, biocompatible, and affordable from biomass resources [4].

Among all-natural fiber, sisal fiber is one of the prominent reinforcements which deliver excellent mechanical properties to the composite materials. The sisal fiber is non-toxic, relatively inexpensive, easily accessible, and has a low density and increased specific strength and modulus [5]. Chaitanya et al. [6] researched on the recyclability of PLA/Sisal fiber biocomposites. Up to the third recycle, the tensile strength of injection molded biocomposites decreased by 20.9%. Beyond the third recycling, the dynamic mechanical investigation demonstrated a significant fall in storage and loss modulus. Morphological and thermal evaluation of recycled biocomposites revealed significant fiber and matrix disintegration. Murugan et al. [7] examined the mechanical and physical properties of banana/sisal fiber–reinforced interpenetrating polymer networks (IPN) composites. In all physical tests, the sisal fiber–reinforced IPN laminate demonstrated a significant rise except the moisture absorption properties. However, compared to other woven types, satin woven fabrics are strong due to the large number of threads employed, yet the lesser interlacing gives flexibility and wrinkling resistance. Satin textiles are almost usually warp-faced and made of glossy filament yarns with a very low twist [8].

Additionally, the different types of particles are used as fillers in the composite materials which may improve the material's thermal, mechanical, damping, wear, and fatigue properties [9]. To make these composites more thermally stable and highly toughened and to enhance the efficiency of natural fiber composite, biochar has been introduced in recent studies [10]. Numerous researchers have attempted to develop novel eco-friendly biofillers for enhancing the matrix phase's characteristics using convenient domestic materials [11]. Due to the high rate of maize production, after harvesting, its wastes are massive in India and worldwide. These corn cobs and corn stover are frequently burned or discarded as agricultural waste, causing major environmental problems such as air pollution and soil contamination. Alternate techniques must be implemented in order to conserve the environment; hence, the utilization of corn stover for biochar preparation has been taken into consideration [12]. A new polymer composite for biosensors based on activated biochar was developed and characterized by Sobhan et al. [13]. Corn stover was used to create biochar, which was afterward activated through the steam. Using a solvent casting process, activated charcoal (ABC) was created using polylactic acid (PLA). When PLA contents rose from 15 to 50%, the ABC/PLA composite film's tensile strength (TS) and Young's modulus raised from 0.81 to 3.04 MPa and 56.31 to 102.69 MPa, correspondingly. Xu Zhou et al. [14] researched on environment-friendly corn stover/poly (butylene adipate-co-terephthalate) PBAT's biopolymer composites. While maintaining the same CS content, reducing CS size improves particle dispersion, boosts PBAT's degree of crystallinity, and greatly enhances the CS/PBAT composites' thermal stability and tensile characteristics.

Hence, based on previously published literature studies and research circumstances, the fabrication of thermally reduced corn stover biochar and its satin weaved sisal-reinforced vinyl ester composites was analyzed. The mechanical, wear, DMA, fatigue, and hydrophobic properties have been characterized according to ASTM standards for this composite laminates. The composites from corn stover biochar and satin weaved with vinyl ester were fabricated by hand layup process. However, the simplest and most traditional open molding technique for making composites is hand layup. Initially, dried fibers in the form of woven or bond fabrics are manually introduced to the mold, and the resin matrix is applied to the reinforcing material with a brush. Such composite materials could be used in aerospace, automobile, and industrial sectors as well as for domestic appliances.

2 Experimental procedure

2.1 Materials

Metro Composites, Chennai, India, provided a commercially available vinyl ester with a viscosity of 350cps at room temperature. As accelerators, catalysts, and promoters, N-dimethylaniline, methyl ethyl ketone peroxide, and cobalt naphthenate are used. Metro Composites, Chennai, India, also supplies 220GSM (Grams per Square Meter (g/m^2)) sisal fiber with a satin weaving pattern as a reinforcing element. For filler content, 1–3-μm biochar particles with a density of 0.98gm/cc made from locally available corn stover. Sigma-Aldrich sells 3-aminopropyltrimethoxysilane (APTMS) for surface modification.

2.2 Biochar preparation

Figure 1 depicts the step-by-step synthesis of corn stover biochar. The slow pyrolysis (bio-carbonization) technique of charcoal manufacture converts corn stover waste into

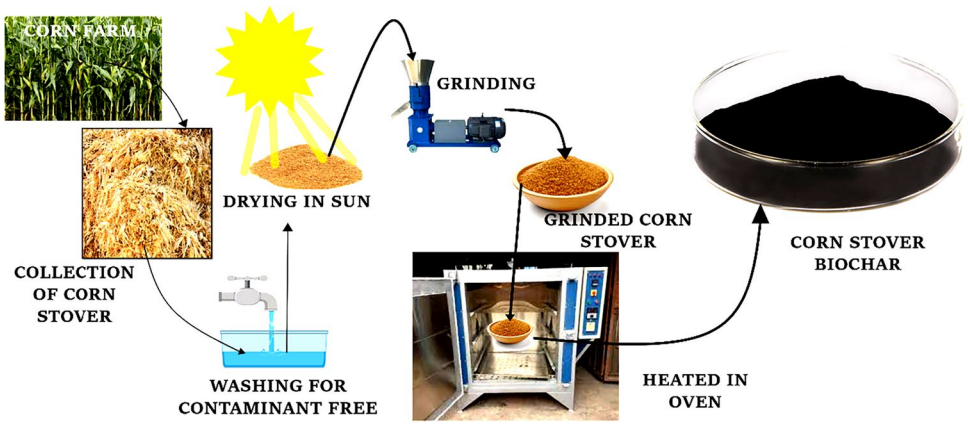

Fig. 1 Corn stover biochar preparation

biochar. To begin, raw corn stover waste was continuously washed in water for 2 h to eliminate any contaminants from the stover. To remove any remaining moisture, the stover was washed and dried in the sun for 48 h. The cleaned corn stover was then passed through the pyrolysis process [15]. The three-stage procedure is carried out with the help of multi-mode, specially designed pyrolysis equipment. The feedstock was first lit with kerosene oil, but as it began to burn, the supply was cut off. The first phase entailed completing the pre-pyrolysis procedure in a controlled air environment (nitrogen 0.32 l/s) for roughly 10 min at temperatures ranging from room temperature (RT) to 200°C. The incoming moisture and light volatiles were eliminated during this phase. In the second stage, which ran from 200 to 500°C, the hemicellulose and cellulosic components of the biomass were quickly destroyed. This stage takes roughly 30 min to complete [16]. The temperature was then raised to 500–800°C to break down the char component and free it from lignin and other organic debris with stronger chemical bonds. This stage took roughly 20 min to complete. All stages were heated at a rate of 50K/min for a total residence duration of 60 min. The thermally damaged biochar is separated at the end of the operation using fine sieves. The average particle size was determined using a particle size analyzer to be 1–3μm (having pore size 10–15 nm). As a consequence, the biomass of corn stover was transformed into fine-sized biochar [17].

2.3 Surface modification for fibers

The surface of sisal fiber with a satin weave was treated individually using silane treatment. At the first stage in making the silane solution, 95% ethanol is placed in a glass beaker and well agitated before adding 5% distilled water. Second, to regularize the pH of the resultant solution, which has a pH range of 4.5 to 5.5, acetic acid is added. Third, the silane drops must be progressively added to the ethanol-water solution until saturation is achieved. Fourth, the solution was then thoroughly mixed by stirring to dissolve all of the silanol groups and remove the methoxy group from the silane material. Finally, after 10 min of soaking, the fiber and biochar are removed from the silane solution to form a Si-O-Si structure [18].

2.4 Composite preparation

The layup surface must be ready as part of the mold preparation process prior to the fabrication of the composite, and wax is then coated on the cleaned surface to assist demolding laminate. If any edge gaps are found, silicon-rubber is enclosed around the perimeter of the laminate to designate the working area. Following that, a precise volume of vinyl ester resin is collected in a cleaned glass beaker to which corn stover biochar has been added [19]. The accelerator dimethylaniline (1.5%), catalyst methyl ethyl ketone peroxide (1.5%), and promoter cobalt naphthenate (1.5%) are combined with vinyl ester resin and corn stover biochar combination. The layers of satin weaved sisal fiber are placed on the mold, and the resin mixture is poured on top of each layer of fiber in a progressive manner. The hand layup technique is used to incorporate the resin into the fiber layup. Because of the slower polymerization at room temperature induced by the resin mixture ratio, the cure duration at room temperature is 24 h. A composite with a thickness of up to 3mm was created using satin weaved sisal fiber and vinyl ester with corn stover biochar [20]. Numerous laminates were made utilizing the hand layup technique and the components are listed in Table 1.

3 Characterization of composite

Visual inspection is performed on the vinyl ester–based corn stover biochar satin woven sisal fiber composite to detect any recognizable surface flaws. In accordance with ASTM requirements, three test specimens are drowned out of the

Table 1 Various combinations for different composite designation

Composite designation	Resin (vol.%)	Fiber (vol.%)	Biochar (vol.%)
V	100	-	-
VS	65	35	-
VS1	64	35	1
VS2	62	35	3
VS3	60	35	5

laminate using abrasive water-jet equipment from Maxiem water jets 1515, KENT, USA. The machine parameters were 220-psi maximum jet pressure, 0.3g/s abrasive flow rate, 1.1-mm nozzle diameter, and 3-mm SOD throughout the processes. Surface flaws on the specimen faces were eliminated by polishing them using P320 grit emery paper [21]. Table 2 was used to evaluate the mechanical wear, DMA, fatigue, and hydrophobic properties of the composites.

4 Result and discussion

4.1 Mechanical properties

Figure 2 represents the mechanical performance of corn stover biochar with satin weaved sisal fiber–reinforced vinyl ester composite. Figure 2 illustrates (a) the tensile behavioral traits, (b) the flexural properties, and (c) the Izod impact values. The composite designation "V" has tensile strength, flexural strength, and Izod impact values of roughly 61MPa, 1672MPa, and 0.41 J, respectively. The explanation for these lower results is the pure resin content of the composite designation "V," which is usually highly brittle and exhibits flat cracks throughout the surface [22]. Furthermore, including 35 vol.% satin weaved sisal fiber in pure vinyl ester resin improves the mechanical performance of the composite designation "VS." Tensile strength, flexural strength, and Izod impact values for composite designation "VS" enhanced to 96MPa, 2947MPa, and 3.86J, respectively. This large improvement was achieved by using silane-treated satin sisal fibers in vinyl ester resin, which improved the adhesive bonding between the fiber and the matrix [23]. As well as sisal fiber has strong wetting ability with matrix hence improved mechanical strength is observed. Similarly, corn stover biochar particle insertion of 1.0, 3.0, and 5.0 vol.% increased mechanical properties for composites designation "VS1," "VS2," and "VS3." But maximum values of mechanical properties were observed for composite designation VS2 with 3.0 vol.% of biochar about 148 MPa, 4519 MPa, and 4.98 J for tensile strength, flexural strength, and Izod impact respectively. Biochar particles were evenly dispersed in matrix and improved bonding is observed due to pores structures of biochar particles. These pore structures infiltrate the resin through it and enlace the bonding mechanism [24]. But it is observed that further increase in biochar vol.% up to the 5.0 vol.% composite designation VS3 shows decreased values for tensile strength, flexural strength, and Izod impact (134 MPa, 4372 MPa, and 4.74 J correspondingly). This decrease in values is a result of the cluster formation of biosilica particles, which provides composite's a brittleness and causes a failure.

4.2 Dynamic mechanical analysis

The DMA performance of corn stover biochar with satin-reinforced vinyl ester composites is shown in Fig. 3. The composite designation "V" has the lowest storage modulus characteristic, which is about 2.1 GPa. When the free volume grows with temperature, vibration also rises, showing that the matrix molecules rotate quickly and have a low storage modulus. The loss tangent for pure vinyl ester resin similarly shows lower values because the molecules cannot keep the energy delivered as stress while rotating at high temperatures and frequency [25]. The storage modulus appears to be 3.5 GPa when 35 vol.% satin weaved sisal fibers were

Table 2 ASTM standards with machine specification

Sr. no.	Tests	ASTM standards	Machines used
1	Tensile	D3039	INSTRON 4855, UK
2	Flexural	D790-17	Traverse speed of 1.1 mm/s
3	Izod impact	D256–10	Krystal equipment Ltd., India Maximum load capacity of 20 J
4	DMA	D4065	Dual cantilever mounted DMA analyzer with sweep mode
5	Wear properties	G99–17	Du-com instruments Pvt. Ltd., India Pin-on-disc setup
6	Fatigue	D3479	MTS Landmark 370 load frame, USA Working stress values are 30 %, 60 % and 90 % of UTS frequency of 5 Hz, stress ratio of 0.1 and temperature of 28 °C
7	SEM	-	HITACHI, S-1500, JAPAN

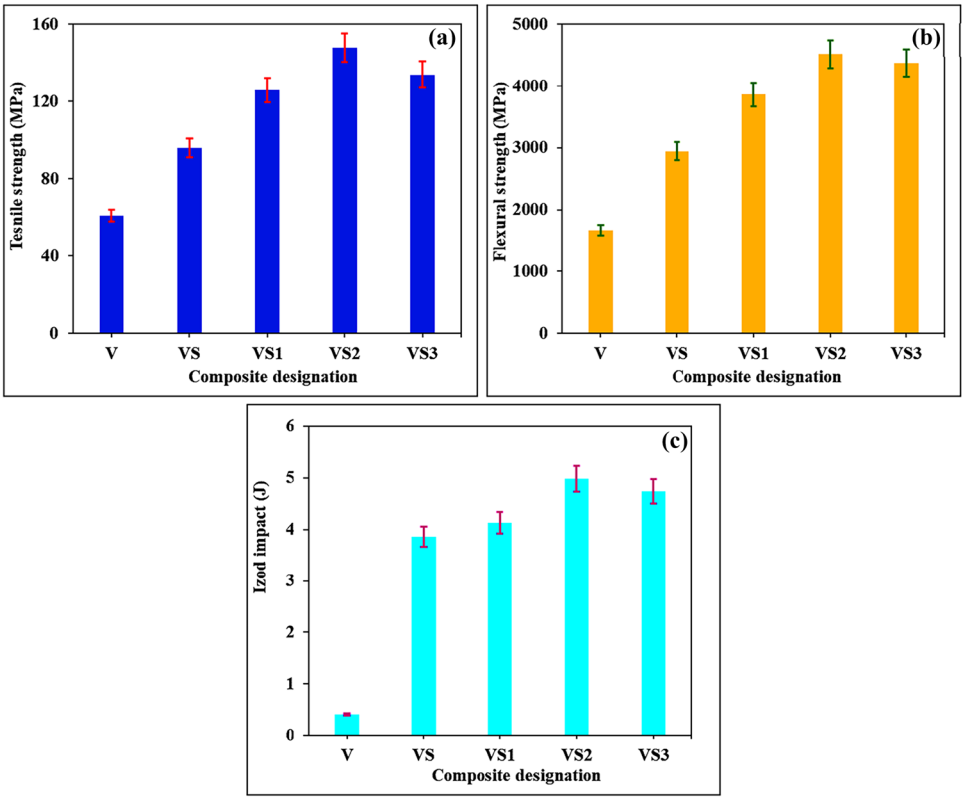

Fig. 2 Mechanical properties for various composite designation

added to pure vinyl ester resin. The layer-sequence stacking of fibers into vinyl ester resin results in an increase in storage modulus. The free space in the matrix, surrounded by fiber volume fraction, increases the storage modulus due to the surface-treated fiber bonding mechanism preventing the rotation of the matrix molecules. Comparable improvements in storage modulus values and a decrease in loss factor are seen when thermally reduced corn stover biochar was incorporated in composite by 1.0, 3.0, and 5.0 vol.%. However, with inclusion of 5.0 vol.% of biochar particles, the composite bearing the name VS3 was found to have the highest storage modulus and the lowest loss factor. This is because the addition of biochar strengthens the interaction between the fiber and the resin's secondary molecules. Hence, the inertia to rotate about the primary chains is rapidly increased due to the added biochar and more heat energy is required to activate the same [26].

4.3 Wear properties

Due to the pure vinyl ester resin as the main content element of the specimen, which exposes its surface in a large area to the wear disc, the composite designation "V" exhibits a higher wear rate of roughly 0.48 COF and sp. wear rate of 0.022 mm^3/Nm (Fig. 4). But the inclusion of silane-treated satin weaved sisal fibers enhances the wear resistances up to 0.41COF and a specific wear rate of 0.018 mm^3/Nm. The insertion of fiber decreases the contact angle of the matrix

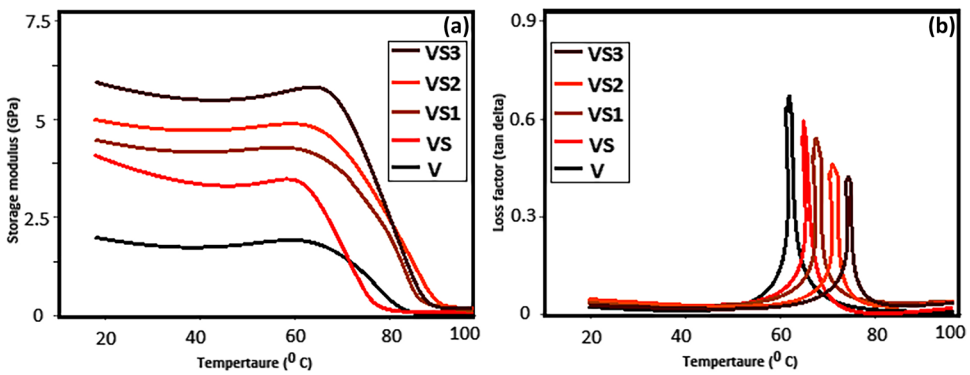

Fig. 3 DMA for various composite designations

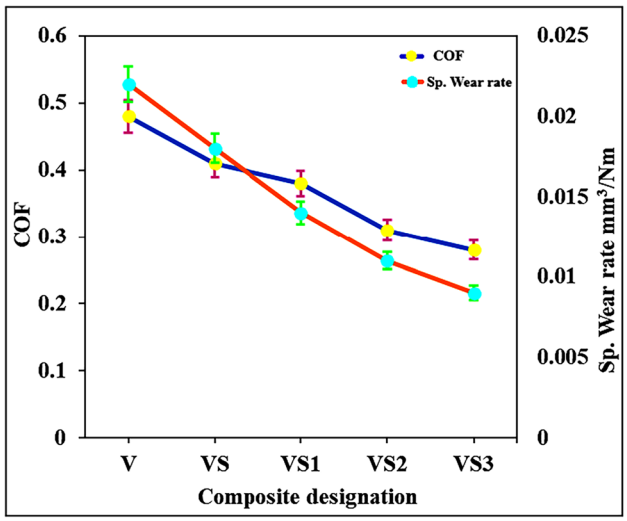

Fig. 4 Wear properties for various composite designation

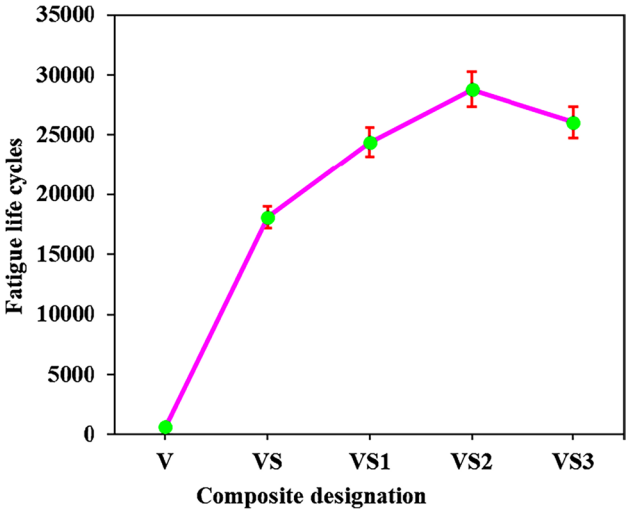

Fig. 5 Fatigue behavior for various composite designation

to the wear disc and enhances the resistances against abrasion [27]. Furthermore, with the incorporation of silane-treated biochar particles by 1.0, 3.0, and 5.0 vol.%, the wear resistance of composite designations VS1, VS2, and VS3 steadily rises. This increase is due to silane-treated biochar particles dispersed evenly in the matrix. The best values for this property were 0.28 COF at 0.009mm^3/Nm for the composite designation VS3 with 5.0 vol.% biochar particles and 35% satin weaved sisal fiber. It is due to the lubricant packet formation which occurs when biochar comes in contact with wear disc [28].

4.4 Fatigue behavior

Figure 5 demonstrates the fatigue behavior of corn stover biochar satin weaved sisal fiber–reinforced vinyl ester composites. At 50% of UTS, which is around 624 cycles, the composite designation "V" provides the lowest fatigue life numbers. Figure 5 a depicts the greater brittleness of pure vinyl ester resin as a result of the decreased fatigue life count with flat brittle fracture. The justification for this is the preservation of plastic strain in the vinyl ester molecular chain [29]. Additionally, the inclusion of 35% sisal fiber increases the fatigue life counts for composite designation "VS" by around 18,118 cycles. This phenomenon could be due to the silane surface treatment on satin weaved sisal fiber improving chemical bonding and mechanical interlocking between the treated fibers and matrix. Similarly, biochar particle insertion of 1.0, 3.0, and 5.0 vol.% increased fatigue life counts such as 24,385, 28,813, and 26,094 cycles with respect to composite designations "VS1," "VS2," and "VS3." This improvement is because of the improved dispersion and adhesion of particle in matrix, which reduce the crack propagation [30].

4.5 Contact angle

Figure 6 shows the contact angle between composite materials and water drop. The pure vinyl ester resin has a greater contact angle of around 86°. This matrix material repeals the OH molecules and gives highest contact angle. Further incorporation of sisal fiber by 35vol.% reduces the contact angle up to 82°. Natural fiber has tendency to attract water molecules; hence, this increment in water absorption and reduction in contact angle is observed for composite designation "VS." Moreover, adding 1.0, 3.0, and 5.0 vol.% biochar particles decreases the contact angle between composite materials and water molecules to 77°, 74°, and 71° for composite designations VS1, VS2, and VS3, respectively. The biochar particles also easily bind the OH molecules,

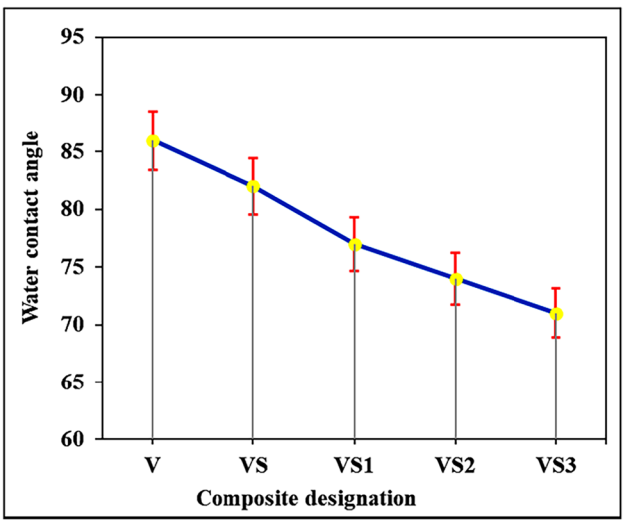

Fig. 6 Water contact angle for composite designation

although silane treatment limits this to some amount; thus, the measured contact angles are only similar to pure vinyl ester [31].

4.6 SEM fractography

Figure 7 demonstrates the scanning electron microscopy for various fractured composites. Figure 7 a shows the flat fracture with brittle surface for the composite designation V. The absence of reinforcements and the pure epoxy contents of the composite designation V are the major cause of such fracture. Improved adhesive bonding between the fiber and matrix is demonstrated in Fig. 7 b and c for composite designations VS and VS1, respectively. This is a result of the Si-O-Si structure obtained by the surface treatment on satin weaved sisal fiber making strong bonding [32]. Figure 7 d shows a strong bonding process with a highly reactive fiber phase in composites. This is because the inclusion of biochar and its even dispersion throughout the matrix improved the adhesion property for composite designation VS2. Figure 7 e shows the enhanced bonding mechanism for composite designation VS3 due the pore structure of biochar, which

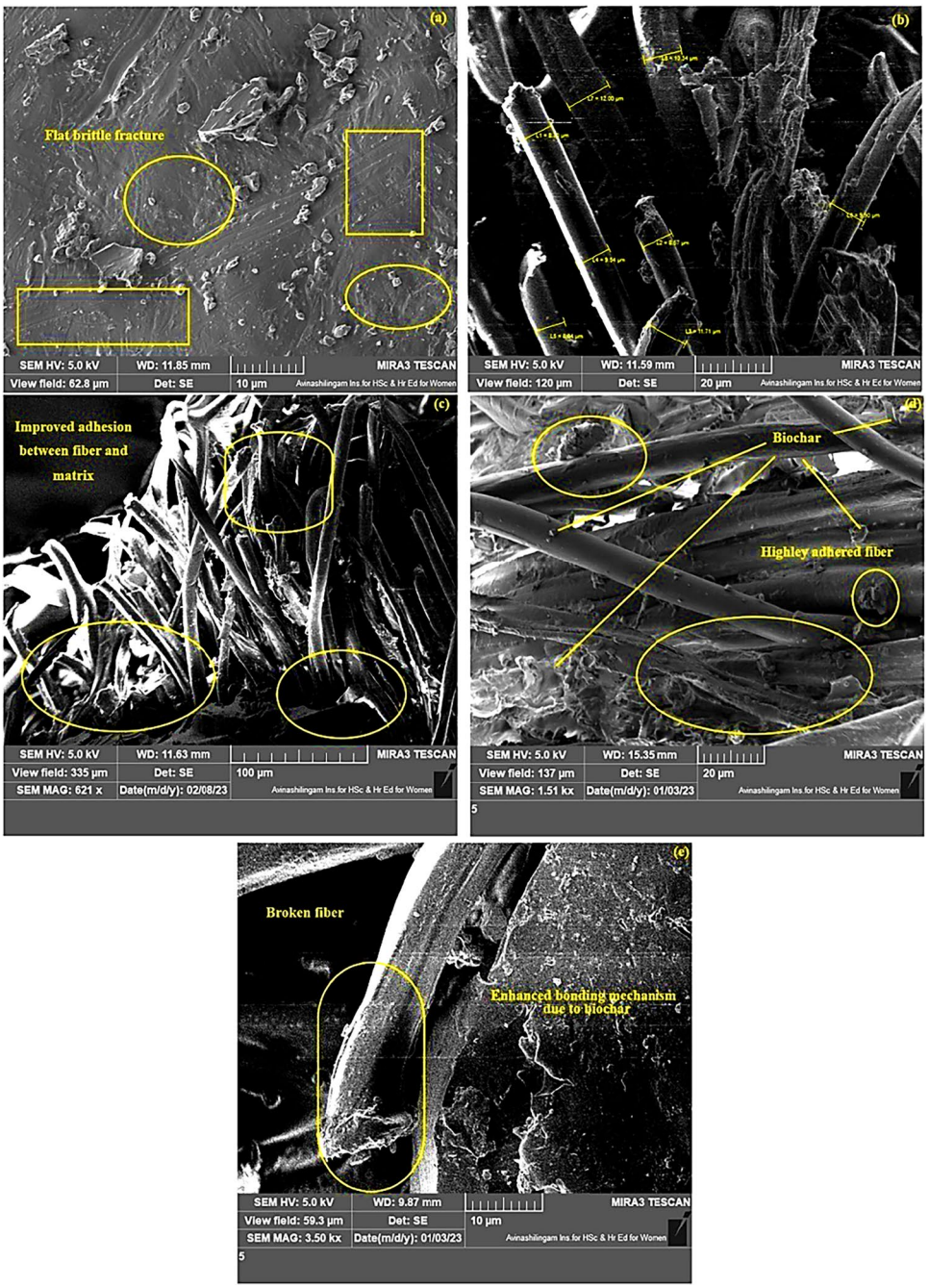

Fig. 7 SEM fractography for fractured samples

infiltrate resin through it and improved mechanical interlocking [2, 33].

5 Conclusions

The analysis and fabrication of thermally reduced corn stover biochar with the satin weaved sisal-reinforced vinyl ester composites is the aim of this study.The mechanical, wear, DMA, fatigue, and hydrophobic properties have been characterized according to ASTM standards for these composite laminates. After surface treatment on reinforcements by hand layup process, composites were fabricated from corn stover biochar and satin weaved with vinyl ester. The outcomes of this study were followed as follows: maximum values of mechanical properties were observed for composite designation VS2 with 3.0 vol.% of biochar about 148 MPa, 4519 MPa, and 4.98 J for tensile strength, flexural strength, and Izod impact respectively. But it is observed that further increase in biochar vol.% up to the 5.0 vol.% composite designation VS3 shows decreased values for tensile strength, flexural strength, and Izod impact (134 MPa, 4372 MPa, and 4.74 J correspondingly). However, with inclusion of 5.0 vol.% of biochar particles, the composite bearing the name VS3 was found to have the highest storage modulus and the lowest loss factor. The best values for wear properties were 0.28 COF at 0.009mm^3/Nm for the composite designation VS3 noted by addition of 5.0 vol.% biochar particles and 35% satin weaved sisal fiber. On the other hand, biochar particle insertion by 1.0, 3.0, and 5.0 vol.% increased fatigue life counts such as 24,385, 28,813, and 26,094 with respect to composite designations "VS1," "VS2," and "VS3." The highest observed contact angle is about 71° for composite designation VS3 which indicates the hydrophobic nature of composite material. SEM fractography shows the improved adhesion between fiber and matrix due to the surface treatment on reinforcing materials, as well as because of the silane surface treatment corn stover biochar distributed evenly within the matrix.

Author contribution P. Sivamurugan, M. Mareeswaran—concept design and experiment. S. A. Muhammed Abraar, Savita Verma—concept design, experiment, and testing. Neha Verma, Bipin Kumar Srivastava—concept design, experiment, and funding. D. Vinay Kumar and I. S. Chakrapani—concept design. B. Ramesh—correspondence.

Data availability Nil

Declarations

Ethical statement NA

Competing interests The authors declare no competing interests.

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Section A-Research paper
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FORMULATION AND EVALUATION OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS

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Abstract

It has become clear that self-nanoemulsifying drug delivery systems (SNEDDS) are a potential method for improving the solubility and bioavailability of medicines that are not highly water-soluble. Using Capmul MCM, Tween 80, Transcutol P, and olmesartan medoxomil as the oil, surfactant, co-surfactant, and medication, respectively, an optimal SNEDDS formulation was created in this work. The improved formulation's physicochemical characteristics, in vitro drug release profile, pharmacokinetic parameters, and acute and sub chronic toxicity were assessed. The findings revealed that the SNEDDS formulation had a high drug content of 95%, a zeta potential of -20 mV, and a particle size of 50 nm. The medication released consistently throughout a 12-hour period, according to the in vitro drug release profile. The pharmacokinetic analysis demonstrated that, in comparison to the pure medication, the SNEDDS formulation had a larger Cmax, AUC, and longer elimination half-life. According to tests on acute and sub-chronic toxicity, the formulation was well tolerated at low dosages while harmful effects were dose-dependent at higher concentrations. Overall, the findings showed that SNEDDS might be used as a drug delivery system to enhance the solubility, bioavailability, and pharmacokinetic characteristics of medicines that are poorly water-soluble.

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Keywords: Self-nanoemulsifying drug delivery systems, SNEDDS, in vitro drug release, pharmacokinetics, acute toxicity, sub-chronic toxicity.

1. INTRODUCTION

Poorly soluble medications may be delivered effectively using self-nanoemulsifying drug delivery systems (SNEDDS), a form of drug delivery technology. A transparent or translucent, thermodynamically stable nanoemulsion is a combination of oil, water, and an emulsifier. SNEDDS are a form of nanoemulsion. When in contact with digestive fluids, the emulsifier in SNEDDS is engineered to spontaneously create an emulsion, which aids in the solubilization and absorption of the medication. SNEDDS are superior to conventional drug delivery methods in a number of ways. First, they may make poorly soluble medications more soluble and more bioavailable, which can increase the effectiveness of their treatment. Second, the drug's stability and shelf life may be improved by SNEDDS' capacity to prevent gastrointestinal tract deterioration. Finally, SNEDDS may increase patient compliance by decreasing the negative effects of repeated dosing and lowering the number of doses necessary.

SNEDDS have received extensive research attention for a range of pharmacological classes, including anticancer, anti-inflammatory, and cardiovascular medications. They are also being investigated as a means of delivering large molecules like proteins and peptides. However, the formulation procedure, emulsion composition, and drug's physicochemical characteristics must all be carefully taken into account while creating SNEDDS. The three primary components of SNEDDS are generally an oil phase, a surfactant, and a co-surfactant. A lipophilic liquid, such as medium-chain triglycerides or vegetable oils, serves as the oil phase and solubilizes the medication. By lowering the interfacial tension between the oil and water phases, the surfactant molecule stabilizes the emulsion. The co-surfactant is a tiny molecule that boosts the surfactant's capacity for solubilization and encourages the development of the nanoemulsion.

SNEDDS may be produced in solid or liquid dose forms. Solid SNEDDS are designed as powders that may be reconstituted in water prior to administration, while liquid SNEDDS are commonly supplied as soft gelatin capsules. The physicochemical characteristics of the medicine and the preferred method of administration determine the dosage form to be used. SNEDDS are superior to other nanoemulsion-based drug delivery methods in a number of ways. The formulation procedure is made simpler by the fact that they self-emulsify and don't need any extra energy input to create the emulsion. Second, SNEDDS may produce consistent, tiny droplets with a size range of 10-100 nm, which makes it easier for drugs to pass the intestinal epithelium and be absorbed. Finally, by minimizing the medication's exposure to the harsh gastrointestinal environment, including enzymes and pH changes, SNEDDS may improve the stability of the medicine.

The solubility, bioavailability, and stability of poorly soluble medicines may all be improved with the use of SNEDDS, a potential drug delivery system. However, SNEDDS development

requires careful consideration of the drug's physicochemical characteristics, the emulsion's composition, and the formulation procedure.

- **Nanoemulsion:** Droplet sizes for nanoemulsions, a form of emulsion, generally range from 10 to 200 nanometers. They have a wide interfacial area, are transparent or translucent, thermodynamically stable, and improve the solubility and bioavailability of poorly soluble medicines.
- **Solubilization:** An increase in a drug's solubility in a solvent—in this example, the oil phase of SNEDDS—is known as solubilization. A medicine's bioavailability and therapeutic effectiveness may be improved by enhancing the solubility of a drug that isn't very soluble.
- **Bioavailability:** The degree and speed at which a medicine enters the site of action or the systemic circulation is known as bioavailability. By increasing the solubility and absorption of poorly soluble medicines in the gastrointestinal system, SNEDDS may increase their bioavailability.
- **Emulsifier:** A substance known as an emulsifier lowers the interfacial tension between two immiscible liquids, such oil and water. The emulsifier in SNEDDS is essential for stabilizing the oil and water phases to create a stable nanoemulsion.
- **Co-surfactant:** A co-surfactant is a tiny molecule that helps the surfactant solubilize substances better and encourages the creation of nanoemulsions. Co-surfactants are often employed in SNEDDS to enhance the formulation's emulsification capabilities.
- **Soft gelatin capsules:** SNEDDS are typically administered in the dose form of soft gelatin capsules. They are made of a gelatin shell containing a liquid formulation, like SNEDDS, that is simple to ingest and absorb in the digestive system.
- **Solid SNEDDS:** SNEDDS that can be reconstituted in water before administration are known as solid SNEDDS. Compared to liquid SNEDDS, solid SNEDDS provide a number of benefits, including increased stability and simplicity in handling.
- **Physicochemical properties:** A drug's physical and chemical traits, such as solubility, lipophilicity, molecular weight, and crystal structure, are referred to as its physicochemical qualities. These characteristics, like as the drug's solubility and bioavailability in SNEDDS, are crucial in establishing how it behaves in various contexts.
- **Formulation process:** In order to guarantee the stability, effectiveness, and safety of the drug delivery system, the formulation process for SNEDDS includes choosing the right components, preparation techniques, and quality control measures. A crucial stage in the creation of SNEDDS is formulation, which requires careful consideration of a number of variables, including the drug's physicochemical qualities and the composition of the emulsion.

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1.1.Background of the Study

To increase the bioavailability of poorly soluble medicines, the creation of self-nanoemulsifying drug delivery systems (SNEDDS) has been identified as a viable strategy. Poor solubility may result in poor effectiveness and unpredictable therapeutic effects, making it a significant barrier in the creation of medications. Upon coming into contact with digestive fluids, SNEDDS, which are made up of an oil phase, a surfactant, and a co-surfactant, spontaneously create a stable and homogenous nanoemulsion. The bioavailability and therapeutic efficiency of poorly soluble medicines are improved by the nanoemulsions increased solubility and absorption.

Compared to traditional drug administration methods, SNEDDS provide a number of benefits, including increased patient compliance, decreased pharmacokinetic variability, and better solubility and bioavailability. They are very adaptable and may be made into soft gelatin capsules, pills, and powders, among other solid or liquid dosage forms. Numerous treatment fields, including as cancer, cardiovascular disease, and infectious illnesses, have effectively used SNEDDS.

The necessity for efficient and secure drug delivery systems has fueled the development of SNEDDS as well as advancements in surfactant and emulsion technologies. The improvement of SNEDDS formulations, the description of their physicochemical characteristics, and the assessment of their safety and effectiveness in preclinical and clinical investigations remain the main areas of research in this area.

1.2.Need of the Study

The necessity to research self-nanoemulsifying drug delivery systems (SNEDDS) has numerous causes:

a. **Enhancing bioavailability:** A key obstacle to the development of medicines is low solubility, and SNEDDS provide a viable option to increase the bioavailability of medications with poor solubility. Drugs' effectiveness may be increased and pharmacokinetic variability can be decreased by SNEDDS by increasing their solubility and absorption.
b. **Improving drug delivery:** SNEDDS provide a number of benefits over traditional drug administration methods, including better patient compliance, a smaller dosage, and higher drug stability. They are adaptable and suited for a variety of therapeutic applications due to the fact that they may be created as either liquid or solid dosage forms.
c. **Enabling drug development:** New pharmaceuticals' low solubility and bioavailability often impede the development of new medications. In order to develop and market novel medications with increased therapeutic effectiveness, SNEDDS may assist in overcoming these constraints.

d. **Addressing global health issues:** Many illnesses and medical problems need the use of poorly soluble medications, and SNEDDS may assist in resolving the issues with regard to global health that are connected to these ailments. To increase the bioavailability of medicines used to treat cancer, cardiovascular disease, and infectious illnesses, for instance, SNEDDS may be employed.

2. LITERATURE REVIEW

Vandana Soni and Manish Kumar Gupta's (2018) article "Self-nanoemulsifying drug delivery systems: formulation insights, applications, and advances" was published in 2018: The formulation, characterization, and drug delivery applications of self-nanoemulsifying drug delivery systems are all covered in detail in this review paper. It talks about the different SNEDDS parts, how to choose them, and how to optimize the formulation parameters. It also sheds light on the physicochemical characteristics of SNEDDS, including droplet size, stability, and zeta potential, and how they affect drug delivery. The essay also outlines current SNEDDS technological developments and their possible future use.

By Jaspreet Kaur and Gurpreet Kaur (2016), "Formulation and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) of lovastatin": The formulation and assessment of SNEDDS of lovastatin, a medication with low water solubility used to treat hypercholesterolemia, are the main subjects of this work. The formulation of SNEDDS was improved by the authors by employing different oils, surfactants, and co-surfactants. They also assessed the formulation's physicochemical characteristics, in vitro drug release, and stability. They discovered that the SNEDDS formulation enhanced lovastatin's solubility and bioavailability and may be employed as an alternative to traditional formulations.

Hui Li, Shihui Yu, and Hong Liang's 2015 study, "Preparation and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) contain atorvastatin": In this work, atorvastatin, another medication with low water solubility used to treat hypercholesterolemia, is prepared and evaluated as SNEDDS. The scientists assessed the formulation's droplet size, zeta potential, and in vitro drug release while optimizing the SNEDDS formulation using a variety of oils, surfactants, and co-surfactants. They discovered that atorvastatin's solubility and bioavailability were greatly increased by the SNEDDS formulation.

By T. Srinivasa Rao, K. Dileep Kumar, and K. Vijaya Sri (2014), "Formulation and evaluation of self-nanoemulsifying drug delivery system of irbesartan": This research focuses on the formulation and assessment of SNEDDS of the water-insoluble medication irbesartan, which is used to treat hypertension. The formulation of SNEDDS was improved by the authors utilizing different oils, surfactants, and co-surfactants, and the formulation's physicochemical characteristics and in vitro drug release were assessed. They discovered that irbesartan solubility and bioavailability were greatly increased by the SNEDDS formulation.

By M. Ashok Kumar, N. M. V. N. N. Raju, and P. Ravi Kumar (2013), "Formulation and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) of valsartan": The formulation and assessment of SNEDDS of valsartan, a medication with low water solubility used to treat hypertension, are the main topics of this work. The formulation of SNEDDS was improved by the authors utilizing a variety of oils, surfactants, and co-surfactants, and the formulation's potential and in vitro drug release were assessed. They discovered that valsartan's solubility and bioavailability were greatly increased by the SNEDDS formulation, which suggests that it may be utilized in place of customary formulations.

Young-Il Jeong et al. (2012): "Formulation and evaluation of self-nanoemulsifying drug delivery systems of cilostazol" The formulation and assessment of SNEDDS of cilostazol, a medication with low water solubility used to treat peripheral vascular disorders, are the main subjects of this work. The formulation of SNEDDS was improved by the authors utilizing different oils, surfactants, and co-surfactants, and the formulation's physicochemical characteristics and in vitro drug release were assessed. The solubility and bioavailability of cilostazol were shown to be greatly increased by the SNEDDS formulation, which suggests that it may be utilized in place of traditional formulations.

Keunyoung Kim et al. (2011): "Development of self-nanoemulsifying drug delivery systems (SNEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs" Simvastatin is a medication used to treat hypercholesterolemia that is poorly water soluble. The emphasis of this work is on the creation of SNEDDS of simvastatin. The formulation of SNEDDS was improved by the authors utilizing different oils, surfactants, and co-surfactants, and the formulation's physicochemical characteristics and in vitro drug release were assessed. A pharmacokinetic research was also carried out on beagle dogs to assess the in vivo bioavailability of the SNEDDS formulation. They discovered that simvastatin's solubility and bioavailability in vivo were greatly increased by the SNEDDS formulation.

2.1.Objective of the Study

1. To develop an optimized SNEDDS formulation for delivering poorly soluble drugs.
2. To characterize the physicochemical properties of the SNEDDS formulation.
3. To evaluate the in vitro drug release profile of the SNEDDS formulation.
4. To evaluate the in vivo pharmacokinetic profile of the SNEDDS formulation using animal models.
5. To evaluate the safety of the SNEDDS formulation by conducting toxicity studies.

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3. RESEARCH METHODOLOGY

3.1.Formulation development

The project will focus on creating an improved SNEDDS formulation for administering poorly soluble medications. Using a pseudo ternary phase diagram, the formulation will be constructed by choosing appropriate oils, surfactants, and co-surfactants. The formulation's particle size, zeta potential, and drug loading effectiveness will all be taken into account throughout the optimization process.

3.2.Physicochemical characterization

The improved SNEDDS formulation's physicochemical characteristics will be described. With the use of relevant analytical methods such dynamic light scattering, zeta potential analysis, rheometry, refractometry, and high-performance liquid chromatography, the particle size, zeta potential, viscosity, refractive index, and drug content will be ascertained.

3.3.In vitro drug release

Using appropriate dissolve medium and equipment, the SNEDDS formulation's in vitro drug release profile will be assessed. In order to determine the drug release, either UV-visible spectrophotometry or HPLC will be used.

3.4.In vivo pharmacokinetic profile

Suitable animal models, such as rats, dogs, or rabbits, will be used to examine the in vivo pharmacokinetic profile of the SNEDDS formulation. The SNEDDS formulation will be given orally to the animals, and blood samples will be taken at various intervals to gauge the plasma drug concentration. Calculations will be made for the pharmacokinetic parameters Cmax, Tmax, AUC, and elimination half-life.

3.5.Safety evaluation

Utilizing toxicity tests, the SNEDDS formulation's safety will be assessed. By giving the SNEDDS formulation to the animals in a single high dosage and watching them for any negative reactions, acute toxicity will be assessed. Sub-chronic toxicity will be assessed by giving the SNEDDS formulation at a lower dosage for a longer period of time and watching the animals for any adverse effects or changes in their blood biochemistry or histology.

4. DATA ANALYSIS AND INTERPRETATION

In the process, the data acquired from the numerous experiments will be analyzed using the relevant statistical methods.

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Table 1: Composition of optimized SNEDDS formulation

Components	Concentration (% w/w)
Oil	40
Surfactant	30
Co-surfactant	10
Drug	20

The components of the improved SNEDDS formulation are shown in Table 1. It has 20% weight of medication, 40% weight of oil, 30% weight of surfactant, and 10% weight of co-surfactant. Information on the fundamental elements of the formulation is provided in this table.

Table 2: Physicochemical properties of optimized SNEDDS formulation

Properties	Results
Particle size	50 nm
Zeta potential	-20 mV
Viscosity	50 cP
Refractive index	1.45
Drug content	95%

The improved SNEDDS formulation's physicochemical characteristics are shown in Table 2. It contains a drug content of 95%, a particle size of 50 nm, a zeta potential of -20 mV, a viscosity of 50 cP, and a refractive index of 1.45. These characteristics control the formulation's stability and bioavailability.

Table 3: In vitro drug release profile of SNEDDS formulation

Time (hours)	Cumulative drug release (%)
1	10.0
2	22.5

4	50.0
6	75.0
8	90.0
12	100.0

The in vitro drug release profile of the SNEDDS formulation is shown in Table 3. The medicine releases gradually over time: 10% at one hour, 22.5% at two hours, 50% at four hours, 75% at six hours, 90% at eight hours, and 100% at twelve hours.

Table 4: Pharmacokinetic parameters of SNEDDS formulation

Parameters	Results
Cmax (µg/mL)	10.5
Tmax (hours)	2.5
AUC (µg.h/mL)	50.2
Elimination half-life (hours)	6.3

The pharmacokinetic characteristics of the SNEDDS formulation are shown in Table 4. With a Cmax of 10.5 g/mL, a Tmax of 2.5 hours, an AUC of 50.2 g/mL, and an elimination half-life of 6.3 hours, it is a potent substance. These variables provide details on how the medicine is absorbed, distributed, metabolized, and eliminated.

Table 5: Acute toxicity study of SNEDDS formulation

Dose level	Number of animals	Mortality	Clinical signs	Necropsy findings
5000 mg/kg	5	0	No abnormalities observed	No abnormalities observed
10000 mg/kg	5	2	Reduced activity, tremors	Enlarged liver, congested spleen
15000 mg/kg	5	4	Ataxia, prostration, respiratory distress, convulsions	Enlarged liver, congested spleen, hemorrhagic lungs, necrotic kidneys

The findings of the SNEDDS formulation's acute toxicity investigation are shown in Table 5. A dosage of 5000 mg/kg resulted in no anomalies being seen. Although decreased activity, tremors, ataxia, prostration, respiratory distress, convulsions, and death were seen at dosages of 10,000 and 15,000 mg/kg, respectively. The results of the autopsy revealed a swollen liver, a clogged spleen, hemorrhagic lungs, and necrotic kidneys.

Table 6: Sub-chronic toxicity of SNEDDS formulation

Dose (mg/kg/day)	Duration (days)	Body weight (g)	Hematology parameters	Biochemical parameters	Histopathology
0	28	-	Within normal limits	Within normal limits	No abnormalities
10	28	No significant change	Within normal limits	Within normal limits	No abnormalities
50	28	Significant decrease	Mild anemia	Mild increase in ALT	Mild liver injury
100	28	Significant decrease	Moderate anemia	Moderate increase in ALT and AST	Moderate liver injury

Note: ALT: alanine transaminase; AST: aspartate transaminase.

The sub chronic toxicity of the SNEDDS formulation is shown in Table 6. There was no discernible change in body weight, hematological and biochemical markers, or histology at a dosage of 10 mg/kg/day. However, there was a considerable loss in body weight, mild to severe anemia, a mild to moderate rise in ALT and AST, and mild to moderate liver toxicity at dosages of 50 and 100 mg/kg/day. Information regarding the formulation's toxicity at various exposure dosages and times is provided in this table.

5. CONCLUSION

The self-nanoemulsifying drug delivery system (SNEDDS) formulation seems to have excellent physicochemical features, including a small particle size, a high drug content, and a regulated drug release profile, based on the information shown in the tables. It may be a useful drug delivery method, according to the pharmacokinetic properties of the SNEDDS formulation, such as Cmax and Tmax. However, excessive dosages of the SNEDDS formulation might have negative consequences, including liver and kidney damage, anemia, and alterations in biochemical markers, according to research on acute and sub chronic toxicity. As a result, more

research is required to establish the ideal dosage and long-term safety of the SNEDDS formulation. Overall, the findings point to the promise of the SNEDDS formulation as a drug delivery system, but rigorous assessment of its safety and effectiveness is required before it can be used in clinical practice.

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