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ORIGINAL ARTICLE

Antioxidant and antimicrobial study of *Schefflera vinosa* leaves crude extracts against rice pathogens

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Abstract Plant extracts are one of the best possible sources of bioactive molecules, and are being used globally as an antioxidants and natural antimicrobial compounds. In current study, *Schefflera vinosa* leaves extract was prepared through Soxhlet extraction procedure using methanol and chloroform as solvents. The extract was investigated for total antioxidant, phenolic and flavonoid contents, free radical scavenging and antimicrobial activities. The free radical scavenging activities were evaluated through 2,2- diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzotiazolin-6-sul fonic acid (ABTS) and Ferric-reducing/ antioxidant power (FRAP) assay. The antimicrobial activity of extract was determined through poisoned food method. The methanolic extract has exhibited high antioxidant, phenolic, and flavonoid activities compared to chloroform extract. Similarly, free radical scavenging activities (ABTS, DPPH and FRAP) were higher in methanolic extract. Further, Fourier-Transform Infrared Spectroscopy (FTIR) used to determine the functional group and Gas chromatography-mass spectrometry (GC–MS) to elucidate volatile composition of the crude extract. Different functional group like N-H, O-H, C-O, C-N, C-H, C = O, C≡C and C-O-H presence indicate the existence of many metabolites in the extracts. GC–MS study identified 61 compounds and subsequently, these molecules were screened virtually using DockThor. Furthermore, antimicrobial study was confirmed against rice pathogens like *Magnaporthe oryzae (M. oryzae) and Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Molecular docking study further suggested that phytomolecules (3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane, and 2-Methoxy-5-methylthiophene) targets Histone Deacetylase (HDAC) of *M. oryzae* and Peptide

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Deformylase (PDF) of *Xoo*, which could inhibit their growth. Hence, this study indicated that *Schefflera vinosa* extracts could be an important ingredient as an antioxidant as well as antimicrobial agent against rice pathogens.

1. Introduction

Plants tissue contains variety of chemical compounds like alkaloids, phenolics, quinones, flavonoids, terpenoids etc. which regulates various biological functions such as antioxidant, antifungal, antibacterial and antimicrobial activities. Conventional drugs (25%) and primary healthcare need up to 80% relies essentially on plants (Johnson and Ayoola, 2015). Plant based antioxidants like carotenes, flavonoids, phenolics, tannins and vitamins are efficient and potent in controlling the destructive processes induced by the free radicals (Gruz et al., 2011). Synthetic/semi-synthetic antibiotics are used to treat various diseases in modern medicine. However, it is costly and extended use develops resistance in pathogens to antibiotics, leading consequential therapeutic failure. This has changed the global interests in using plant extract and their derived compounds for therapeutic and other uses like in agriculture (Hassine et al., 2014). Various botanicals from different sources have shown excellent curative potential against chronic diseases in humans (Kalita et al., 2012). Precisely, plants represent essentials pool of such chemical compounds which are required for novel drug discovery.

Often human cells fail to produce sufficient quantity of antioxidants as required and hence, they depend on synthetic supplements like Butylated Hydroxy Toluene (BHT) and Butylated Hydroxyanisole (BHA), to mitigate the oxidative stress. However, prolonged consumption often produce toxicity which led to mutagenic and carcinogeniceffects (Bendiabdellah et al., 2012). Thus, natural sources of antioxidants are in high demands and plants offer the best option of safe dietary antioxidants which react with free radicals and protect cells without triggering any side effect (Prasad et al., 2010). Over the years, growing awareness about life style diseases and understanding the importance of preventative healthcare measures, the demand of natural antioxidants in nutraceuticals, pharmaceutics, cosmetics and food industry has escalated immensely.

The two most devastating diseases of rice includes rice blast and bacterial blight caused by *Magneporthe oryzae* (*M. oryzae;* fungi) and *Xanthomonas oryzae pv. oryzae* (*Xoo*; bacteria), respectively (Kumar et al., 2020). Generally, chemical pesticides are used to manage these diseases and minimize yield loss. However, hazardous effects of synthetic pesticides have resulted into serious health implications on human, non-targeted organism and environment enormously. Unfortunately, the indiscriminate and unscientific use has developed resistance in pathogen and resurgence of serious diseases. Presently, a target specific and least toxic pesticides are required for better management of plant diseases and environment simultaneously. Therefore, the current global change has shifted more towards organic foods due to increasing awareness of consumers. Considering the welfare of environment and consumes health, many synthetic pesticides have been either banned or withdrawn. Thus, botanical pesticides or biopesticides are getting prodigious attentation as an alternative to synthetic pesticides specifically to control agricultural pests. Botanicals are considered to conserve eco-friendly profile. It is also cost-effective, less toxic and highly biodegradable compared to conventional pesticides (Campos et al., 2019).

Raw materials for botanical pesticides are enormous, if used strategically, particularly less exploited plants like *Schefflera vinosa*. It belongs to Araliaceae family, popularly known as Devsemal. Traditionally, extracts of this plants have been used in treating various diseases like asthma, dropsy, arthritis, kidney problem, liver and heart disease (Kennedy et al., 2010). Few compounds of *S. vinosa* like Quercetin 3-O-β-D-rhamnoside has been reported as antiparasitic (Ndjonka et al., 2013). However, there is a dearth of knowledge about its antimicrobial activities against phytopathogens. In Amarkantak region (Madhya Pradesh, India) it is found at an average elevation of 3000 ft and hence, a different phytochemical profile is expected.

In the present study, we performed phytochemical profiling and antimicrobial studies of *S. vinosa* crude extract. Functional group and volatile compounds present in crude extract were also identified through FTIR and GC–MS studies respectively. *In silico* molecular docking and virtual screening was also performed for best ligands identification from GC–MS result which could potentially bind with Histone Deacetylase (HDAC) receptor of *M. oryzae* and Peptide Deformylase (PDF) receptor of *Xoo* and inhibit their growth.

2. Experimental section

2.1. Strains and reagents

Virulence strains of *Xoo* and *M. oryzae* were collected from the National Institute for Plant Biotechnology (NIPB), Pusa Campus, New Delhi, India. Potassium ferricyanide, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt] ABTS, Trichloroacetic acid, Phosphate buffer, 3,5-Di-*tert*-4-butylhydroxytoluene (BHT) was obtained from Sigma (St. Louis, MO, USA). Ascorbic acid, quercetin, gallic acid, potassium persulfate, ammonium molybdate, sodium phosphate, sulphuric acid, Folin – Ciocalteu, aluminum chloride, potassium acetate, sodium carbonate, methanol, chloroform, potato dextrose agar, sucrose, peptone, n-glutamic acid was purchased from Hi-Media, India. All reagents used in the study were of analytical standard.

2.2. Collection and authentication of plant material

Leaves of *S. vinosa* (Cham. & Schltdl.) were collected in the month of February 2018 from Indira Gandhi National Tribal

University (IGNTU), Amarkantak, Madhya Pradesh (India) campus [(geographical coordinates (22° 48′ 23.06″ N 81° 44′ 58.17′E)]. The taxonomic identification of specimen was performed and stored at Herbarium, Department of Botany, IGNTU with Voucher specimen no. AM/DOB/018.

2.3. Extraction and yield quantification

Plant experimental material was prepared from the cleaned dried leaves ground into fine powder through mechanical grinder. Further, 15 g of powder was mixed in 120 ml methanol (80%) and 120 ml chloroform (95%). Then, it was kept on rotary shaker for 48 hrs at room temperature (RT). The slurry obtained was centrifuged at 10,000 rpm for 15 min. The supernatants were filtered out into a 150 ml volumetric flask and to evaporate it was incubated at 40°C in water bath. The crude extracts were collected and preserved in refrigerator for performing experiments. The percentage yield of crude extract was calculated as: WC/WP × 100 where WC = weight of crude extract WP = powdered plant material weight.

2.4. Phytochemical composition and antioxidant activity

Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant content (TAC) of both methanolic extract (ME) and chloroform extract (CE) were determined following standard protocols (Marinova et al., 2005; Kumar et al., 2011; 2013). Gallic acid, quercetin and ascorbic acid was taken as a standard for TPC, TFC and TAC respectively.

Antioxidant ability of the extract was calculated through free radical scavenging assay (FRSA) methods. DPPH and $ABTS^{+\cdot}$ free radical scavenging activity were evaluated following standard protocols (Kumar et al., 2013). Ascorbic acid and BHT were taken as standard for DPPH and ABTS+· FRSA respectively. The hydroxyl radical scavenging effect and DNA nicking experiments of both the extracts were also performed to validate the FRSA of the extracts (Lee et al., 2002).

2.5. DNA damage protection activity

Fenton's reagent generated hydroxyl (·OH) radicals damages the native structure of plasmid DNA (supercoiled). When plant extracts are added, it scavenges the ·OH radicals and preserve the supercoiled nature of plasmid DNA, is confirmed through agarose gel electrophoresis, termed as DNA nicking assay. In this study, ME and CE ·OH radicals scavenging ability was tested using *pGEM-T* plasmid as explained by Lee et al., 2002. The reaction mixture was made in 2 separate micro centrifuge tube (one for ME and another for CE) using 5 µl of plasmid DNA (150 ng/µl) and 10 µl of Fenton's reagent (30 mM H_2O_2 + 50 mM ascorbic acid and 80 mM FeCl3). Subsequently, 5 µl of each extract were added in both micro centrifuge tube. Next, tubes were incubated at 37 °C for 45 min followed by adding 2.5 µl of loading dye (0.25% bromophenol blue, 50% glycerol). DNA damage outcomes were analyzed by running 1% agarose gel electrophoresis and banding patterns were seen by ethidium bromide using Gel Doc system.

2.6. Antimicrobial activity

The antimicrobial activity of ME was evaluated against *Xoo* and *M. oryzae* on PDA solid medium amended with 25 mg/ml, (1 ml) in petri dishes following poisoned food method (Mohana and Raveesha, 2007). *M. oryzae* and *Xoo* inoculated plates were raised at 23 °C and 28 °C respectively up to 14 days. Afterward, the radial microbial growth was recorded on 4^{th}, 8^{th} and, 14^{th} days after inoculation (DAI) for both *Xoo* and *M. oryzae* using Vernier caliper. The development of inhibition zones was noted, documented and taken as an indication for the extract activity (Balouiri et al., 2016). All experiments were performed in three replicates. PDA media without extract amended was used as a control.

2.7. FTIR and GC–MS spectroscopy study

The FTIR spectral analysis of crude extract was performed at RT through ATR instrument, Thermo Nicolet iS5 (Thermo Scientific, Madison, WT, USA) following standard protocol (Schulz and Baranska, 2007). All reading were recorded in the spectral range of 4000–500 cm^{-1} at 4 cm^{-1} resolution. The air spectral generated background noise was eliminated and ATR crystal surface were repeatedly cleaned using 70% ethanol prior to each experiment.

Gas chromatography-mass spectrometry (GC–MS) study of crude extract was accomplished through GC–MS system (GCMS-QP2010, Shimadzu, Kyoto, Japan) following standard protocol (Semwal and Painuli, 2019). This instrument is fortified with an auto injector (AOC-20i) and head spacer (AOC-20 s). Approximately 1.0 µl of extract (1 mg/ml) was injected into the silica capillary column (Rtx-5) using Helium (1.2 ml/min) as carries gas. Unique peaks were detected through MS detector and compounds were identified based on retention time. Finally, spectra of each compounds were equated with library data of WILEY8 and NIST14. One full cycle of GCMS was completed in 33 min.

2.8. In-silico studies

2.8.1. Protein receptors and ligands preparation

The 3D structure of histone deacetylase (HDAC) (RPD3) from *M. oryzae* (PDB ID 4LXZ), and peptide deformylase from *Xoo* (PDB ID 5CY8) were downloaded from the Protein Data Bank (PDB) (http://www.wwpdb.org/). PyMOL (https://pymol.org/2/) was used to remove all non-protein molecules (Joshi et al., 2020). The configuration of the ligands was drawn using ChemDraw (https://perkinelmerinformatics.com/products/research/chemdraw/) and were translated into 3D SDF format (Mendelsohn, 2004). Subsequently, the SDF format were cross checked with ligands downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Usha et al., 2013). Docking analysis was performed to search a compound and predict the best binders based on scores. The active site prediction (ASP) of the targeted protein was done through the Computed Atlas of Surface Protein Topography (CASTp 3.0) (Tian et al., 2018).

2.8.2. ADME-toxicity prediction

The pharmacodynamics and pharmacokinetic profile of promising molecules was predicted using open online tool SwissADME (http://www.swissadme.ch/). It is very useful to screen molecules on the basis of absorption, metabolism, distribution, excretion, and toxicity, which virtually indicate the drug like properties of phytochemicals (Daina et al., 2017). Probable drug like and no drug like characteristic of a compounds were also evaluated using Lipinshi Rule of Five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

2.8.3. Virtual screening and LigPlot studies

Virtual screening was performed by rigid docking against the active site of specific pathogen molecules through DockThor (https://www.dockthor.lncc.br/v2/). Total binding energy between hydrogen bonds, cation-π bonds, π-π bonds and ionic interaction between ligands and proteins were studied. The distance between interacting amino acids and the subjected molecules active sites were also analyzed. Further, the most suitable and top pose ligands were selected based of its confirmation and binding energy (Kandeel and Kitade, 2013). Finally, a table was extracted, withholding lowest free binding energy (kcal/mol) and best pose ligands following standard protocol (Lee et al., 2012).

Subsequently, LigPlot (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) analysis was performed to find out protein–ligand interface after docking. It automatically produces diagrams of interacting molecules like protein–ligand interaction in PDB format. Generally, the interaction is facilitated by H-bonds and hydrophobic interactions. The H-bond and hydrophobic interactions are indicated by dashed lines between involved atoms and arc with spokes respectively. The hydrophobic contacts represented by arc and spoke radiate towards contacting ligands while contacted atoms are represented as spoke radiating back. Additionally, it explains the in-depth interaction pattern of participating amino acids between the docked ligands and the residues of active site proteins (Bharatham et al., 2008).

2.9. Calculation and statistical analyses

ME and CE biochemical assay were executed in triplicates independently. The percentage inhibition of free radicals was enumerated exploring formula % inhibition = $[(A_{Control} - A_{Extract})/A_{Control}] \times 100)]$. The results were stated as the mean ± standard deviation (SD) of triplicates. The statistical analysis was executed employing one-way ANOVA ($p < 0.05$). The statistical investigation and graph were made using Python script, origin 8.5.

3. Results and discussion

3.1. Extraction yield, total phenolic, total flavonoid and total antioxidant

Efficient extraction method is very crucial in getting better yield and activities. The percentage yield of ME was better (10.85%), compared to CE 07.30%. Few compounds of phenolics and flavonoids forms complexes with carbohydrates and proteins in methanol, which are more extractable. This could be region of higher yield in methanol, compared to chloroform. Further, both of the extract (ME and CE) were subjected for TPC, TFC and TAA determinations. Phenolics are plant secondary metabolites and regulates antioxidant activities in several ways such as chelating redox active metal ions, disabling metals from radical chains and dodge hydroperoxide alteration into ROS (Sahreen et al., 2010). The TPC in ME was (52.83 ± 0.02 mg GAE/g dw) and in CE (10.66 ± 0.001 mg GAE/g dw). Higher quantity of flavonoid in any plant manifests themselves as good source of antioxidant. This augments the overall antioxidant activity and protect the cells from lipid peroxidation (Sharififar et al., 2009). The TFC in ME was (49.08 ± 0.007 mg QE/g dw) and in CE (42.72 ± 0.005 mg QE/g dw). The TPC and TFC were entirely synchronous. It appears that extract containing higher amount of phenolics also contains higher amount flavonoids and both (TPC and TFC) ultimately augment the antioxidant activity of an extract and guard cells from lipid peroxidation (Sharififar et al., 2009). The TAA in ME and CE was (328.80 ± 0.003 mg AAE/g dw) and (189.40 ± 0.001 mg AAE/g dw) respectively. ME demonstrated significantly higher amount of TPC, TFC and TAA compared to CE extract, which further revealed the correlation among TPC, TFC and TAA (Table 1).

3.2. Evaluation of free radical scavenging activity

Various methods (DPPH, FRAP and ABTS) were adapted to evaluate the free radical activity (FRA) of extract. DPPH is one of the most accepted screening methods to understand the antioxidant property of plant extract, because this experiment takes short time and less amount of sample. Its violet colour solution changes to yellow colour product (diphenylpicryl hydrazine) post extract addition in the concentration dependent manner. This experiment revealed that ME has superior FRSA than CE and control. Both, ME and CE were proficient to reduce DPPH· radicals by 49.44 ± 0.1% and 29.85 ± 0.04% respectively at 300 μg/ml. The IC_{50} values of ME and

Table 1 Phytochemical composition, and antioxidant activity of Methanol (ME) and chloroform crude extract (CE) of *Schefflera vinosa* leaves.

	Phytochemical composition	
	Methanol extract	Chloroform extract
Total phenol content (mg GAE/g)	52.83 ± 0.02	10.66 ± 0.001
Total flavonoid content (mg QE/g)	49.08 ± 0.007	42.72 ± 0.005
Antioxidant activity		
DPPH radical scavenging activity	311.63 ± 0.91	521.11 ± 0.52
Reducing power (Abs. at 700 nm)	246.00 ± 0.01	191.00 ± 0.01
ABTS radical scavenging activity	18.76 ± 0.43	20.64 ± 0.49
Total antioxidant activity (mg AAE/g)	328.80 ± 0.003	189.40 ± 0.001.

CE were 311.63 ± 0.91 and 521.11 ± 0.52 µg/ml respectively. The inhibition proportion of positive control (ascorbic acid) was 97.27% at 300 µg/ml and IC_{50}value of standard DPPH was 38.75 ± 0.23 µg/ml. The DPPH scavenging ability of *S. vinosa* leaves could be due to abundance of polyphenols and flavonoids in the extract.

The polyphenols antioxidant activity was estimated by its reducing power ability. It has been used to measure the antioxidant activity of extract as a quick and simple method (Kumar et al., 2013). In FRAP assay the values for ME and CE were 246.00 ± 0.01 mg AAE/gdw and 191.00 ± 0.01 mg AAE/gdw, respectively. FRAP activity of ME was better, compared to CE, studied in concentration dependent manner (60–220 µg/ml), suggesting better potential of ME over CE to react with free radicals and stop the reaction. Here, we inferred that the occurrence of polyphenols in the extract plays important role in free radical scavenger via donation e^-/H; thus, relay on the reducing power capacity of the extract.

The reaction between ABTS and $K_2S_2O_8$ leads to convert ABTS into blue colour radical cation ($ABTS^{\cdot+}$), which absorbs light at 734 nm (Prior et al., 2005). The $ABTS^{\cdot+}$ has been stated to be reactive to many antioxidants including phenols, thiols and vitamins (Walker and Everette, 2009). In this study, ME and CE both exhibited better inhibition of ABTS radical formation by 92.25 ± 0.80% and 91.64 ± 0.39% at 260 µg/ml extract concentration. Accordingly, the changes in the IC_{50} value of ME (18.76 ± 0.43 µg/ml) and CE (20.64 ± 0.49 µg/ml) was observed. Hence, ME could be a potential inhibitor of free radical and therefore, could be a suitable choice in averting chain reaction during lipid peroxidation and could also aid in developing possible nutraceuticals (Table 1; Fig. 1).

3.3. Plasmid DNA damage protection activity

Free radicals are well known to cause DNA damage to various extent and aggravate several diseases. We examined the plasmid DNA (pGEM-T) damage protection activity of ME and CE against oxidative damage caused by Fenton's reagent. Fenton's reagent generated Hydroxyl (·OH) radicals, which is known to cause breaks in the DNA molecule results in to the production of relaxed or linear plasmid DNA (Lee et al., 2002). This reduces the flow of damaged DNA in agarose gel compared to intact native DNA during electrophoresis. However, the addition of ME and CE together with Fenton's reagent has prevented the supercoiled plasmid DNA from nicking, signifying the neutralizing action of extract on Fenton's reagent-based DNA impairment. Therefore, both ME and CE have showed considerable protective activity by minimizing the effect of the oxidative stress on the plasmid DNA from the destructive reaction of ·OH radicals formed by Fenton's reagent (Fig. 2). Possibly, plants enriched with various phenolic compounds and other antioxidant molecules are able to protect their genetic material against the free radical induced DNA damages (Salar et al., 2017; Paramaguru et al., 2013; Aryal et al., 2019). Similar properties (extract mediated plasmid DNA protection) have been also reported in other plants as well (Singh et al., 2021, Mansoori et al., 2021)

3.4. Antimicrobial activity against Xoo and M. oryzae

The antibacterial and antifungal activity of ME and CE were performed against *Xoo* and *M. oryzae* on PDA media using poisoned food method. PDA media amended with 25 mg/ml (ME) showed inhibitory effect on *M. oryzae* growth. In ME, mycelial growth of 0.1 ± 0.05 cm and 1.8 ± 0.10 cm was recorded on 4th and 8th DAI (, which was significantly lower than control blank (CB) (1.9 ± 0.05 cm and 5.8 ± 0.05 cm) and control methanol (CM) (1.9 ± 0.15 cm and 5.8 ± 0.15 c m), respectively. Similarly, on 14th DAI, the inhibition of fungus growth was prominent in ME (3.8 ± 0.10 cm), compared to CB (7.9 ± 0.05 cm and CM (7.9 ± 0.15 cm (Table S1A; Fig. S1). In terms of % inhibition, it ranged between 45.78% – 51.89% in ME.

Interestingly, similar inhibitory trend of ME was also noted against *Xoo* with respect to the CB and CM. ME was very competent against *Xoo* as it inhibited bacterial growth up to 0.7 ± 0.01 cm and 1.5 ± 0.05 cm on the 4th & 14th DAI respectively (Table S1B and Fig. S1). In terms of % inhibition, 53.12–55.88% was ascertained in the case of *Xoo* on 14th DAI. Slightly better % inhibition was observed in case of *Xoo* then *M. oryzae*. Previous study suggests that the subsistence of several secondary metabolites like polyphenols, tannins, flavo-

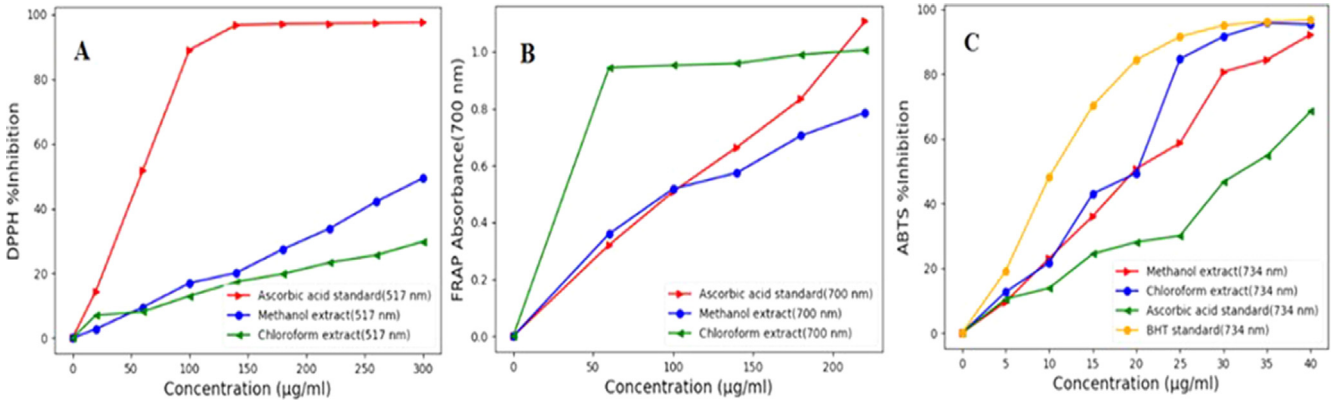

Fig. 1 Antioxidant activity of methanol and chloroform leaf extract of *Schefflera vinosa* (A) DPPH radical scavenging activities (B) Ferric reducing capacity (FRAP) (C) ABTS radical scavenging activity of extract at varying concentrations. Values represented as mean ± SD (n = 3, P < 0.05).

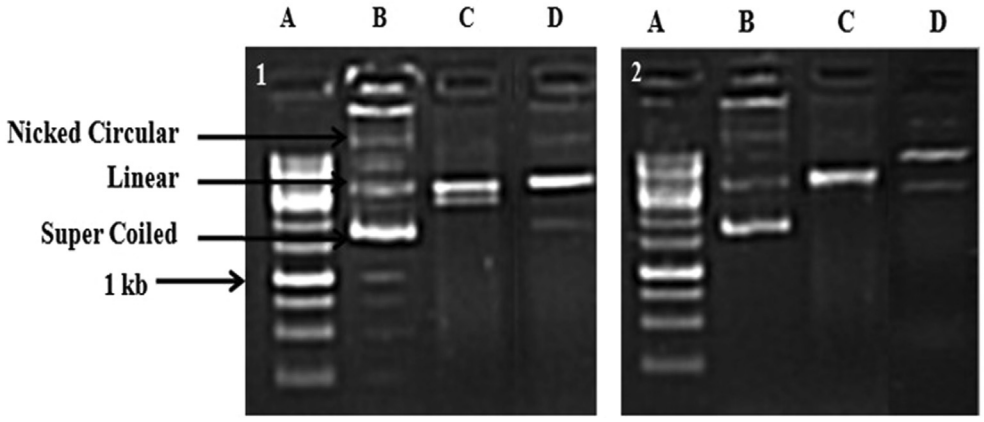

Fig. 2 DNA damage protecting activity of methanol and chloroform leaf extract of *Schefflera vinosa* against hydroxyl radical induced DNA damage of pGEM-T plasmid. Lane 1: Ladder 1 kb; Lane 2: pGEM-T plasmid DNA; Lane 3: DNA + Fentoń's reagent; Lane 4: DNA + Fentoń's reagent + extract (5 mg/ml). Gel 1: ME and Gel 2: CE.

noids, phenols and saponins in plant extracts forms complexes with bacterial proteins and may inhibit enzymatic activity which could result in inhibition of bacterial growth (Khan et al., 2011). The solubility of extract constituents also determines the efficacy of extract against microorganisms. The superior antimicrobial properties of extracts could be associated to the quantity of some enzymes like peroxidases in the plant along with other phytochemicals (Mansoori et al., 2021). Our result clearly demonstrates the efficiency of ME against *Xoo* and *M. oryzae*, which is the first steps towards realizing its therapeutic usage against rice pathogens.

3.5. FTIR and GC–MS characterization

FTIR spectroscopy experiment was executed to detect the unique chemical fingerprint existing in the extract. The functional group of active component present in an extract was determined based on spectrum obtained in the IR radiation region. FTIR spectrum of ME (Fig. 3A) and CE (Fig. 3B) revealed the presence of several functional group including phenols, amines, alkyne alcohols, alkenes, aldehyde carboxylic acids, aliphatic compounds, aromatic compounds and carbonyl compounds. At various frequencies, potent bands were detected which specify the occurrence of N-H, O-H, C-O, C-N, C-H, C=O, C≡C and C-O-H skeletal vibrations. The IR spectrum of both ME and CE were similar, which could be due to existence of common functional group in active compounds (Table S2). However, few significant differences were observed like peak numbers and skeletal vibration at various wavenumbers (cm−1). Convincingly, FTIR detected the existence of phenolic compounds, proteins, primary & secondary amines, aromatic ethers and phenols,

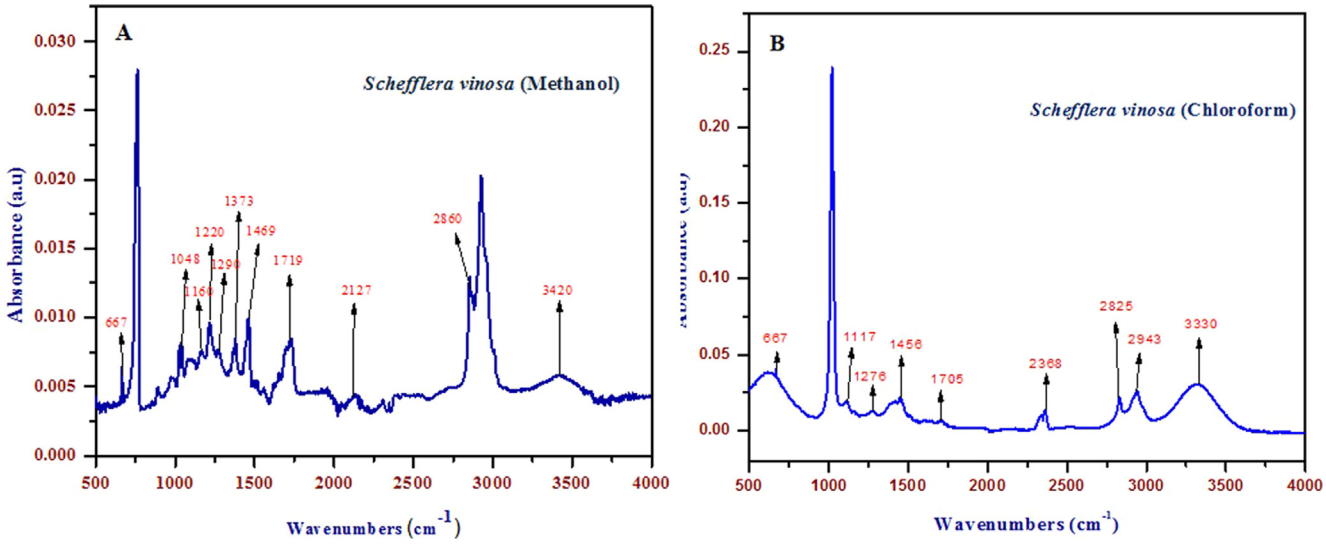

Fig. 3 FTIR spectrum analysis of methanol and chloroform leaf extract of *Schefflera vinosa*. (A) Chromatogram of ME (B) Chromatogram of CE. Both ME and CE chromatogram represented similar infra-red spectrum (IR) profile probably due to presence of polar nature of molecules in extracts. Intense bands were observed at various frequencies which indicate the existence of several metabolites like N-H, O-H, C-O, C-N, C-H, C=O, C≡C and C-O-H in the extract.

aldehyde and carbonyl compounds and fatty acids, that are most likely to responsible for the antimicrobial properties of the *S. vinosa* extracts. However, FTIR alone could not detect all active compound of the active constituents of the extract and hence, GC–MS was carried out.

GC–MS is considered as one of the best method available for the detection of nonpolar components, volatile molecules, fatty acids and lipids. In the current study, in ME, 61 peaks were identified based on peak area and retention time (Table 2). Subsequently, acquired compounds mass from GC–MS spectra (Fig. S2) were matched with database compounds of WILEY8.LIB and NIST08.LIB. The major component of the extract comprises Kinic acid (33.53%), 5-Methyl-2(3H)-furanone (3.54%), Formic acid, allyl ester (2.34%), Guaiacol (7.93%), 1-Oxaspiro [3.5] nona-5,8-dien-7-one, 3-methylene (7.24%), 2-Methoxy-4-vinylphenol (6.72%), Syringol (5.86%), Cytosine riboside (7.74%), and 2,2′-Bioxirane (1.70%). Several identified compounds possess various biological affairs including antioxidant, antifungal, anticancer, antibacterial, insecticidal, antidiabetic, anti-inflammatory etc. Many identified compounds of GC–MS are extensively used in aroma, pharma, detergent, perfume, food and beverage industries, as flavor, medicine, antiseptic, propellent, disinfectant and pesticides (Swamy and Sinniah, 2015; Semwal and Painuli, 2019). These chemical compounds are also considered as an integral part of plant defense system and hence, referred as phytoanticipins (Salehi et al., 2019). Thus, identification of different phytochemicals from ME displayed the endowed medicinal properties of *S. vinosa*. These finding could help to evaluate the multipurpose use of *S. vinosa* plant more rationally specifically as a potential antimicrobial natural source.

3.6. Protein receptors and ligands preparation

Receptors and ligands are prepared to be used as an input for DOCK calculation and prediction of ligand orientation in receptor active site. *In vitro* study clearly demonstrated the antimicrobial activity of ME. Hence, there could be some compounds in the extract participating in inhibition of pathogens like *Xoo* and *M. oryzae*. Previous study suggests that bacterial and fungal growth can be inhibited by targeting Peptide deformylase (PDF) and Histone Deacetylase (HDAC) (Fieulaine et al., 2016; Joshi et al., 2020; Mansoori et al., 2021). Legends (GC–MS identified compounds) were prepared to create 3D geometries and to assign proper bond order. Heteroatoms were removed, charged groups were neutralized, ionization states were generated and geometry were optimized prior to virtual screening to identify stronger binders. Two best legends namely 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and 2-Methoxy-5-methylthiophene were prepared in this study. Subsequently, all non-protein molecules were removed from receptors before final screening from HDAC and PDF receptor. Prepared legends and receptors are illustrated in Fig. 4.

3.7. Active site prediction (ASP) of target proteins

Active site pocket contains both functional and non-functional residues. Functional residues participate in ligand binding whereas non-functional residues do not participate in ligand binding. Sometimes mutation in active site alter the residues binding the legends and this could prevent the use of active site directed drug development. Moreover, active site pockets are required to bind legends with receptors (Andreotti et al., 2011). In current study, CASTp server was used to predict every single pockets along with cavities. Total surface area and volume covered by these pockets and cavities were also measured. Pockets are considered to contains the binding sites for enzymes (Yang et al., 2015). The CASTp server predicted 50 pockets in ADHC receptor whereas 23 pockets were found in PDF receptors. The amino acids created the active sites of ADHC (PDB ID 4LXZ) protein includes Met35, Gly143, Leu144, His145, His146, Gly154, Phe155, Cys156, Asp181, His183, Phe210, Asp269, Leu276, Gly305, Gly306, and Tyr308. The total area and volume covered by active site was 70.367 and 24.564 respectively. In case of PDF receptor (PDB ID 5CY8), virtual screening exposed the presence of various active amino acid residues viz Gly44, Val45, Gly46, Gln51, Arg68, Tyr69, Trp96, Glu97, Gly98, Cys99, Leu100, Ile102, Pro103, Gly104, Leu105, Arg106, Phe134, Arg137, Val138, His141, Glu142, His145, and Asp164. PDF receptor covered an active site region 224.868 and volume 96.21919 (Fig. S3). Interestingly, Gly, Leu, His, Phe, Cys, Asp, and Tyr were found to be present in both receptors and could be involved in specific molecular events. The presence of these amino acids residues in active sites might be determining the functioning of receptors.

3.8. Virtual screening assessment of receptors using ligand profiles

An automatic DockThor docking software (https://dockthor.lncc.br/v2/) was used for intermolecular interactions study between the ligand and the target proteins. It conducts energetic grid-based ligand docking and looks for beneficial associations between tiny ligand molecules and usually larger receptor molecule. All 61 ligands were screened against Histone Deacetylase (HDAC) RPD3 and PDF receptor (Table 2). On the basis of the highest docking score and lowest binding energy, the best ligand was identified and hypothesized as the most effective ligand against each target receptor. The best legends identified were 9-eicosene (-7.773 kcal mol^{-1} for PDF and -7.612 kcal mol^{-1} for HDAC), Methyl 10,12-pentacosadiynoate (-7.919 kcal mol^{-1} for PDF and -7.915 k cal mol^{-1} for HDAC), E-15-Heptadecenal (-7.573 kcal mol^{-1} for PDF and -7.535 kcal mol^{-1} for HDAC), 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane (-7.697 kcal mol^{-1} for PDF and -8.012 kcal mol^{-1} for HDAC), Flexricin P-4 (-7.575 kcal mol^{-1} for HDAC), Cyclohexene-d1 (-7.543 kcal mol^{-1} for PDF), 5H-Cyclopenta[b]pyridine (-7.817 kcal mol^{-1} for PDF), Tetradec-7-ene (-7.555 kcal mol^{-1} for PDF) and Butyl 2-ethylhexyl phthalate (-7.890 kcal mol^{-1} for HDAC) (Table S3). Based on the above study, it can be assumed that these molecules are antimicrobial in nature and could be used in agriculture and pharmaceuticals industries after experimental confirmation to develop environmentally friendly antimicrobial agents to control plant diseases specifically rice diseases.

Table 2 Bioactive compounds identified in the methanolic crude extract of *Schefflera vinosa* leaf in GC–MS.

Sr No	R. time	Area/ Height (A/ H)	Compound name	Mol. formula	Mol. weight (g/mol)	Biological functions
1	22.306	2.49	9-eicosene	$C_{20}H_{40}$	280	Anti-phytoapthogenic, Antifungal, Anti-inflammatory, Anti-cancer
2	11.221	3.03	3-(Dimethylsiloxy)-3,3-dimethyl-1-propene	$C_7H_{15}OSi$	144	Anti-fouling
3	3.701	4.03	l-Alanine, N-methoxycarbonyl-, methyl ester	$C_6H_{11}NO_4$	161	Anti-malarial, Anti-inflammatory, Antiandrogenic
4	15.831	2.38	4-(1,3-Butadienyl)-3,5,5-trimethylcyclohex-2-en-1-one	$C_{13}H_{18}O$	190	Anti-tumor, anti-microbial
5	26.787	2.96	Phytol	$C_{20}H_{40}O$	296	Anti-inflammatory, Anticancer Antimicrobial, Diuretic
6	24.235	2.45	Flexricin P-4	$C_{21}H_{38}O_4$	354	Anti-cancer, Anti-fungal
7	15.716	3.62	Methyl 10,12-pentacosadiynoate	$C_{26}H_{44}O_2$	388	Anti-microbial
8	8.255	5.95	Cyclohexene-d1	C_6H_9D	83	Anti-tumor
9	12.171	4.24	2-Chlorocycloheptanol	$C_7H_{13}CIO$	148	Anti-cancer, Anti-microbial
10	19.728	3.01	3-Hydroxy-.beta.-damascone	$C_{13}H_{20}O_2$	208	Anti-inflammatory, Anti-cancer
11	16.959	3.82	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	Anti-fungal
12	15.831	3.28	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone	$C_{13}H_{18}O_2$	206	Antihistamine
13	25.175	2.65	E-15-Heptadecenal	$C_{17}H_{32}O$	252	Anti-phytopathogenic, Anti-inflammatory
14	17.329	2.98	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-3-en-2-one	$C_{13}H_{18}O$	190	Anti-oxidant
15	18.905	3.52	6-Methoxycoumaran-7-ol-3-one	$C_9H_8O_4$	180	Anti-cancer
16	14.274	4.49	5H-Cyclopenta[b]pyridine	C_8H_7N	177	Urinary incontinence, anxiety, obesity
17	13.035	17.07	1-Oxaspiro [3.5] nona-5,8-dien-7-one, 3-methylene-	$C_9H_8O_2$	148	Anti-inflammatory, Hypoglycemic, thyroid inhibiting properties
18			2-Vinylbicyclo [2.1.1] hexan-2-ol	$C_8H_{12}O$	124	No activity recorded
19	14.889	4.53	Alpha [5-Methyl-2,3,4,5-tetrahydro-2-furyl] glucine	$C_7H_{13}NO_3$	159	Urinary incontinence, helps to cure renal heart disorder, anti-inflammatory
20	15.982	4.68	1,3-Oxathiane, 5-isopropyl-2-methyl-	$C_8H_{16}OS$	160	Anti-proliferative, Food improvement agent
21	3.941	3.35	Ethylene glycol, TMS derivative	$C_5H_{14}O_2Si$	134	Antifreeze, Anti-bacterial
22	16.767	2.95	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	Antimicrobial
23	5.968	6.16	7-Methyl-2-oxepanone	$C_7H_{12}O_2$	128	Antimicrobial
24	7.290	3.78	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	Antioxidant
25	11.381	3.28	Pentanedioic acid, ethyl methyl ester	$C_8H_{14}O_4$	174	Antimicrobial
26	10.522	3.26	3-Mercaptohexanol	$C_6H_{14}OS$	134	Flavouring agent, Ripening agent
27	17.120	7.15	Benzeneacetic acid, .alpha.-hydroxy-, pentyl ester	$C_{13}H_{18}O_3$	222	Neuroprotective, antibacterial, anticancer
28	8.977	2.90	Pentanamide, 5-hydroxy-	$C_5H_{11}NO_2$	117	Antimicrobial, Antitubercular, Antidiabetic, Antiviral, Anti-inflammatory
29	8.710	3.02	N-Carbobenzoxy-DL-leucine	$C_{14}H_{19}NO_4$	265	Anticancer
30	11.967	6.21	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	115	Cholesterol acyltransferase
31	8.800	5.11	2-Methoxy-5-methylthiophene	C_6H_8OS	128	Anti-bacterial lung infection
32	3.219	1.66	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118	Antidermatophytic, Antidiabetic, Anti-proliferative
33	10.611	2.67	2-Methylbutanoic anhydride	$C_{10}H_{18}O_3$	186	Alkylating agent
34	11.118	4.92	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$	144	No record found
35	12.299	5.94	5-Hydroxymethyl-dihydro-furan-2-one	$C_5H_80_3$	116	Anti-tumor
36	3.453	4.18	1,3-Dioxan-5-ol	$C_4H_8O_3$	104	Antibacterial, Neuroprotective
37	10.125	5.03	2-Methylcyclohexane-1,3-dione	$C_7H_{10}O_2$	126	Anti-cancer
38	9.971	4.98	Diazene, bis(1,1-dimethylethyl)-	$C_8H_{18}N_2$	142	Anti-phytopathogenic
39	26.193	5.88	Octanoic anhydride	$C_{16}H_{30}O_3$	270	Anti-bacterial and Anti-fungal
40	5.382	5.09	Protoanemonin	$C_5H_4O_2$	96	Anti-fungal, Anti-cancerous, Flavor and fragrance agents

Table 2 (*continued*)

Sr No	R. time	Area/ Height (A/ H)	Compound name	Mol. formula	Mol. weight (g/mol)	Biological functions
41	7.439	7.71	Silane, ethoxydimethyl[3-(oxiranylmethoxy)propyl]-	$C_{10}H_{22}O_3Si$	218	Anti-fungal
42	8.035	7.14	Formic acid, allyl ester	$C_4H_6O_2$	86	Flavoring agents, Anti-bacterial
43	8.428	3.82	3-Methyl-1,2-cyclopentanedione	$C_6H_8O_2$	112	Scavenging peroxynitrite, Anti-inflammatory
44	19.141	2.38	Tetradec-7-ene	$C_{14}H_{28}$	196	Fluid loss additive, detergent, Antimicrobial
45	9.356	4.88	2,5-Anhydro-1,6-dideoxyhexo-3,4-diulose	$C_6H_8O_3$	128	Phytoremediation
46	4.368	4.24	4-Hydroxy-3-methylbutan-2-one	$C_5H_{10}O_2$	102	Peptide Deformylase Inhibitors
47	7.742	4.36	Cyclohexane-1,2-dione	$C_6H_8O_2$	112	flavouring agent, Antibacterial activity
48	4.167	1.96	Methyl pentanoate	$C_6H_{12}O_2$	116	Fragrances, Detergent, Antibacterial
49	6.304	5.10	5-Methyl-2(3H)-furanone	$C_5H_6O_2$	98	Flavoring agent, Antimicrobial
50	4.484	3.50	Furan, 2,5-dimethyl-	C_6H_8O	96	Biofuel, Scavenger for singlet oxygen
51	3.267	4.40	2,2′-Bioxirane	$C_4H_6O_2$	86	Curing polymers, Prevent spoilage
52	3.520	3.89	Ketocyclopentane	C_5H_8O	84	Antidiabetic
53	16.959	3.82	Eugenol acetate	$C_{12}H_{14}O_3$	206	Antibacterial, Anti-virulence
54	15.045	4.89	Syringol	$C_8H_{10}O_3$	154	Antimicrobial, Bacteriostat
55	17.738	25.30	Cytosine riboside	$C_9H_{13}N_3O_5$	243	Antiviral, Antimicrobial, Anticancer
56	24.782	2.76	Butyl 2-ethylhexyl phthalate	$C_{20}H_{30}O_4$	334	Antifungal, Medical tubing, Used in blood storage bags
57	7.917	4.30	4-Hydroxybenzenesulfonic acid	$C_6H_6O_4S$	174	Dental antiseptics, Fungal degradation
58	14.613	10.61	hydroquinone	$C_6H_6O_2$	110	Dermatologic agent, Antibacterial, Anti-cancer, Anti- influenza
59	20.959	29.85	Kinic acid	$C_7H_{12}O_6$	192	Neuropharmacological agent
60	9.712	7.32	Guaiacol	$C_7H_8O_2$	124	Bone repair, Anti-bacterial, Antifungal activity
61	14.380	3.86	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	Anti-proliferative

3.9. ADME-toxicity properties prediction

ADMET study is used for determining the disposition of a novel molecules holding greater pharmacokinetic and pharmacodynamic abilities within an organism. On the basis of docking review, the elite ligands were selected for ADMET prediction including water solubility, absorption in human intestinal, blood brain barrier, liver injury by drug consumption, mutagenicity, cytotoxicity etc. (Table S4). Two molecules namely 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trime thylsiloxy) tetrasiloxaneand 2-Methoxy-5-methylthiophene were undertaken for this study. These compounds have not shown cardiotoxicity, mutagenicity, cytotoxicity and mitochondrial Toxicity at all. Therefore, these molecules can be targeted for therapeutic uses and have been proved to be the potential lead molecules for antimicrobial agent. Lipinski Rule of Five was performed to check the drug-likeness quality of these two compounds. Rule of 5 violation was zero for 2-Methoxy-5-methylthiophene and it was only 2 for 3-Isopro poxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane compounds. Log P value for 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane and 2-Methoxy-5-methylthiophene was 8.62 and 2.28 respectively. The bioavailability score of these three molecules is 0.55 (far less than acceptable score of 3), which means the uptake by pathogens will be very high (Table S5). Thereforethese compounds impact on physiological and ecological system will be minimal and can be used in field condition. Additionally, the chances of unwanted dispersal of these molecules in the environment is negligible. From these findings, it can be inferred that *S. vinosa* leaves extract could be used to control rice blast and bacterial blight in filed conditions without any harm to surrounding flora and fauna with more specificity.

3.10. LigPlot analysis

LigPlot investigation was executed to analyzed the H-bond pattern and hydrophobic interactions between ligands and receptors. Hydrogen bond/s play a significant role and greatly impact the drug specificity, metabolization, and adsorption. One of the ligand 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane forms nine H-bond (Fig. 5A) with different amino acids [Asp104(C), Tyr308(C), His145(C), Asp179(C), Asp269(C), Asp181(C), Asp186(C), His146(C), His183(C)], and it also interacted with a metal ion (Zn^{2+}) that is essential for activity (zn401) with the amino acid residues of the HDAC. Similarly, HDAC complex also exhibits five hydrophobic interactions with Pro34(C), Phe155 (C), Gly386(C), Phe210(C), and Gly386(C) and the overall binding energy of ligand and receptor was −8.0 kcal mol^{-1}. Similarly, another ligand 2-Methoxy-5-methylthiophene shows multiple residue interaction such as two H-bond formation with Glu142(A) and Tyr69(A) and eight hydrophobic interactions with Glu97(A), His141(A), Gly98(A), Val45(A), Gly46 (A), Arg68(A), Leu100(A), and Gly44(A) (Fig. 5B) with PDF receptor and the binding energy yield was

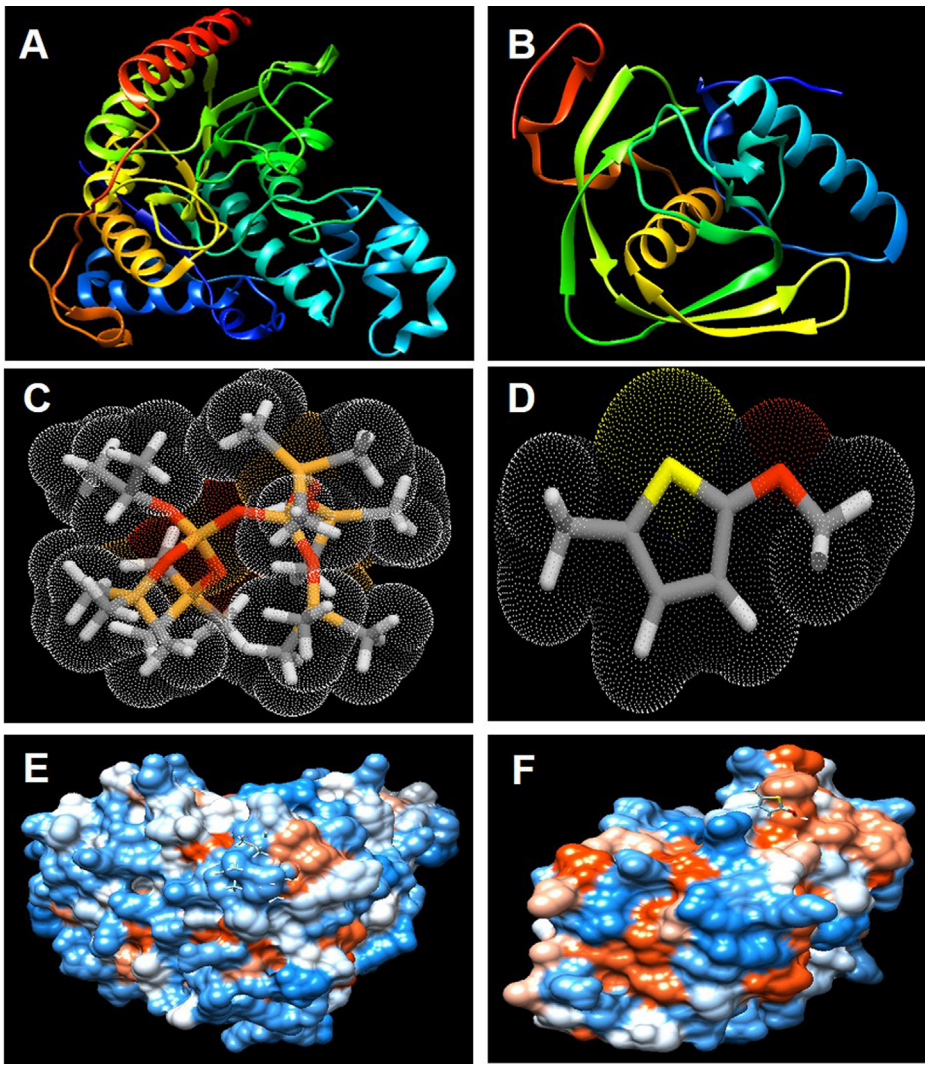

Fig. 4 Molecular docking view: (A&B) Predicted models of receptor enzymes HDAC (4LXZ protein) and PDF (5CY8 protein) of *M. oryzae* and *Xoo*; (C&D) Prepared ligands of GC–MS molecules 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and 2-Methoxy-5-methylthiophene; (E&F) ligand and protein (receptor) interaction with 4LXZ protein with 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and 5CY8 protein with 2-methoxy-5-methylthiophene.

−7.9 kcal mol^{-1}. Hydrophobic interactions were closure with both the ligands and favorable interaction with Tyr was observed, which are important for specific ligand recognition (Moreno-Castillo et al., 2018). Overall, it could be inferred from the result that the interaction between Isopropoxy-1,1,1 ,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane (ligand) and HDAC and 2-Methoxy-5-methylthiophene (ligand) and PDF is strong and stable.

4. Conclusions

Current study showed that ME has better antioxidant and antimicrobial properties over CE. The differences observed in ME and CE activities could be due to variable concentration of phytochemical components in the extract which imparts antioxidant and scavenging activities. A positive corelation between polyphenols and FRSA was also established. Nicking assay further proved the FRSA of *S. vinosa* through protecting the DNA from the possible damage induced by Fenton's reagent. Thus, it can be used to avert free radical based disease development and amelioration. *In vitro* study clearly demonstrated the antimicrobial activities of ME in concentration dependent manner against *Xoo* and *M. oryzae*. FTIR and GC–MS confirmed the existence of various secondary metabolites which could be accountable for the antimicrobial quality of the extract. Molecular docking study showed promising binding affinity of phytochemicals like 3-Isopropoxy-1,1,1,7,7 ,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxaneand 2-Methoxy-5-methylthiophene could be used to target bacterial and fungal receptors like HDAC and PDF to control the rice pathogens. Interestingly, the ADME-toxicity prediction revealed that *S. vinosa* extract is safe to use in the field due to its negligible chances of unwanted dispersal in the environment. Further investigations such as identification and isolation of reliable specific antimicrobial and antioxidant molecules is required and recommended for its broad-spectrum usage.

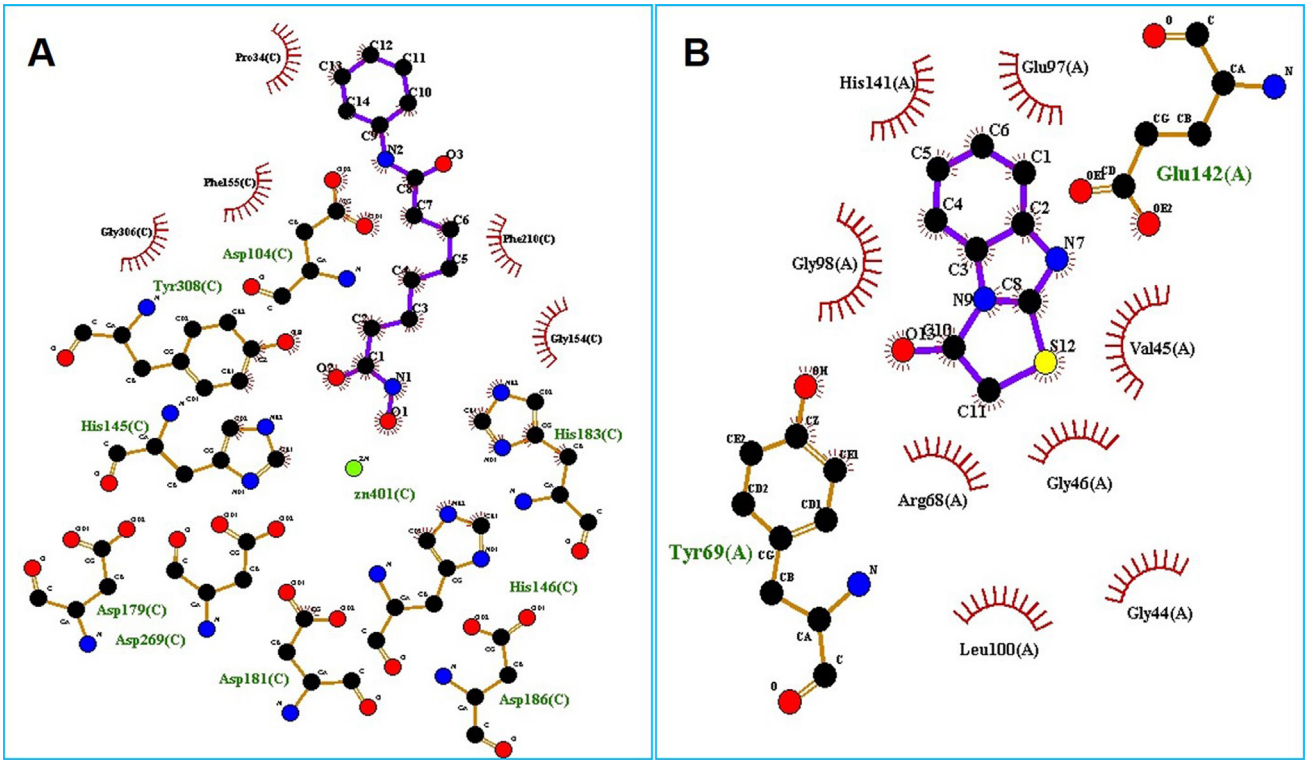

Fig. 5 2D representation of Ligplot intermolecular interaction analysis: (A) PubChem CID 553025 with 4LXZ of *M. oryzae* (B) PubChem CID 141615 with 5CY8 of *Xoo*.

Ethics statements

Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects

Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: All datasets presented in this study are included in the article/ supplementary material.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103243.

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