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Phytochemical Composition and Potential Bioactivity With ADMET Analysis of Selected Species From Myrtaceae Family

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ABSTRACT

Compounds derived from natural sources continue to serve as chemical scaffolds for designing prophylactic/therapeutic options for human healthcare. This study aimed to evaluate the phytochemical composition, antioxidant potential, in vitro anti-inflammatory, antibacterial and Herbicidal activity of methanolic extracts from leaves of four medicinal plants of family *Myrtaceae*: *Melaleuca leucadendron* (MLeuME), *Melaleuca linarrifolia* (MLinME), *Melaleuca bracteata* (MBME) and *Callistemone viminalis* (CVME). These plants are traditionally used in various therapies in India and across the countries, but comparative analyses are limited. Major compounds identified in *Melaleuca leucadendron* were (*E,Z*)-nerolidol (24.7%), betulinic acid (13.6%), ribitol (8.6%), ursolic acid (6.3%), mannitol (5.0%), oleanolic acid (1.7%), threitol (1.4%) and glycerol (1.3%). The major compounds in *Melaleuca linnarifolia* were betulinic acid (9.9%), quinic acid (6.4%), glycerol (1.2), methyl gallate (1.0%), gallic acid (1.1%), *Myo*-inositol (2.0%) and ursolic acid (1.0%). The prominent compounds in *Melaleuca bracteata* were glycerol (1.2%), methyl eugenol (2.5%), quinic acid (3.1%), oleanolic acid (1.1%), betulinic acid (13.4%) and ursolic acid (4.3%), whereas shikimic acid (13.1%), quinic acid (4.5%), gallic acid (7.1%), oleanolic acid (3.0%), betulinic acid (7.3%) and ursolic acid (6.7%) were identified as the major compounds in *Callistemon viminalis*. Our results showed that *Callistemon viminalis* had the highest phenolic content (30.26 mg/g GAE). The strongest antioxidant, anti-inflammatory and antibacterial activity was shown by *Melaleuca linnarifolia* methanolic extract. Herbicidal activity was shown by all extracts in a dose-dependent manner. All the tested extracts demonstrated strong anti-inflammatory and bactericidal activities. Methanolic extract of *Melaleuca bracteata* exhibited a strong allelopathic effect, indicating the most potent herbicidal potential among the tested plants. The results of molecular docking studies suggest that most of the phytocompounds show high binding affinity with the protein Human peroxiredoxin 5, indicating their strong antioxidant potential. Furthermore, ADME (absorption, distribution, metabolism and excretion), related physicochemical and pharmacokinetic properties have been assessed that support our in vitro findings. Our findings highlighted the four species as promising candidates for therapeutic applications, owing to their strong antioxidant and biological activities, as well as their potential as a source of natural antioxidants. These results provide a foundation for further research into the safe and effective use of these plants in the food and pharmaceutical sectors.

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1 | Introduction

Plants are an essential source of medicines and play a vital role in global health, as their therapeutic and curative properties have gained a prominent place in healthcare systems worldwide [1–4]. Medicinal plants are valued not only for treating diseases but also for maintaining overall health. Their cultural acceptability, compatibility with the human body, adaptability and fewer side effects have led many countries to rely on herbal medicine for primary healthcare [5–7]. For centuries, plants have been widely used as sources of natural compounds for treating human diseases. In recent years, growing research on medicinal plants and their active constituents has renewed interest in their role in modern medicine [8–11]. Myrtaceae is a large, well-defined family comprising about 140 genera and 4000 species. The genus *Melaleuca*, a member of this family, includes around 230 species that are native to Australia but are now widely distributed worldwide. The economic importance of *Melaleuca* species lies primarily in their essential oils, with *Melaleuca alternifolia* oil being the most widely used. Additionally, honey produced from *Melaleuca* blossoms is highly valued [12]. Research on *Melaleuca* has primarily emphasized the chemical composition and antimicrobial properties of its essential oils, while studies on non-volatile constituents have revealed mainly phenolic and terpenoid compounds. Numerous bioactive molecules—including phenolics, flavonoids, phenylpropanoids, polyphenols, free phenolic acids and triterpenoids (lupane, ursane and oleanane derivatives)—have been identified across the genus. Unique benzylic phloroglucinol–terpene hybrids (meroterpenoids) have also been reported from *Melaleuca leucadendron*. Collectively, *Melaleuca* species exhibit a wide range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, neuroprotective, anticancer and hepatoprotective effects [13]. *Callistemon*, another member of Myrtaceae family, comprises 34 species and is characterized by cylindrical, brush-like flowers resembling a bottlebrush. *Callistemon viminalis* (weeping bottlebrush) is a small tree or shrub native to Australia, typically reaching about 4 m in height in its natural habitat. Ecologically, *Callistemon* species are widely planted as farm trees for forestry plantations, ornamental use, and weed control [14]. In traditional Chinese medicine, *C. viminalis* is used in pill formulations for the treatment of haemorrhoids [15]. Several researches have been carried out on *C. viminalis* extracts, and showed that the plant is rich in phenolics, triterpenoids, flavonoids, saponins, steroids, alkaloids, tannin, carbohydrates, amino acids and protein compounds [16, 17]. n-hexane extract from leaves of *Callistemon viminalis* showed potential activity against skin pathogen *S. aureus, Streptococcus pyogenes* and the enteric *Bacillus cereus* [18]. Crude water extract of *C. viminalis* showed effective inhibition of *Pseudomonas aeruginosa* (*P. aeruginosa*) [19]. In this study, we selected plants from Myrtaceae family growing in the wild in Uttarakhand and compared their phytochemical composition and other biological activities. Many studies have been done on essential oils from these species in past, but the comparative study and chemical profiling of extracts were not done in past giving us an opportunity to investigate these species.

2 | Material and Methods

2.1 | Plant Material

The fresh leaves of different species (*Melaleuca leucadendron, Melaleuca linnarrifolia, Melaleuca bracteata* and *Callistemon viminalis*) were collected from the Nainital District of Uttarakhand. A voucher specimen has been deposited in the herbarium of the Department of Biological Sciences of G.B. Pant University of Agriculture and Technology, Pantnagar. Plants were identified by Dr. D.S. Rawat, Taxonomist and Professor, Botany Department, G.B. Pant University of Agriculture and Technology, Pantnagar.

2.2 | Preparation of Methanolic Extract

Fresh leaves of all the listed plants were collected separately, shade dried, powdered and extracted with methanol and the solvent was removed under vacuum. Methanol was used for plant extraction due to its high polarity and ability to dissolve a broad range of phytochemicals, including phenolics and flavonoids. Its efficient penetration into plant tissues improves extraction yield, while its low boiling point enables easy removal without thermal degradation [20]. The cold extraction process was used and the ground plant material was soaked in the methanol for 10 days. The solvent was evaporated using a vacuum rotary evaporator. The methanol extracts were evaporated at 40°C ± 5°C. The extracts were kept in refrigerator for further use and their yields are presented in Table 1.

2.3 | GC–MS Analysis

The GC–MS analysis was performed on an Agilent 7890A chromatograph with Agilent 5975C mass detector, on a capillary non-polar column HP-5MS (30 m × 0.25 mm × 0.25 μm) at a helium flow rate of 1 mL/min. An injection of a 1-μL sample was performed using an Agilent 7693A autosampler. The injector worked in a split (1:10) mode at an injector temperature of 300°C. The initial column temperature was 50°C, rising to 325°C, at 3°C/min; the final temperature was held for 10 min. The ion source and quadrupole temperatures were 230°C and 150°C, respectively. Electron ionization mass spectral (EIMS)

TABLE 1 | Percent yield of methanolic extract of MLuME, MLinME, MBME and CVME.

S. no.	Extracts	Weight of part (g)	Yield of extract	
			Yield (g)	%Yield
1.	M.LeuME	985	55	5.6
2.	MLinME	500	30	6.0
3.	MBME	850	35	4.1
4.	CVME	750	45	6.0

was obtained at ionization energy 70 eV. The detection was performed in a full scan mode from 41 to 800 a.m.u. After integration, the content (%) of each component in the total ion current (TIC) was calculated. The identification of compounds was based on a comparison of MS spectra with computer mass library NIST 2024 along with the relative retention indices (RI, non-polar column). The experimental retention indices (RI) of the compounds were compared to the RI of the *n*-alkane standard mixture (C_{10}–C_{40} Sigma Aldrich). The compounds were identified with the help of NIST-MS, FFNSC Wiley Library and comparing the data with literature reports and GC retention indices [21].

2.4 | Total Phenolics Assay

The total phenolics were measured using the Folin–Ciocalteu method [22, 23]. In brief, the reaction mixture contained 1 mL of the sample extract with 1 mL of 80% methanol and 8 mL of distilled water. Further to each sample, 0.5 mL of 1 N Folin–Ciocalteu reagent was added and mixed. After 5 min of incubation, 1 mL of saturated Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance of the test sample was recorded at 650 nm using a UV spectrophotometer (Thermo Scientific Evolution 201 series). The standard curve was drawn using various concentrations of gallic acid, and results were expressed as mg of gallic acid per gram of sample taken in dried weight.

2.5 | Estimation of Flavanols

Aluminium chloride colorimetric assay was applied for estimation of flavanols [23]. A stock solution was prepared by adding 10 mg of extract with 10 mL of 80% methanol. The reaction mixture contained 0.1 mL of stock solution with 1.25 mL of water and 0.75 mL of 5% $NaNO_2$. The mixture was incubated for 5 min and after incubation, 0.15 mL of 10% $AlCl_3$ was added to the mixtures. After 6 min 0.5 mL of 1 N NaOH and 275 μL of distilled water was added. The absorbance was measured at 510 nm. The flavanol content was evaluated by using standard curve of catechin and the concentrations were calculated with the help of calibration curve and expressed in mg/100 g of the dry weight of material.

2.6 | Estimation of Ortho Dihydric Phenols

A stock solution of extracts was prepared by dissolving 10 mg of extracts in 10 mL of desired solvent. Briefly, the reaction mixture contained 0.1 mL of the extract solution with 0.4 mL water and 1 mL of 0.05 N HCl, 1 mL of Arnow's reagent (10 g of sodium nitrite and 10 g of sodium molybdate were mixed in 100 mL of distilled water), 10 mL of water and 2 mL of 1 N NaOH. The solutions were mixed thoroughly and absorbance was measured at 515 nm. The ortho dihydric phenols content was determined using a standard curve, prepared with the help of a working standard catechol solution at different concentrations. The concentrations were calculated from the calibration curve and expressed in mg/100 g of material [24].

2.7 | Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the extracts was determined by the phosphomolybdate method. Briefly, 0.1 mL aliquot of various concentrations of the plant extracts was mixed with 1 mL of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). The test tubes were covered with aluminium foil and incubated in a water bath at 95°C for 90 min. Then the extracts were cooled to room temperature, and the absorbance of the mixture was determined at 765 nm against a blank containing 1 mL of the reagent solution. Ascorbic acid was used as standard. The assay was carried out in triplicate. The total antioxidant capacity (TAC) was expressed as mg equivalents of ascorbic acid per gram (EAA/g) [25].

2.8 | Antioxidant Activity

2.8.1 | DPPH Radical Scavenging Activity

The DPPH (Diphenyl Picrylhydrazyl) radical-scavenging method was used to evaluate the anti-oxidant capacity of *extracts* following the experimental protocol described by Fraternale et al. [26]. Briefly, the reaction solution was mixed with 80 μL of 0.5 mM DPPH (Sigma-Aldrich) ethanol solution and 40 μL of extract at different final concentrations (400 μL final volume). After shaking, mixtures were left for 1 h in the dark. Absorbance was measured at 517 nm using ethanol as blank. The negative control was prepared with only DPPH (80 μL of 0.5 mM) diluted in ethanol (400 μL final volume). Lower absorbance indicates high free radical scavenging ability. DPPH inhibition (expressed in IC%) was used to evaluate the antioxidant activity by the following equation:

$$\text{IC}\,\% = \frac{A_0 - A_S}{A_0} \times 100$$

where A_0, absorbance value of the control sample; A_s, absorbance value of the test sample; IC, inhibitory concentration. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (BHT) to calculate the IC_{50} values for standard and samples. A lower IC_{50} value indicated more radical scavenging activity.

2.8.2 | Metal Chelating Activity

The metal chelating activity of Fe^{2+} was examined by the methods recently being practiced and based on the principle of the Fe^{2+} chelating ability [27]. The absorbance of the ferrous iron-ferrozine complex formed was measured at 562 nm. The reaction was performed by adding 0.1 mL of 2 mM $FeCl_2.4H_2O$, 0.2 mL of 5 mM ferrozine, and 4.7 mL of methanol with different concentrations of test samples. After incubation, the absorbance of the test samples was measured at 562 nm. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (citric acid) to calculate the IC_{50} values for standard and samples.

2.8.3 | Reducing Power

The reducing power was determined by the method developed earlier with some modifications [28]. The different concentrations of extracts were mixed with 2.5 mL of phosphate buffer (200 mM, pH = 6.6) and 2.5 mL of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. After 20-min incubation at 50°C ± 1°C, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer (1.0 mL) was mixed with 5 mL distilled water and 1 mL of 0.1% ferric chloride, and the absorbance of the resultant solution was measured at 700 nm using a UV spectrophotometer (Thermo Scientific Evolution 201 series). All the readings were taken in triplicate and BHT was used as the standard. The reducing power of samples was calculated using the formula given below:

$$RP_{50}\% = \frac{A_0 - A_S}{A_0} \times 100$$

where A_0, absorbance value of control sample; A_s, absorbance value of test sample. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant to calculate the RP_{50} values for standard and samples. The lower RP_{50} value indicates greater reducing power ability.

2.8.4 | Assay of Nitric Oxide Scavenging Activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride and 2 mL orthophosphoric acid). It is based on the principle that SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. 2 mL of SNP (10 mM) in phosphate buffer saline (PBS) pH 7.4 was mixed with different concentrations of extracts and incubated at 25°C for two and half hours. The samples were reacted with 1 mL of Griss reagent. Absorbance was measured at 546 nm. Ascorbic acid was taken as a standard [29]. Nitric oxide scavenging activity was calculated by following the equation.

$$IC\% = \frac{A_0 - A_S}{A_0} \times 100$$

where A_0, absorbance value of control sample; A_s, absorbance value of test sample; IC, inhibitory concentration. Percent inhibition was plotted against concentrations. The standard curve was drawn using standard antioxidant (Ascorbic acid) to calculate the IC_{50} values for standard and essential oils.

2.8.5 | Super Oxide Radical Scavenging Activity

1 mL of Nitroblue tetrazolium (156 μM), 1 mL Nicotinamide adenine dinucleotide (reduced) (468 μM) and 0.1 mL of phenanzine methosulphate solution (PMS) in 0.1 M of phosphate buffer solution (pH 7.4) were added to different concentrations of extracts, then incubated at 25°C for 5 min and absorbance was read at 560 nm against blank containing all reagents except PMS. Ascorbic acid was taken as standard [30]. Super oxide radical scavenging activity was calculated by following equation.

$$IC\% = \frac{A_0 - A_S}{A_0} \times 100$$

where A_0, absorbance value of control sample; A_s, absorbance value of test sample; IC, inhibitory concentration. Percent inhibition was plotted against concentrations. The standard curve was drawn using a standard antioxidant (ascorbic acid) to calculate the IC_{50} values for the standard and samples.

2.9 | Determination of In Vitro Anti-Inflammatory Activity

2.9.1 | Inhibition of Albumin Denaturation

In vitro anti-inflammatory activity was determined by the method of Chandra et al. [31]. The experiment involves the addition of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of test samples to make a final volume of 5 mL. A similar volume of double-distilled water served as a control. The mixtures were incubated at (37°C ± 2°C) in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium was used as a reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\,\text{Inhibition} = 100 \times (Vt / Vc - 1)$$

where Vt, absorbance of the test sample; Vc, absorbance of control. The concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition concerning control against treatment concentration.

2.10 | Antibacterial Activity

Antimicrobial activities of extracts were evaluated by the agar well diffusion method on Nutrient agar (NA) [32, 33]. The antimicrobial screening was tested against *Salmonella typhimurium, Escherichia. coli, Staphylococcus aureus* and *Bacillus megaterium*. The inhibition zones were reported in millimetres (mm). 20 mL of molten nutrient agar was poured into the Petri plates and left to solidify. Wells were prepared using a cork borer. Nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 50 μL of the test samples and incubated at 37°C for 24 h. After the incubation period, the diameter of the growth inhibition zones was measured in millimetres. Eighteen to twenty-four hours of single colonies on agar plates were used to prepare the bacterial suspension containing 1.0×10^6 colony-forming units (CFU)/mL. Reference commercial antimicrobial (Rifamycin) was used to compare the antimicrobial potential. Tests were performed in triplicate.

2.11 | Herbicidal Activity

The seed germination inhibition activity of extracts was tested on *Raphanus sativus* seeds by the method used by Sahu and Devkota [34] with slight modification. The seeds of *Raphanus sativus* were selected due to easy germination, easy handling, and showed a pronounced effect after the application. Filter paper was used in Petri dishes. For sterilization of the medium from dust particles or fungal attack on Petri plates, cleaned ethanol-dipped cotton was used to wipe petri plates and then filter paper was placed. Different concentrations of extract were applied to the test plant.

2.11.1 | Source of *Raphanus sativus* Seeds

The *Raphanus sativus* (radish) seeds were purchased from the seed store of Pantnagar.

2.11.2 | Source of Pendimethalin

Standard herbicide (Pendimethalin) was suggested and supplied by a scientist of the Weed Control Laboratory, C.R.C, G.B.P.U.A.& T. Pantnagar.

2.11.3 | Preparation of Test Solution for Screening of Herbicidal Activity

A stock solution of 10,000 ppm for all the extracts was prepared in sterilized distilled water. The stock solutions were serially diluted to four concentrations, namely 250 ppm, 500 ppm, 750 ppm and 1000 ppm respectively for testing seed germination inhibition activity. Pendimethalin (3 mg/L) was used as standard.

2.11.4 | Bioassay

The seeds of *Raphanus sativus* were sterilized using 5% hypochlorite solution for 15 s prior to use. The petri plates were covered with ordinary filter papers on which test solution was poured. In each petri plate, ten sterilized seeds were taken and allowed to germinate at 25°C ± 1°C in an incubator with 12 h of photoperiod. Sterilized distilled water was taken as control. The whole experiment was performed in triplicate. After 72 h, the number of germinated seeds was counted for each concentration, and percent seed germination inhibition values were calculated.

2.12 | Molecular Docking

In silico method was used to dock small molecules (ligand) to macromolecules (protein) in order to discover potent compounds having necessary biological effects [35]. The molecular docking study of the selected compounds from different Myrtaceae species was performed to determine antioxidant potential against 1HD2 (Human peroxiredoxin 5), which exhibited broader activity against reactive oxygen species [36]. The three-dimensional structure of Human peroxiredoxin 5 proteins was obtained from the RCSB Protein Data Bank with PDB ID: 1HD2. The three-dimensional structure of the selected protein was downloaded into SDF format. The phytocompounds for the docking study were selected based on their higher percentage contents, and their concerned structures were obtained from the PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 2 February 2026) in SMILES format. The selected compounds were phytol (CID: 5,280,435), mannitol (CID: 6251), quinic acid (SID: 516,576,382), glycerol (CID: 753), methyl eugenol (CID: 7127), gallic acid (CID: 370), *myo*-inositol (SID: 516,572,213), linolenic acid (CID: 5,280,934), betulinic acid (CID: 64,971), ursolic acid (CID: 64,945), (*E,Z*)-nerolidol (CID: 5,284,507), shikimic acid (CID: 8742), oleanolic acid (CID: 10,494), ribitol (CID: 516,576,661). The three-dimensional structure of Human peroxiredoxin-5, obtained in SDF format, was used as the target macromolecule for molecular docking analysis. Docking studies were carried out for selected major phytochemical compounds using the CB-Dock 2 web-based platform. The protein structure was uploaded to the CB-Dock 2 server, while ligand structures were sourced from the PubChem database and provided in SMILES format. Auto blind docking was performed to detect probable binding pockets and estimate ligand–protein binding affinities, expressed as Vina scores (kcal/mol). After completion of the docking process, the protein–ligand complexes were downloaded for further evaluation. The docked poses and molecular interactions were visualized and analysed using the BIOVIA Discovery Studio 2025 Client.

2.13 | ADMET Analysis

ADMET analysis was performed for the determination of pharmacokinetics (ADME: Absorption, Distribution, Metabolism and Excretion) of major phytochemicals. The chemical structures of the selected constituents of the extracts were constructed using ChemDraw Ultra 8.0. The generated structures were converted to SMILES format and analysed for drug-likeness and pharmacokinetic properties using the Swiss ADME online platform (http://www.swissadme.ch/, accessed on 10 February 2026), following the established protocol [37]. Further, the ProTox-3.0 web server (https://tox.charite.de) accessed on (Date 11 February 2026) was employed to predict the toxicity profile of the compounds. This tool estimates potential toxic effects based on multiple parameters, including organ-specific toxicity such as oral toxicity, hepatotoxicity and various toxicological endpoints such as cytotoxicity, carcinogenicity, mutagenicity and immunotoxicity.

2.14 | Statistical Analysis

The mean values, ± standard deviations, were calculated using MS Excel 2007. Linear regression analysis was used to calculate IC_{50} for each extract. One-way analysis of variance (ANOVA) and Tukey's post hoc tests were performed using SPSS Statistics 16 software, and $p < 0.05$ was considered to indicate a statistically significant difference.

3 | Results and Discussion

3.1 | Phytochemical Composition

The GC–MS analysis of leaf methanolic extract of *Melaleuca leucadendron* (MLeuME), *M. linnarrifolia* (MLinME), *M.*

TABLE 2 | Comparative chemical composition of *Melaleuca leucadendron*, *Melaleuca linarrifolia*, *Melaleuca bracteata* and *Callistemon viminalis*.

S. no.	Compound name	RI^{Exp}	RI^{Lit}	MLeuME	MLinME	MBME	CVME
1.	1,8-cineole	1032	1031	—	0.1	—	—
2.	Lactic acid	1071	1073	0.2	t	—	t
3.	Glycolic acid	1086	1083	—	0.1	—	t
4.	Alanine	1113	1114	—	0.1	—	t
5.	Valine	1227	1227	—	0.1	—	—
6.	Glycerol	1294	1293	1.3	1.2	1.2	0.4
7.	Proline	1304	1303	—	0.7	0.5	0.2
8.	Succinic acid	1324	1324	—	—	0.1	0.1
9.	α-terpineol	1324	1320	—	0.6	—	—
10.	Glyceric acid	1351	1348	—	0.1	—	t
11.	Methyl cinnamate	1379	1378	—	—	0.1	—
12.	Methyl eugenol	1403	—	—	—	2.5	—
13.	Eugenol	1476	1478	0.1	t	—	—
14.	Malic acid	1512	1512	0.1	0.3	0.5	1.0
15.	Pyroglutamic acid	1530	1530	—	—	—	0.3
16.	1,2,3,4-tetrahydroxybutane	1534	—	0.2	—	—	—
17.	Threitol	1541	1540	1.4	0.3	0.2	—
18.	Pyrogallol	1554	1555	—	—	—	0.1
19.	(*E*)-nerolidol	1562	1565	0.2	—	—	—
20.	Caryophyllene oxide	1578	1586	0.6	—	—	—
21.	Tetronic acid	1595	1595	—	—	0.1	—
22.	2,3,4-trihydroxybutyric acid	1595	1595	—	—	—	0.1
23.	Humulene epoxide II	1604	1607	0.1	—	—	—
24.	2-piperidine carboxylic acid	1618	—	—	—	—	0.1
25.	Caryophylladien-5- α -ol	1640	1636	0.1	—	—	—
26.	(*Z*)-nerolidol	1647	1643	0.2	—	—	—
27.	Arabinoic acid			—	—	—	0.1
28.	Globulol	1674	1671	—	0.1	—	—
29.	(*E*,*Z*)-nerolidol	1692	1692	24.7	—	—	—
30.	Methyl trimethyl gallate	1720	—	—	—	0.3	—
31.	Xylitol	1749	1750	0.2	—	—	—
32.	Ribitol	1762	1764	8.6	0.3	0.3	0.1
33.	Arabinitol	1765	1770	—	0.1	—	0.3
34.	Lyxofuranose	1768	1770	—	0.6	0.7	—
35.	Pentitol	1779	—	—	0.3	—	—
36.	Ribonic acid	1809	1805	0.2	0.1	—	0.1
37.	(2*E*,6*Z*)-farnesol	1814	1810	0.3	—	—	—
38.	Neophytodiene	1838	1840	0.1	—	—	—

(Continues)

TABLE 2 | (Continued)

S. no.	Compound name	RI^{Exp}	RI^{Lit}	MLeuME	MLinME	MBME	CVME
39.	Shikimic acid	1848	1844	—	—	—	13.1
40.	1,5-anhydro-D-sorbitol	1896	—	—	1.2	—	—
41.	Quinic acid	1902	1901	—	6.4	3.1	4.5
42.	Methyl gallate	1911	1921	—	1.0	0.3	—
43.	psicose	1940	1943	0.1	—	—	—
44.	p-coumaric acid	1944	1947	0.1	—	—	—
45.	Mannitol	1975	1973	5.0	0.5	0.2	0.1
46.	Glucitol	1982	1980	0.1	—	—	—
47.	Gallic acid	1985	1985	0.3	1.1	0.7	7.1
48.	3-vanil-1,2-dihydroxypropane	1990	1990	—	—	0.1	—
49.	Cyclohexanepentol	2005	2004	—	—	—	0.4
50.	Inositol	2027	—	—	0.1	0.8	—
51.	*scyllo*- inositol	2047	2040	—	0.3	0.1	0.3
52.	Palmitic acid	2052	2052	0.9	0.4	—	0.4
53.	Methoxy cyclohexane	2074	2069	—	0.1	—	—
54.	Methyl cyclohexenepentol	2113	2113	—	0.9	—	—
55.	*myo*-inositol	2129	2129	1.2	2.0	—	0.3
56.	Phytol, TMS	2183	2187	t	0.1	—	0.1
57.	Linoleic acid	2213	2215	0.1	t	t	0.2
58.	α-linolenic + oleic acids	2219	2222	0.3	0.2	0.2	0.3
59.	Octadecanoic acid	2246	2248	t	t	—	—
60.	*epi*-catechin	2907	2906	—	—	—	0.1
61.	Catechin	2934	2936	—	—	—	0.1
62.	Kaempherol	3110	3114	—	—	—	0.1
63.	Quercetin	3235	3239	—	—	—	0.1
64.	Ellagic acid	3324	3328	—	—	—	0.6
65.	β-sitosterol	3341	3345	0.6	0.5	0.4	0.6
66.	Betulinol	3559	3564	0.4	0.2	0.5	—
67.	Oleanolic acid	3571	3576	1.7	0.3	1.1	3.0
68.	Betulinic acid	3591	3599	13.6	9.9	13.4	7.3
69.	Ursolic acid	3619	3620	6.3	1.0	4.3	6.7
	Total			69.3%	31.3%	31.7%	48.3%

Note: t, trace compounds present in < 0.05%.

bracteata (MBME) and *Cllistemone viminalis* (CVME) has been presented in Table 2. The GC–MS analysis revealed the presence of more than 35 compounds contributing to 69.6% of the total volatile composition from the extract of *Melaleuca leucadendron*. (*E,Z*)-nerolidol (24.71%) was identified as the major compound in the extract. The other major constituents were betulinic acid (13.6%), ribitol (8.6%), ursolic acid (6.3%), mannitol (5.0%), oleanolic acid (1.7%), threitol (1.4%), glycerol (1.3%) and *myo*-inositol (1.2%), whereas palmitic acid (0.9%), β-sitosterol (0.6%), betulinol (0.4%), caryophyllene oxide (0.6%) etc. were detected in minor quantity besides other compounds. Figure 1 presents the structure of major compounds identified from the methanolic extract of studied species. The GC/MS analysis of leaf methanolic extract of *M. linariifolia* revealed the presence of over 30 compounds. The major compounds identified were betulinic acid (9.9%), quinic acid (6.4%), 5-hydroxy-2-piperidine carboxylate (3.3%), *myo*-inositol (2.0%), 1,5-anhydro-D-sorbitol (1.2%), methyl gallate

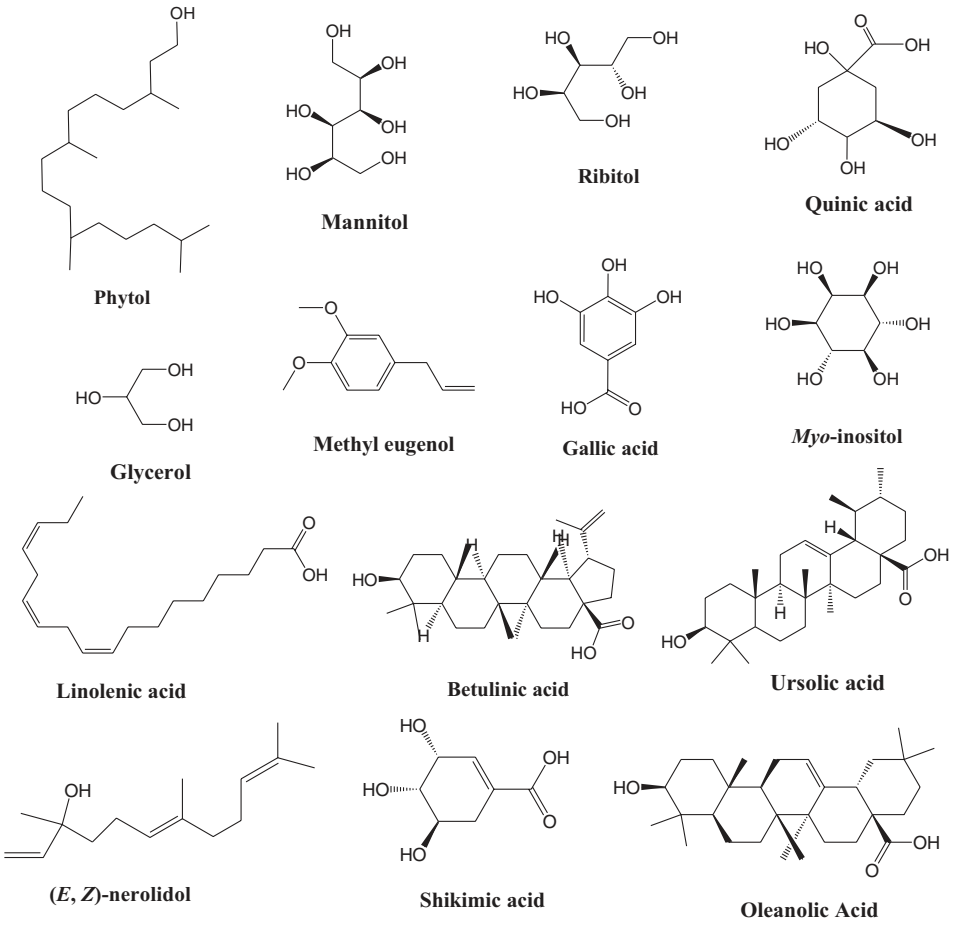


FIGURE 1 | Structure of major compounds identified in the extracts of Myrtaceae species.

(1.0%), gallic acid (1.1%), ursolic acid (1.0%) and glycerol (2.1%). Among minor components methoxy cyclohexapentol (0.9%), proline (0.7%), α-terpineol (0.6%), mannitol (0.5%), β-sitosterol (0.5%), palmitic acid (0.4%) and oleanolic acid (0.3%), *scyllo*-inositol (0.3%), pentitol (0.3%), ribitol (0.3%), threitol (0.3%), malic acid (0.3%) were identified. Compounds like eugenol, lactic acid, linoleic acid and octadecanoic acid were found in trace amount in the extract of *M. linariifolia*. Over 25 compounds were identified contributing 32.1% of the total composition from methanolic extract of *M. bracteata*. The major compounds identified were betulinic acid (13.4%), ursolic acid (4.3%), quinic acid (3.1%), methyl eugenol (2.5%), glycerol (2.1%) and oleanolic acid (1.1%) whereas inositol (0.8%), gallic acid (0.7%), malic acid (0.5%), betulinol (0.5%), proline (0.5%) and β-sitosterol (0.4%), were the minor compounds. The GC/MS analysis revealed the presence of more than 35 compounds constituting 48.3% of total composition of *Callistemon viminalis* extract. The major components identified were shikimic acid (13.1%), gallic acid (7.1%), betullinic acid (7.3%), ursolic acid (6.7%), quinic acid (4.5%), oleanolic acid (3%) and malic acid (1%). The minor components identified were β-sitosterol (0.6%), ellagic acid (0.6%), palmitic acid (0.4%) and glycerol (0.4%). Compounds like lactic acid, glycolic acid, alanine, glyceric acid and 1-eicosanol were detected in trace amounts.

Previously, 1,8-cineole (eucalyptol) was reported as the principal component from essential oil of *M. leucadendron*, including α-terpineol, terpinene-4-ol, linalool, α-pinene, β-pinene, limonene and caryophyllene as other significant constituents [38, 39]. The methanolic and ethanolic extracts of *M. leucadendron* have been found to contain flavonoids, tannins, phenolic acids and saponins [40]. These non-volatile phytochemicals enhance the plant's pharmacological potential by providing antioxidant and cytoprotective effects. The composition of the extract may vary with factors such as geographical origin, climatic conditions, harvest time and extraction technique [41]. The essential oil and plant extracts of *M. linariifolia* contain a wide range of monoterpenes and sesquiterpenes, which are the main contributors to its biological activity and fragrance [42]. The non-volatile phenolic compounds play a key role in scavenging free radicals and protecting against oxidative stress. Despite these variations, the terpinen-4-ol-rich chemotype remains the most characteristic profile of *M. linariifolia*. The essential oil of *M. bracteata* was found rich in methyl eugenol, comprising 70%–95% of the total oil content [43, 44]. Other components from *M. bracteata* essential oil include methyl chavicol (estragole), 1,8-cineole, linalool, β-caryophyllene, α-pinene, limonene and terpinen-4-ol [45]. In addition to volatile constituents, methanolic and ethanolic extracts of *M. bracteata* contain flavonoids, tannins and phenolic acids (e.g., gallic acid, caffeic acid), which enhance the extract's radical scavenging and anti-inflammatory potential [46]. Previously, the essential oils of *C. viminalis* leaves and flowers were found rich in monoterpenes and sesquiterpenes, where the major constituents include 1,8-cineole (30%–60%), α-pinene (10%–20%), limonene (5%–10%), α-terpineol (3%–7%), terpinene-4-ol

(2%–5%) and β-pinene (2%–5%) [47, 48]. These compounds impart a characteristic eucalyptus-like aroma and contribute to the oil's antibacterial and antifungal properties. In addition to the volatile components, methanolic and ethanolic extracts of *C. viminalis* leaves and flowers have been shown to contain various non-volatile phytochemicals, including flavonoids (such as quercetin and kaempferol), tannins, saponins, phenolic acids and alkaloids [49]. These polar compounds exhibit strong antioxidant activity due to their free radical scavenging potential. To our knowledge, the extracts of studied species has not been analysed by GC–MS in previous studies. For this reason, comparisons were made among species of the Myrtaceae family. Phytochemical analysis vividly proved that our extracts are rich repository of marker compounds, their presence in high concentrations explains the higher level of antioxidant and anti-inflammatory activities.

3.2 | Total Phenols

Methanolic extracts of *Melaleuca leucadendron*, *Melaleuca linarrifolia*, *Melaleuca bracteata* and *Callistemon viminalis* were studied in terms of their total phenolic content with the help of respective calibration curves of standard (gallic acid) obtained calorimetrically. Table 3 and Figure 3 presented the phenolic content in methanolic extracts of the species under study. The phenolic content in methanol extracts of various species of the family Myrtaceae ranged from 9.07 ± 0.01 to 35.12 ± 0.01 mg/g Gallic acid equivalent as given in Table 3. The highest phenolic content among species of Myrtaceae family was found in the methanolic extract of *Callistemon viminalis* (CVME) (30.26 ± 0.03 mg/g GAE) followed by *Melaleuca linariifolia* (MLinME) (17.18 ± 0.01 mg/g GAE). The methanolic extract of *Melaleuca bracteata* (MBME) (11.07 ± 0.01 mg/g GAE) and *Melaleuca leucadendron* (MLeuME) (10.82 ± 0.01 mg/g GAE) showed a lower amount of phenolic content.

TABLE 3 | Total phenol, flavonoid and ortho dihydric phenol content in methanol extract of MLeuME, MLinME, MBME and CVME.

S. no	Sample name	Total phenols (mg/g GAE)	Flavonoids (mg/g CTE)	Ortho dihydric phenol (mg/g CLE)
1.	MLeuME	10.82 ± 0.01	26.88 ± 0.04	3.97 ± 0.02
2.	MLinME	17.18 ± 0.01	29.09 ± 0.04	0.89 ± 0.03
3.	MBME	11.07 ± 0.01	5.24 ± 0.02	4.55 ± 0.01
4.	CVME	30.26 ± 0.03	5.18 ± 0.08	4.82 ± 0.02

Note: Data are represented by means ± SD of 3 independent experiments.

3.3 | Total Flavonoids

In the present study, the flavonoid content was estimated in methanolic extracts of different species from the family Myrtaceae. Table 3 and Figure 1 presented the flavonoid content in methanolic extracts of species taken for the present study. The highest flavonoid content was found in the methanolic extract of *Melaleuca linariifolia* (29.09 ± 0.04 mg/g CTE) followed by *Melaleuca leucadendron* (26.88 ± 0.04 mg/g CTE), whereas the methanolic extract of *Melaleuca bracteata* (5.24 ± 0.02 mg/g CTE) and *Callistemon viminalis* (5.18 ± 0.08 mg/g CTE) showed a low amount of flavonoids.

3.4 | Ortho Dihydric Phenol

The Ortho dihydric phenol content in methanolic extracts of species from Myrtaceae family was studied and presented in Table 3 and Figure 2. The highest amount of ortho dihydric phenols was observed in *Calistemon viminalis* extract (4.82 ± 0.02 mg/g CLE) whereas least was found in *Melaleuca linariifolia* extract (0.89 ± 0.03 mg/g CLE). *Melaleuca brecteata* and *Melaleuca leucadendron* exhibited 4.55 ± 0.01 mg/g CLE and 3.97 ± 0.02 mg/g CLE ortho dihydric phenol respectively in their methanolic extract. Kanwal et al. [50] reported total phenolic and flavonoid content (37.45 ± 7.40 mg GAE/g & 18.43 ± 6.34 mg R.E/g) from the n -hexane extract of *Callistemon viminalis*. Mobdj et al. [51] evaluated Phenol and Flavanoid content from the different solvent extracts of *Melaleuca leucadendron* and reported significant quantity of phenols ranging from 86.48 ± 0.075 to 110.72 ± 0,075 EAG/g extract and flavonoid from 61.323 ± 0.827 to 100,285 ± 1578 mg EQ/g extract.

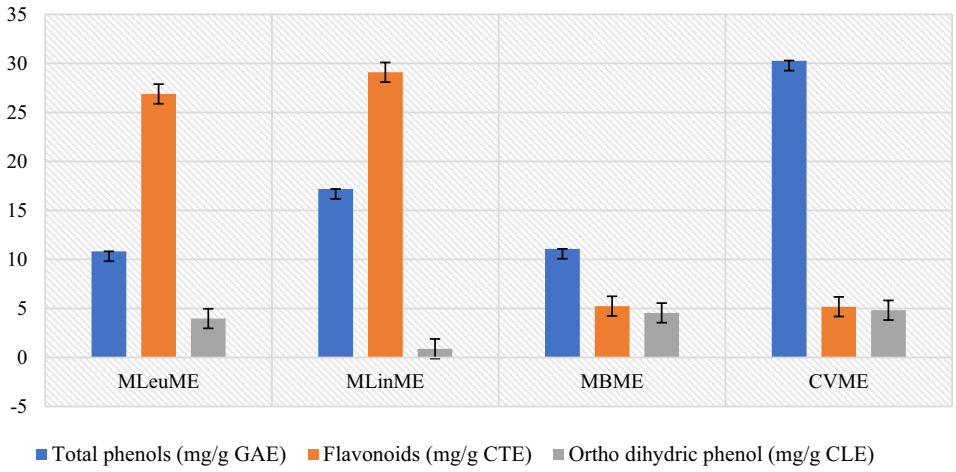


FIGURE 2 | Total phenolics, flavonoid and ortho dihydric phenol in the extracts of Myrtaceae species.

TABLE 4 | IC_{50} values of methanolic extracts for antioxidant activity.

S. no	Sample	DPPH radical scavenging activity (IC_{50})	Metal chelating activity (IC_{50})	Reducing power assay (IC_{50})	Nitric oxide radical scavenging activity (IC_{50})	Super oxide radical scavenging activity (IC_{50})
1.	MLeuME	61.89 ± 0.484^{f}	202.87 ± 0.604^{e}	263.17 ± 1.284^{a}	266.55 ± 1.376^{a}	148.66 ± 0.818^{c}
2.	MLinME	40.85 ± 2.501^{h}	186.87 ± 1.010^{f}	97.24 ± 0.194^{d}	101.81 ± 0.819^{e}	88.92 ± 0.561^{f}
3.	MBME	96.19 ± 1.529^{d}	241.11 ± 0.635^{c}	90.28 ± 2.554^{e}	140.12 ± 1.147^{d}	74.59 ± 0.168^{g}
4.	CVME	131.49 ± 0.238^{a}	174.47 ± 0.986^{g}	197.45 ± 0.800^{b}	138.75 ± 0.852^{d}	86.50 ± 0.690^{f}
5.	Catechin*	36.11 ± 0.191^{i}	—	—	—	—
6.	Gallic acid*	31.32 ± 0.959^{j}	—	—	—	—
7.	EDTA*	—	107.15 ± 2.799^{j}	—	—	—
8.	Citric Acid*	—	92.41 ± 0.834^{k}	—	—	—
9.	BHT*	—	—	59.34 ± 0.474^{g}	—	—
10.	Ascorbic Acid*	—	—	—	60.13 ± 0.077^{g}	62.26 ± 0.766^{h}

Note: Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.
*Reference standard.

3.5 | Antioxidant Activity

The in vitro antioxidant potentials of methanolic extracts of *Melaleuca leucadendron* Linn, *Melaleuca linariifolia* Sm., *Melaleuca bracteata* F. Muell. and *Callistemon viminalis* (Sol. ex Gaertn.) were studied in terms of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, nitric oxide and superoxide radical scavenging activity, chelating activity with Fe^{2+} and reducing power in comparison with the synthetic and natural antioxidants.

3.5.1 | DPPH Radical Scavenging Activity

The DPPH method is the most preferred method to determine the antioxidant potential because it is fast, easy and reliable. DPPH is a stable, synthetic radical that does not disintegrate in solvents like water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen. The DPPH free radical, at its maximum wavelength of 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule [52]. The DPPH radical is converted into DPPH-H (diphenyl hydrazine) molecules in the presence of an antioxidant agent, and it can be observed by the change in colour from violet to golden yellow.

The percent DPPH radical scavenging activity of methanolic extracts at different concentrations has been presented in Table 4. The DPPH radical scavenging activity of all the methanolic extracts varied in a dose dependent manner. Moderate to strong DPPH radical scavenging activity was observed in the extracts in comparison to the standard gallic acid and catechin. The leaf extract of *M. linariifolia* showed strong radical scavenging activity ($IC_{50} = 40.85 \pm 2.50\,\mu g/mL$) followed by the extract of *M. leucadendron* ($IC_{50} = 61.90 \pm 0.49\,\mu g/mL$). The extracts of *M. bracteata* and *C. viminalis* exhibited moderate activity with average IC_{50} values of $96.20 \pm 1.53\,\mu g/mL$ and $131.53 \pm 0.24\,\mu g/mL$ respectively. The radical scavenging activity of extracts was compared with standards (gallic acid and catechin).

3.5.2 | Metal Chelating Activity

Excess free irons have been implicated in the induction and formation of free radicals in biological systems. Metal ions are also responsible for initiating lipid peroxidation and start a chain reaction that leads to the deterioration of food. The catalysis of metal ions also correlates with incidents of cancer and arthritis [53]. Fe^{2+} ions, the most effective pro-oxidants, are commonly found in food systems [54]. Iron is essential for life as it is used for oxygen transport, respiration, and for many enzymatic activities.

Decrease in the red colour of ferrozine-Fe^{2+} complex indicates high radical scavenging activity of the compound. The chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [55]. In the present study, the chelating ability of the methanolic extracts towards ferrous ions was evaluated and percent metal chelating activity has been shown in Table 4. The methanolic extracts presented metal chelating activity in a dose dependent manner. Among the methanolic extracts of species of Myrtaceae family, the leaf methanolic extract of *M. linariifolia* exhibited maximum ($IC_{50} = 186.87 \pm 1.010\,\mu g/mL$) chelating potential followed by leaf extract of *M. leucadendron* ($IC_{50} = 201.87 \pm 0.604\,\mu g/mL$) and extract of *M. bracteata* ($IC_{50} = 241.11 \pm 0.635\,\mu g/mL$) compared to standard chelating agent EDTA ($IC_{50} = 107.15 \pm 2.799\,\mu g/mL$) and citric acid ($IC_{50} = 92.41 \pm 0.834\,\mu g/mL$). The methanolic extract of *Callistemon viminalis* ($IC_{50} = 174.47 \pm 0.986\,\mu g/mL$) showed moderate iron chelating activity ($IC_{50} = 174.47 \pm 0.986\,\mu g/mL$).

3.5.3 | Reducing Power Assay

In reducing power assay, the presence of reductants in the test sample was tested by reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) and monitored by the formation of pearl Prussian blue at 700 nm. Fe (III) reduction is generally used as an indicator of electron donating activity, which is an indicator of phenolic antioxidant action. The reducing ability of a compound depends on the presence of reductones (antioxidants), which shows the antioxidant activity by breaking the free radical chain [56].

The methanolic extracts of all the species under study exhibited reducing potential in a dose dependent manner at all tested concentrations and are presented in Table 4. Among the methanolic extracts, the maximum reducing potential ($RP_{50} = 90.28 \pm 2.554\,\mu g/mL$) was demonstrated by leaf extract of *M. bracteata* followed by leaf extract of *M. linariifolia* ($RP_{50} = 97.24 \pm 0.194\,\mu g/mL$), whereas *M. leucadendron* exhibited relatively weak reducing activity ($RP_{50} = 263.17 \pm 1.284\,\mu g/mL$). The extract of *Callistemon viminalis* demonstrated moderate reducing activity ($RP_{50} = 197.45 \pm 0.800\,\mu g/mL$).

3.5.4 | Nitric Oxide Radical Scavenging Activity

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citrulline with the formation of NO via a five-electron oxidative reaction [57, 58]. Excess concentration of NO is associated with several diseases.

The methanolic extracts showed NO radical scavenging activity in a dose dependent manner at all tested concentrations. Table 4 presented the IC_{50} values of methanolic extracts of species taken for present study. Among the species of family Myrtaceae, the leaf methanolic extract of *M. linariifolia* (MLinME) exhibited maximum scavenging activity with average IC_{50} of $101.81 \pm 0.819\,\mu g/mL$ followed by leaf extract of *M. bracteata* ($IC_{50} = 140.12 \pm 1.147\,\mu g/mL$) and *Callistemon viminalis* ($IC_{50} = 138.75 \pm 0.852\,\mu g/mL$) whereas moderate activity ($IC_{50} = 266.55 \pm 1.376\,\mu g/mL$) was shown by leaf extract of *M. leucadendron* compared to standard Ascorbic acid ($IC_{50} = 60.13 \pm 0.077\,\mu g/mL$).

3.5.5 | Superoxide (SO) Radical Scavenging Activity

Various biological reactions resulted in the formation of superoxide anion, which is very toxic for living beings [59]. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen reduces NBT through coupling reactions [60]. Superoxide anion radical is responsible for the formation of other reactive oxygen species, such as hydrogen peroxide or singlet oxygen.

All the extracts exhibited SO radical scavenging activity in a dose dependent manner. Table 4 revealed the IC_{50} values of methanolic extracts. The maximum superoxide radical scavenging was shown by leaf methanolic extract of *M. bracteata* ($IC_{50} = 75.59 \pm 0.168\,\mu g/mL$), followed by extract of *M. linariifolia* ($IC_{50} = 88.92 \pm 0.561\,\mu g/mL$) and *M. leucadendron* ($IC_{50} = 148.66 \pm 0.818\,\mu g/mL$). The leaf extract of *Callistemon viminalis* exhibited moderated ($IC_{50} = 86.50 \pm 0.690\,\mu g/mL$) superoxide radical scavenging activity.

Previously, Surh and Yun [61] studied the antioxidant activity of methanolic extract of *Melaleuca leucadendron* and reported significant antioxidant activity. Kersh et al. [40] studied the Phytochemical composition and antioxidant activities of methanolic extract of *Melaleuca leucadendron* and reported strong antioxidant activity with high phenolic content. Ganesan et al. [62] reported significant antioxidant activity in the extract of *Melaleuca styphelioides*.

In another investigation Silva et al. [63] reported significant antioxidant activity of ethanolic extract of flowers of *Melaleuca leucadendron*. Wang et al. [44] reported antioxidant, antibacterial and herbicidal activities of the essential oil of *M. bracteata* from China. Our findings are consistent with previous studies indicating that species of the Myrtaceae family are strong candidates as sources of potential antioxidants. The antioxidant activity of the extracts may be attributed to the presence of key marker compounds such as nerolidol [64], ursolic acid [65], oleanolic acid [66], quinic acid [67], gallic acid [68] and shikimic acid [69], which are widely distributed across the extracts. The synergic effect of other minor compounds like phytol, α-linolenic acid, α-tocopherol, linoleic acid, palmitic acid, lactic acid, β-sitosterol can also be responsible for the antioxidant property of the extracts. The redox properties of phenolic compounds play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [70]. The results confirm the remarkably good radical scavenging activity of extracts. The strong radical scavenging activity of *M. bracteata* could be attributed to its major compound methyl eugenol as this molecule have reported antioxidant activity [71]. These results revealed that extracts under study act as an electron donor and react with free radicals, terminate the radical chain reaction and act as reducing agent.

3.6 | Total Antioxidant Activity

The total antioxidant activity of the methanolic extracts was measured spectrophotometrically through the phosphomolybdenum method, which is based on the subsequent formation of Mo (IV) to Mo (V) with a maximum absorption at 695 nm (Table 5). The higher the absorbance, the stronger is the antioxidant activity. The highest activity was found in the methanolic extract of *Melaleuca bracteata* ($65.24 \pm 1.07\,\mu g/mL$) followed by *Callistemon viminalis* ($35.45 \pm 0.49\,\mu g/mL$) and *Melaleuca leucadendron* ($33.34 \pm 0.44\,\mu g/mL$).

3.7 | In Vitro Anti-Inflammatory Assay

Tables 6, 7 and Figure 3 presented the percent inhibition of protein denaturation and IB_{50} values of methanolic extracts at different concentrations. The methanolic extracts exhibited anti-inflammatory activity in a dose-dependent manner with maximum inhibition at the highest concentration. Among the species of the family Myrtaceae, the highest inhibitory activity

TABLE 5 | Total antioxidant activity of various methanolic extracts.

S. no	Sample	TAC (μg/mL)
1.	MLeuME	33.34 ± 0.44^{f}
2.	MLinME	20.52 ± 0.32^{h}
3.	MBME	65.24 ± 1.07^{c}
4.	CVME	35.45 ± 0.49^{e}

Note: Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.

($IB_{50} = 136.92 \pm 3.96$ ppm) was found in the extract of *Melaleuca linariifolia* while the *Melaleuca bracteata* extract showed minimum activity ($IB_{50} = 343.05 \pm 1.129$ ppm) when compared to the standard diclofenac sodium ($IB_{50} = 109.73 \pm 2.413$ ppm). A moderate activity ($IB_{50} = 194.73 \pm 1.490$ ppm) was recorded for the extract of *Melaleuca leucadendron*. The extract of *Callistemon viminalis* exhibited significant anti-inflammatory activity ($IB_{50} = 252.26 \pm 1.300$ ppm). Albouchi et al. [72] reported anti-inflammatory activity in the extract of *Melaleuca styphelioides*. Ebrahim et al. [73] reported anti-inflammatory, antioxidant and anti-aging activities in the polyphenol rich fraction of *Melaleuca*

TABLE 6 | In vitro anti-inflammatory activity of extracts.

		% Inhibition of protein denaturation					
S. no	Sample	75 ppm	125 ppm	250 ppm	500 ppm	750 ppm	1000 ppm
1.	MLeuME	18.93	25	83.29	109.15	142.82	186.50
		19.05	25.12	84.03	109.77	143.31	184.28
		19.05	25.37	84.15	109.52	142.82	185.51
		19.01	25.16	83.82	109.48	142.98	185.43
		±0.071	±0.189	±0.468	±0.311	±0.285	±1.116
2.	MLinME	24.13	52.35	72.89	100.61	111.50	125.37
		23.01	51.73	72.89	100.61	111.75	126.11
		23.63	50.74	72.77	100.74	111.63	125.86
		23.59	51.60	72.85	100.66	111.63	125.78
		±0.558	±0.811	±0.071	±0.071	±0.123	±0.378
3.	MBME	28.83	38.11	55.94	60.02	70.04	77.97
		28.46	37.74	56.18	60.14	70.29	77.59
		28.71	37.99	56.06	59.77	70.42	77.59
		28.67	37.95	56.06	59.98	70.25	77.72
		±0.189	±0.189	±0.123	±0.189	±0.189	±0.214
4.	CVME	18.93	37.12	63.24	85.51	90.84	105.56
		18.44	37.12	62.99	85.39	90.84	105.69
		19.05	37.12	62.99	84.90	91.08	106.06
		18.81	37.12	63.07	85.27	90.92	105.77
		±0.327	±0.00	±0.142	±0.327	±0.142	±0.257
		97.15	128.71	195.04	219.43	229.45	291.21
		98.26	128.21	197.15	219.80	229.82	291.83
		97.40	128.25	195.99	219.30	229.62	291.46
		±0.772	±0.434	±1.067	±0.567	±0.189	±0.327
5.	Diclofenac sodium*	31.93	44.30	85.14	86.50	101.4851	114.10
		32.05	43.56	84.65	86.88	101.11	114.72
		31.55	44.05	84.90	86.75	101.36	114.48
		31.84	43.97	84.90	86.71	101.32	114.43
		±0.257	±0.378	±0.247	±0.189	±0.189	±0.311

*Reference standard.

TABLE 7 | IB_{50} value for anti-inflammatory activity of various extracts.

S. no	Sample	IB_{50} values (ppm)			Mean IB_{50} value (ppm) ± SD
		I	II	III	
1.	MLeuME	196.45	193.83	193.91	194.73 ± 1.490^{d}
2.	MLinME	132.38	138.67	139.70	136.92 ± 3.96^{e}
3.	MBME	342.07	344.28	342.78	343.05 ± 1.129^{a}
4.	CVME	251.07	253.65	252.06	252.26 ± 1.300^{c}
5.	Diclofenec sodium*	106.99	111.54	110.66	109.73 ± 2.413^{f}

Note: Values are mean ± standard deviation, Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.
Abbreviations: CVME, *C. viminalis* methanolic extract; MBME, *M. bracteata* methanolic extract; MLeuME, *M. leucadendron* methanolic extract; MLinME, *M. linariifolia* methanolic extract.
*Reference standard.

rugulosa. Elsayed et al. [74] reported potential hepatoprotective, antioxidant and anti-inflammatory activities against paracetamol-induced liver inflammation by aqueous methanol extract of *M. rugulosa* leaves. Ijinu et al. [75] reported anti-inflammatory activity of essential oil of *Melaleuca citrina* from India. Compounds such as (*E,Z*)-nerolidol, betulinic acid [76], gallic acid [68] and shikimic acid [69] have been reported to exhibit anti-inflammatory activity. In the present study, *M. linarrifolia* demonstrated the highest anti-inflammatory activity, which may be attributed to the presence of betulinic acid, gallic acid, quinic acid, or possibly the synergistic effects of minor constituents in the extract. Additionally, the antioxidant properties of this species may be associated with its anti-inflammatory activity.

3.8 | Antibacterial Activity

The results of antibacterial activity of extracts have been presented in Table 8 and Figure 3a–d. The results revealed moderate to good antimicrobial activity against the tested microorganism like *Salmonella typhimurium*, *Escherihia coli*, *Staphylococcos aureus* and *Bacillus megaterium*.

3.8.1 | Antibacterial Activity of Methanolic Extracts

The methanolic extracts of plants undertaken for present study have been screened for their antibacterial potential and compared with standard Rifamycin. The methanolic extracts exhibited a poitive correlation between Concentration and Zone of Inhibition. The tested exracts exhibited strong activity against *S. typhimurium*, with inhibition zone of *M. linalriifolia* (ZI = 18.33 mm), *M. bracteata* (ZI = 18.67 mm) and *M. leucadendron* (ZI = 16.33 mm). However, the methanolic extract of *C. viminalis* (ZI = 13 mm) exhibited least activity compared to Rifamycin (ZI = 35.33 mm). A stronger senstivity was also observed against *E. coli*, where the extract of *M. bracteata* demonstrated good inhibition (ZI = 20 mm), followed by *M. leucadendron* (ZI = 17.33 mm) and *Callistemon viminalis* (ZI = 16.67 mm) as compared to Refamycin (ZI = 37 mm) at a concentration of 1000 ppm.

Similarly, the extracts exhibited strong inhibition against *S. aureus* and *B. megaterium* with maximum inhibition of 23.67 mm and 24 mm by *Melaleuca linarrifolia* and *Callistemon viminalis*, respectively.

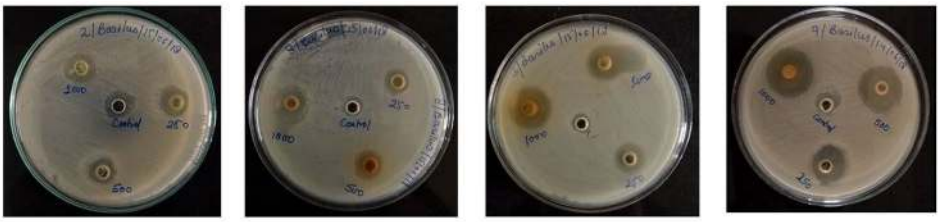
Previously, Kanwal et al. [50] reported antibacterial activity of n-hexane extract of *Callistemon viminalis* against *Staphylococcus aureus* (71.30% ± 4.44%), *Bacillus subtilis* (68.55% ± 2.70%), *Pseudomonas aeruginosa* (57.86% ± 6.02%) and *Salmonella typhi* (53.90% ± 5.05%). Mpala et al. [77] reported potential of *M. alternifolia* extract against bacterial species like *A. hydrophilia*, *C. freundi*, *P. mirabilis*, and *B. cereus* with MIC values as low as 147 μg/mL. The presence of marker compounds like (*E,Z*)-nerolidol [64], ursolic acid [65], quinic acid [67], etc., having reported antibacterial activity could be attributed to the antimicrobial potential of tested extracts. Our results strongly suggest the use of Myrtaceae species as a source of potential antimicrobial agents.

3.9 | Herbicidal Activity

Weeds comprise a small and taxonomically different group of plants. They exhibited relatively high adaptability to different habitats, taking advantage of the favourable conditions that occur in agricultural systems and competing with crops. Excessive application of synthetic herbicides has contributed to increased resistance in weeds, leading to gradual degradation of soil and the environment, and posing hazards to human health. The secondary metabolites of plant species display an excellent potential to develop new herbicide formulations or as a cornerstone towards identifying active components to obtain natural herbicides. Thus, numerous studies have been done for plant-derived compounds in order to obtain synthetic herbicide substitutes for weed control. In view of this fact, the methanolic extract of selected species of the family Myrtaceae was tested for herbicidal activity.

3.9.1 | Herbicidal Activity of Methanolic Extracts

The methanolic extract of Myrtaceae species was screened for herbicidal activity. The herbicidal potential was evaluated by observing seed germination inhibition and inhibition of root and



MLeuME MLinME MBME CVME

(a) Antibacterial activity of studied extracts against *Bacillus megaterium*



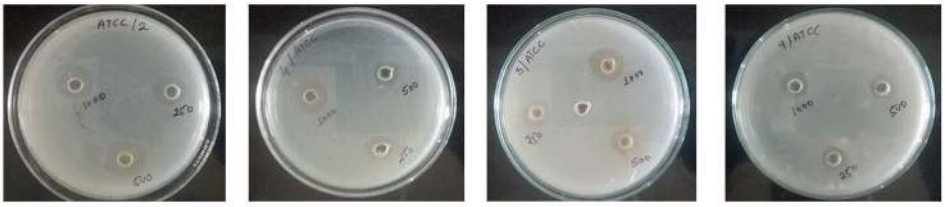
MLeuME MLinME MBME CVME

(b) Antibacterial activity of studied extracts against *Escherichia coli*



MLeuME MLinME MBME CVME

© Antibacterial activity of studied extracts against *Staphylococcus aureus*



MLeuME MLinME MBME CVME

(d) Antibacterial activity of studied extracts against *Salmonella Typhimurium*

FIGURE 3 | Antibacterial activity of extracts against tested pathogens.

shoot length of radish (*Raphanus sativus*) seeds. The seeds were treated with four different concentrations (250 ppm, 500 ppm, 750 ppm and 1000 ppm) of extracts. Pendimethalin was used as a reference herbicide. The control and reference herbicide were also tested for comparison.

The methanolic extracts under study exhibited dose dependent inhibition and a significant reduction of seed germination at the highest concentration of 1000 ppm. Among the species of family Myrtaceae, the highest inhibitory effect against seed germination was shown by the methanolic extract of *Melaleuca bracteata* causing 96.66% inhibition at concentrations of 1000 ppm, followed by *Melaleuca linariifolia* (86.66% ± 5.77%) and *Melaleuca leucadendron* (80.0% ± 0.00%). The methanolic extract of *Callistemon viminalis* exhibited strong inhibition at 1000 ppm by inhibiting 93.33% inhibition of seed germination.

TABLE 8 | Antibacterial activities of the methanolic extracts of plants of family Myrteacae.

S. no	Sample	Test bacteria			
		S. typhimurium	*E. coli*	*S. aureus*	*B. megaterium*
1.	**MLeuME**				
	250 ppm	12 ± 1.00^{b}	12.67 ± 2.00^{c}	12.33 ± 0.58^{a}	13.00 ± 2.00^{a}
	500 ppm	13 ± 1.00^{b}	15.67 ± 1.53^{b}	12.67 ± 2.30^{a}	14.33 ± 1.15^{a}
	1000 ppm	16.33 ± 0.58^{a}	17.33 ± 2.08^{a}	16 ± 1.00^{a}	15 ± 2.65^{a}
2.	**MLinME**				
	250 ppm	12 ± 1.73^{b}	13 ± 2.00^{a}	19.33 ± 0.58^{b}	19.67 ± 0.58^{b}
	500 ppm	14.33 ± 1.53^{ab}	13.67 ± 1.53^{a}	19.66 ± 1.53^{b}	20.67 ± 0.58^{b}
	1000 ppm	18.33 ± 1.53^{a}	14.67 ± 0.58^{a}	23.67 ± 0.58^{a}	23.67 ± 0.58^{a}
3.	**MBME**				
	250 ppm	12.67 ± 0.58^{b}	10.67 ± 0.58^{b}	14.33 ± 0.58^{c}	14.67 ± 0.58^{c}
	500 ppm	13.66 ± 1.15^{b}	12 ± 0.00^{b}	16.67 ± 0.58^{b}	16.67 ± 0.58^{b}
	1000 ppm	18.67 ± 1.53^{a}	20 ± 1.00^{a}	20.33 ± 0.58^{a}	21 ± 1.00^{a}
4.	**CVME**				
	250 ppm	11 ± 1.00^{a}	12.33 ± 1.15^{b}	18.33 ± 0.58^{b}	19.67 ± 0.58^{b}
	500 ppm	12.66 ± 1.15^{a}	13.67 ± 0.58^{b}	20.67 ± 0.58^{ab}	23.33 ± 0.58^{a}
	1000 ppm	13 ± 1.00^{a}	16.67 ± 1.15^{a}	22.67 ± 1.52^{a}	24 ± 1.73^{a}
5.	Rifamycin* (1000 ppm)	35.33 ± 0.58^{a}	37 ± 1.00^{a}	29.67 ± 1.15^{a}	34.67 ± 0.58^{a}

Note: Values are mean ± standard deviation, Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.
Abbreviations: CVME, *C. viminalis* methanolic extract; MBME, *M. bracteata* methanolic extract; MLeuME, *M. leucadendron* methanolic extract; MLinME, *M. linariifolia* methanolic extract.
*Reference standard.

TABLE 9 | Mean percent seed germination inhibition values of methanolic extracts.

Extracts/Standard	% Inhibition of seed germination			
	250 ppm	500 ppm	750 ppm	1000 ppm
MLeuME	6.66 ± 5.77^{d}	33.33 ± 5.77^{c}	46.66 ± 5.77^{b}	80.00 ± 0.00^{a}
MLinME	16.66 ± 5.77^{d}	46.66 ± 5.77^{c}	63.33 ± 5.77^{b}	86.66 ± 5.77^{a}
MBME	26.66 ± 5.77^{c}	46.66 ± 5.77^{b}	60.00 ± 10.00^{b}	96.66 ± 5.77^{a}
CVME	16.66 ± 5.77^{c}	26.66 ± 5.77^{c}	56.66 ± 5.77^{b}	93.33 ± 5.77^{a}
Pendimethalin*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Note: Values are mean ± standard deviation, Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.
Abbreviations: CVME, *Callistemon viminalis* methanolic extract; MBME, *Melaleuca bracteata* methanolic extract; MLeuME, *Melaleuca leucadendron* methanolic extract; MLinME, *Melaleuca linariifolia* methanolic extract.
*Standard herbicide.

3.9.2 | Effect of Methanolic Extracts on Root and Shoot Growth

The inhibitory effects of the methanolic extracts on root and shoot growth in terms of length and percentages of growth inhibition relative to control are shown in Tables 9–12 and Figures 4–7.

All the extracts significantly inhibited root growth in a concentration-dependent manner. Among the tested species, *M. linariifolia* causes 97.07% inhibition of root growth at 1000 ppm, *M. bracteata* inhibited 95.75%, *M. leucadendron* inhibited 93.43% while the extract of *C. viminalis* inhibited 92.42% of root growth at 1000 ppm of concentration.

The inhibitory effects of the extracts on root growth were greater than on shoot growth. The strong herbicidal activity of methanolic extract could be attributed to the presence of polyhydroxy and polyphenolic compounds such as quinic acid, gallic acid, methyl eugenol, glycerol and ursolic acid etc.

3.10 | Molecular Docking Study

From in vitro studies, it was found that most of the extracts exhibited strong antioxidant activity. It was also examined whether the major phytoconstituents from all extracts physically bind with antioxidant protein (Human peroxiredoxin 5). The tested extracts showed good free radical inhibition, and the enzyme Human peroxiredoxin 5 was selected, as it exhibits broader activity against reactive oxygen species [78, 79]. Among fourteen selected major phytocompounds, ursolic acid (CID: 64945) and oleanolic acid (CID: 10,494) demonstrated the best binding affinity with Human peroxiredoxin 5, with the highest binding affinity (−7.4 kcal/mol) (Table 13). By introspecting the multiple dock poses, the best docked pose was selected with the lowest binding energy. The best docked pose of ursolic acid exhibited Van der Waal interactions with 1HD2 containing amino acid residues such as Gly A:92, Arg A:95, Gly A:17, Glu A:16, Leu A:96, Gly A:82, Gly A:85, Ala A:90, Arg A:86, Gly A:91 and for Oleanolic acid it exhibited Van der Waal interactions with 1HD2 containing amino acid residues such as ALA A:90, GLY A:65, GLY A:62, ARG A:86, GLU A:91, ARG A:95, GLY A:92, GLY A:17 as represented in Figure 8. Highest binding affinity was found for ursolic acid and oleanolic acid (−7.4 kcal/mol), followed by betulinic acid (−6.6 kcal/mol), quinic acid (−6.0 kcal/mol), shikimic acid (−5.8 kcal/mol), gallic acid (−5.7 kcal/mol), *myo*-inositol (−5.7 kcal/mol), phytol (−5.4 kcal/mol), linolenic acid (−5.3 kcal/mol), (*E*,*Z*)-nerolidol (−5.0 kcal/mol), mannitol (−4.9 kcal/mol), methyl eugenol (−4.6 kcal/mol), ribitol (−4.6 kcal/mol) and glycerol (−3.6 kcal/mol). The most negative binding free energy demonstrated a more significant interaction between the protein and the ligand. The binding energies of the volatiles docked with Human peroxiredoxin 5 (Figure 8) were found to be in the range −7.4 to −3.6 kcal/mol. Based on the study, it was observed that the major phytochemical constituents interacted favourably with the receptor protein, most of which are the Van der Waals interactions

TABLE 10 | IC_{50} values of methanolic extracts of various species under study.

Extracts	IC_{50} values in triplicate (ppm) I	II	III	IC_{50} (ppm)
MLeuME	700	706.52	738.63	715.05 ± 20.68^{a}
MLinME	568.18	597.82	597.82	587.94 ± 17.11^{bc}
MBME	535.71	511.36	572.91	539.99 ± 0.99^{cd}
CVME	625	625	669.64	639.88 ± 25.77^{ab}

TABLE 11 | Mean percent inhibition values of root length of methanolic extracts.

Extracts/Standard	% Inhibition of root length 250 ppm	500 ppm	750 ppm	1000 ppm
MLeuME	69.98 ± 0.699^{b}	87.67 ± 5.338^{a}	93.03 ± 3.277^{a}	93.43 ± 3.446^{a}
MLinME	74.54 ± 11.125^{b}	83.93 ± 8.661^{ab}	90.80 ± 7.973^{ab}	97.07 ± 5.073^{a}
MBME	46.96 ± 2.624^{d}	57.57 ± 2.624^{c}	87.87 ± 2.624^{b}	95.75 ± 0.524^{a}
CVME	42.42 ± 9.741^{c}	57.57 ± 7.160^{bc}	69.69 ± 1.749^{ab}	92.42 ± 0.874^{a}
Pendimethalin*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Note: *Pendimethalin indicates Standard herbicid.

TABLE 12 | Mean percent inhibition values of shoot length of methanolic extracts.

Extracts/Standard	% Inhibition of shoot length 250 ppm	500 ppm	750 ppm	1000 ppm
MLeuME	21.67 ± 3.677^{d}	44.05 ± 5.100^{c}	63.48 ± 10.649^{b}	88.22 ± 5.100^{a}
MLinME	35.21 ± 5.100^{b}	49.94 ± 2.698^{b}	76.44 ± 5.100^{a}	91.16 ± 8.833^{a}
MBME	35.21 ± 5.100^{c}	52.88 ± 5.100^{b}	87.04 ± 4.44^{a}	93.52 ± 1.020^{a}
CVME	14.60 ± 5.100^{d}	35.21 ± 5.100^{c}	71.73 ± 6.370^{b}	94.11 ± 5.100^{a}
Pendimethalin*	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Note: Values are mean ± standard deviation. Data are represented by means ± SD of 3 independent experiments. Columns with the same letter did not differ significantly according to ANOVA analysis.
Abbreviations: CVME, *Callistemon viminalis* methanolic extract; MBME, *Melaleuca bracteata* methanolic extract; MLeuME, *Melaleuca leucadendron* methanolic extract; MLinME, *Melaleuca linariifolia* methanolic extract.
*Standard herbicide.

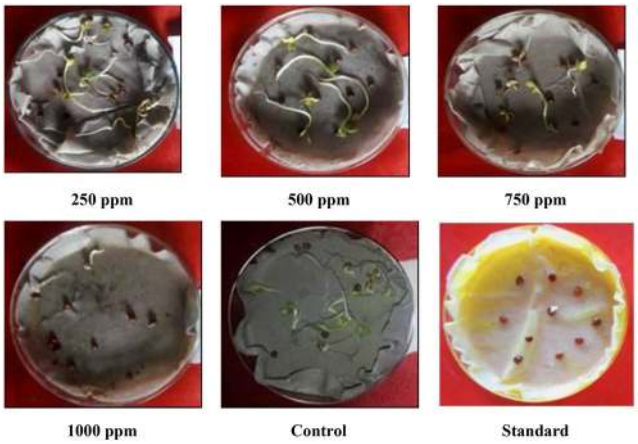


FIGURE 4 | Herbicidal effect of methanolic extract of *M. leucadendron* against *R. sativus* seeds at different concentrations.

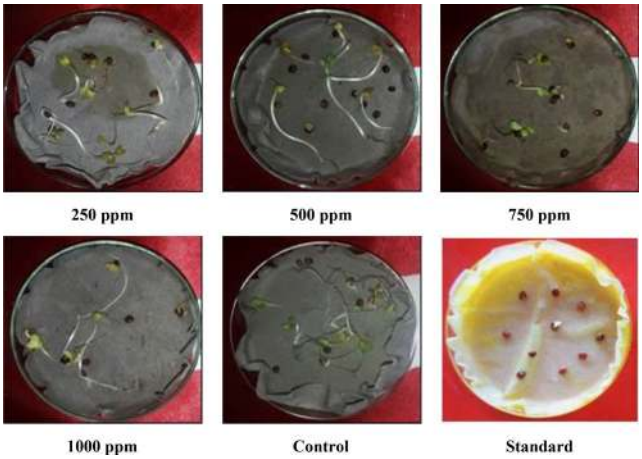


FIGURE 5 | Herbicidal effect of methanolic extract of *M. linnarrifolia* against *R. sativus* seeds at different concentrations.

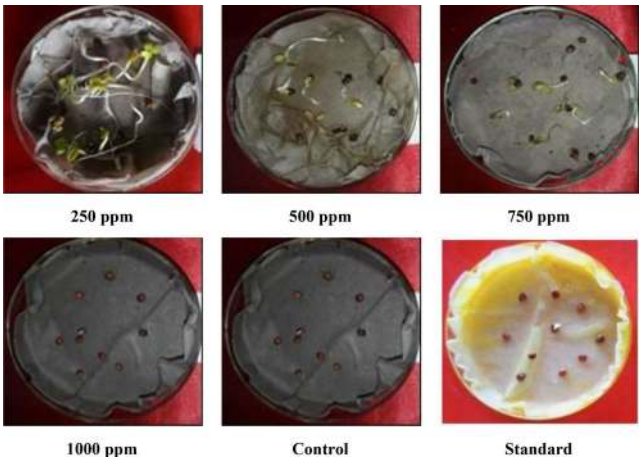


FIGURE 6 | Herbicidal effect of methanolic extract of *M. bracteata* against *R. sativus* seeds at different concentrations.

& conventional hydrogen bonds. From our molecular docking findings, it can be suggested that all ligands interact favourably with the target proteins. The analysis of ligand recognition reveals that the compounds can be used as good

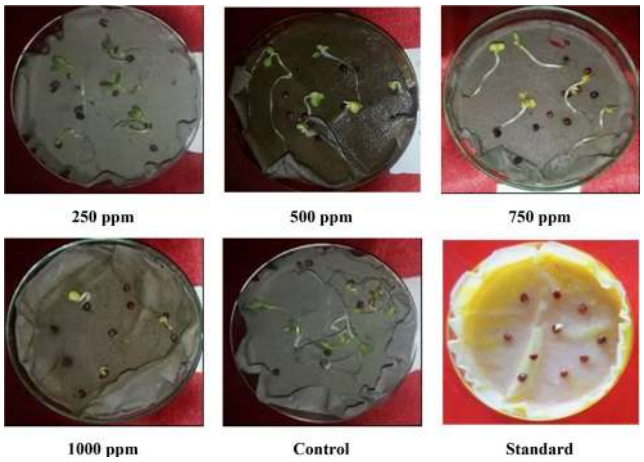


FIGURE 7 | Herbicidal effect of methanolic extract of *C. viminalis* against *R. sativus* seeds at different concentrations.

TABLE 13 | Binding energies of major phytochemicals obtained from molecular docking analysis.

S. no	Compound name	Binding affinity (kcal/mol)
1.	Phytol	−5.4
2.	Mannitol	−4.9
3.	Quinic acid	−6.0
4.	Glycerol	−3.6
5.	Methyl eugenol	−4.6
6.	Gallic acid	−5.7
7.	*Myo*-inositol	−5.7
8.	Linolenic acid	−5.3
9.	Betulinic acid	−6.6
10.	Ursolic acid	−7.4
11.	(*E,Z*)-nerolidol	−5.0
12.	Shikimic acid	−5.8
13.	Oleanolic acid	−7.4
14.	Ribitol	−4.6

antioxidant agents. Figure 9 represents the binding energy (−kcal/mol) of selected phytocompounds from different species of the Myrtaceae family, complexed with 1HD2.

3.11 | ADMET Analysis of Major Phytochemicals

The ADME characteristics—namely Absorption, Distribution, Metabolism and Excretion—of the selected phytocompounds were predicted using the Swiss ADME web-based tool (http://www.swissadme.ch/), accessed on 8 February 2026. Their pharmacokinetic behaviour and drug-likeness were evaluated in accordance with established guidelines, including Lipinski's, Egan's and Veber's rules, which are commonly used to assess drug suitability. In line with the accepted drug-likeness criteria,

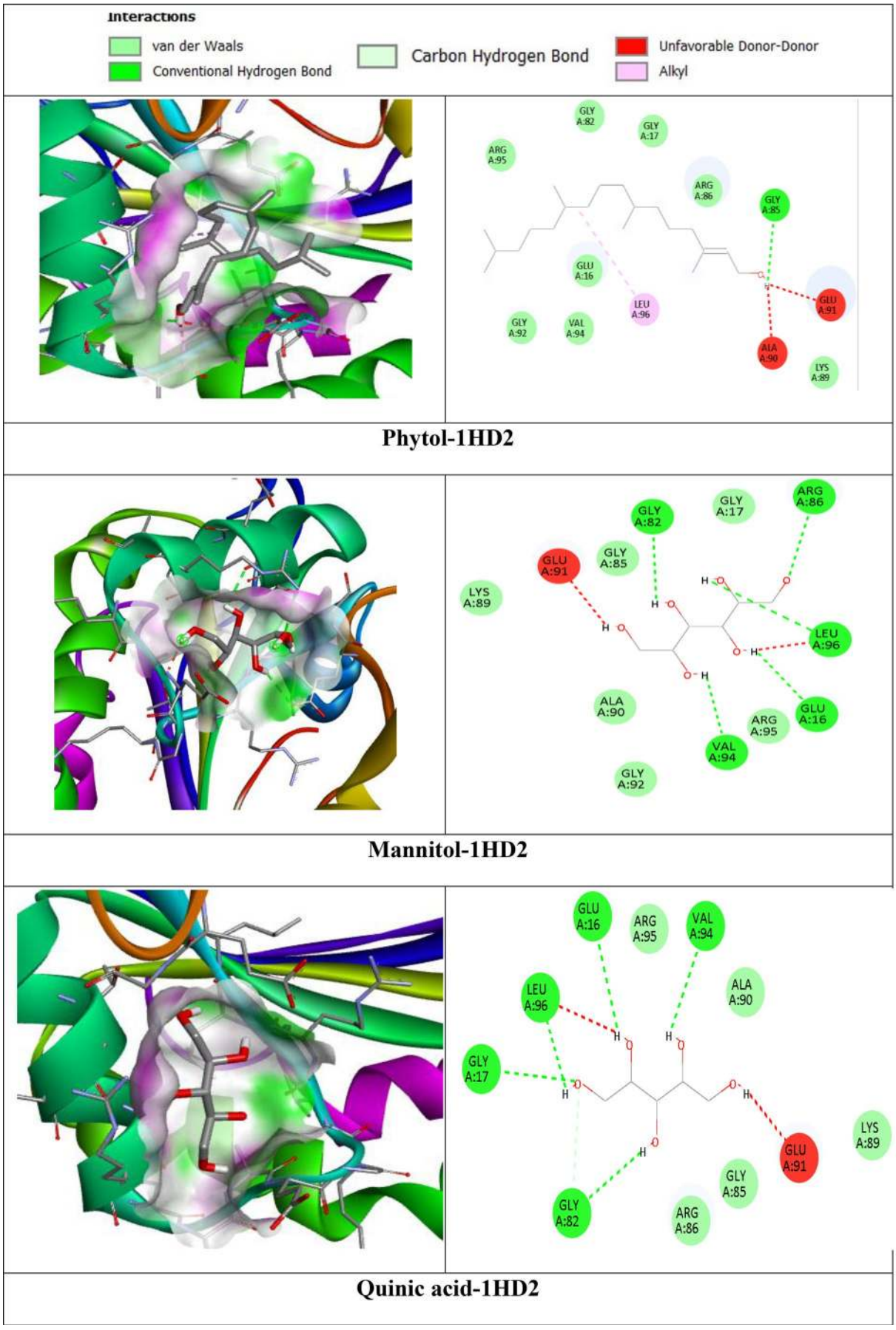
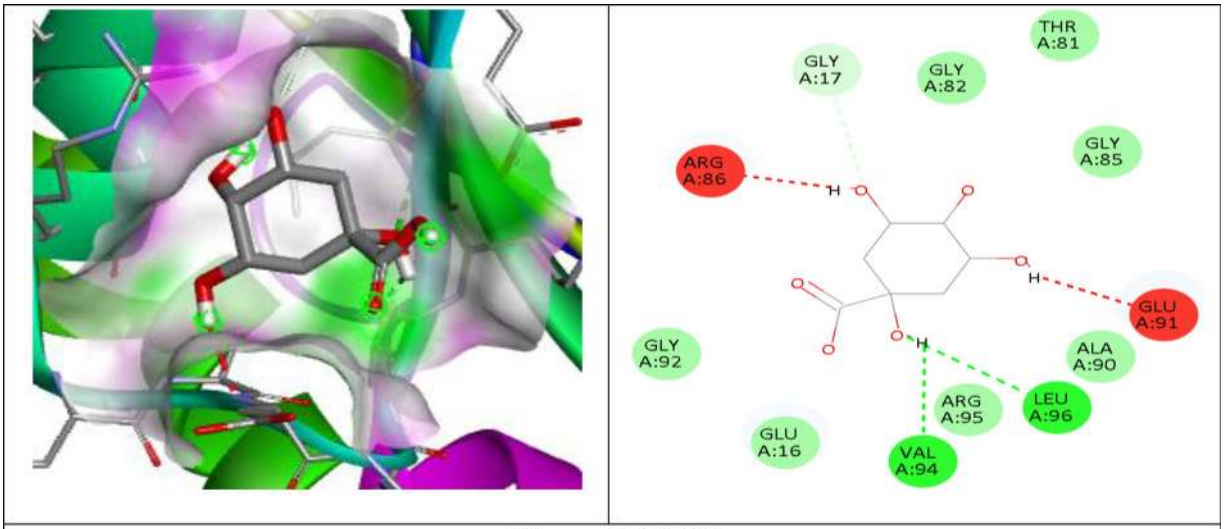
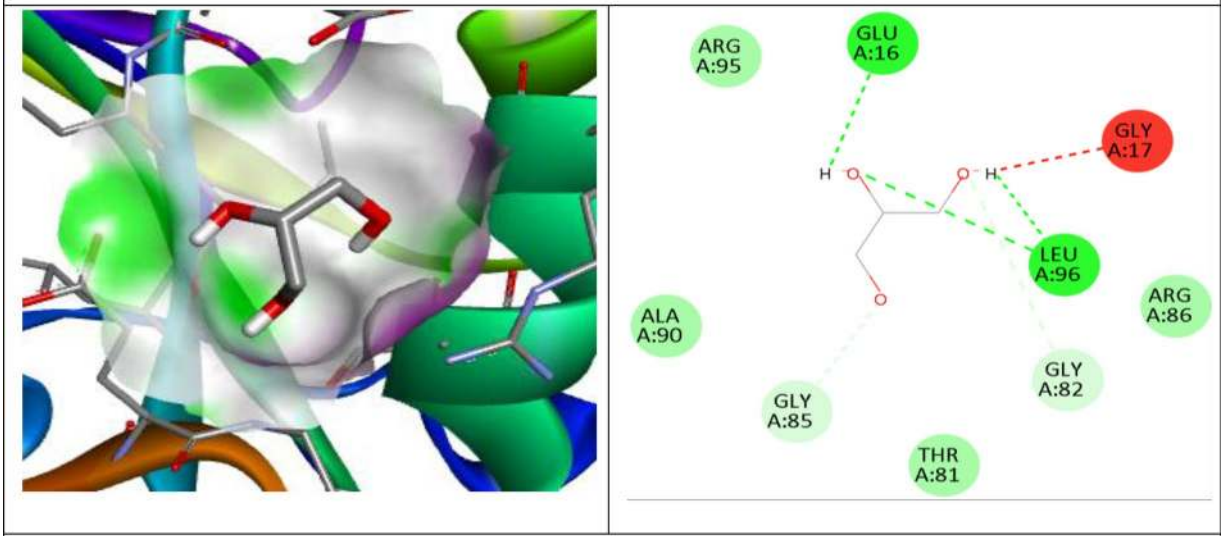


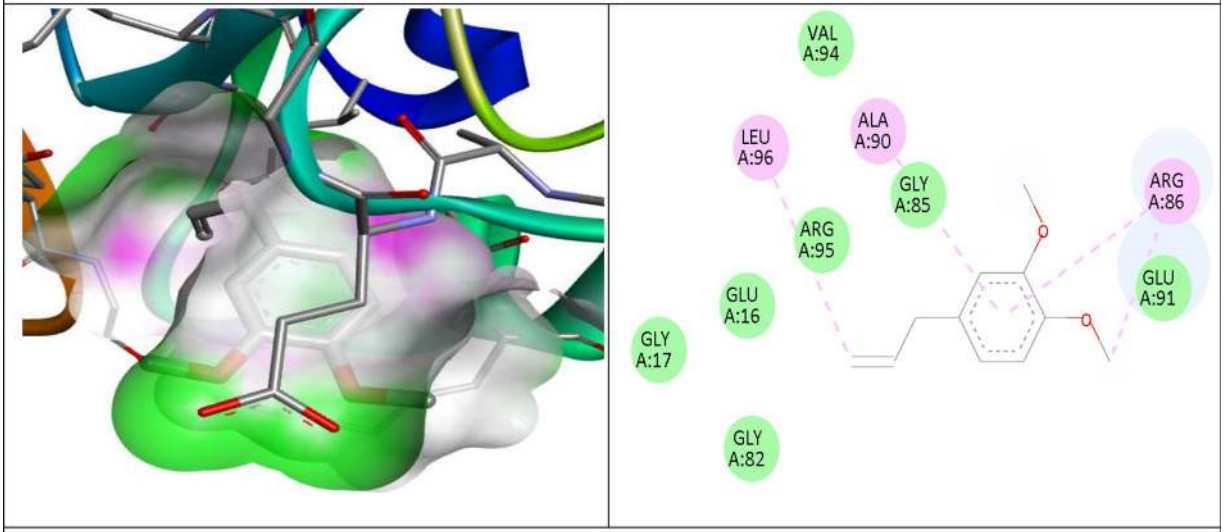
FIGURE 8 | Molecular docking studies of major phytochemicals (2D and 3D interactions) from different species of Myrtaceae family.



Glycerol-1HD2



Methyl eugenol-1HD2



Gallic acid-1HD2

FIGURE 8 | (Continued)

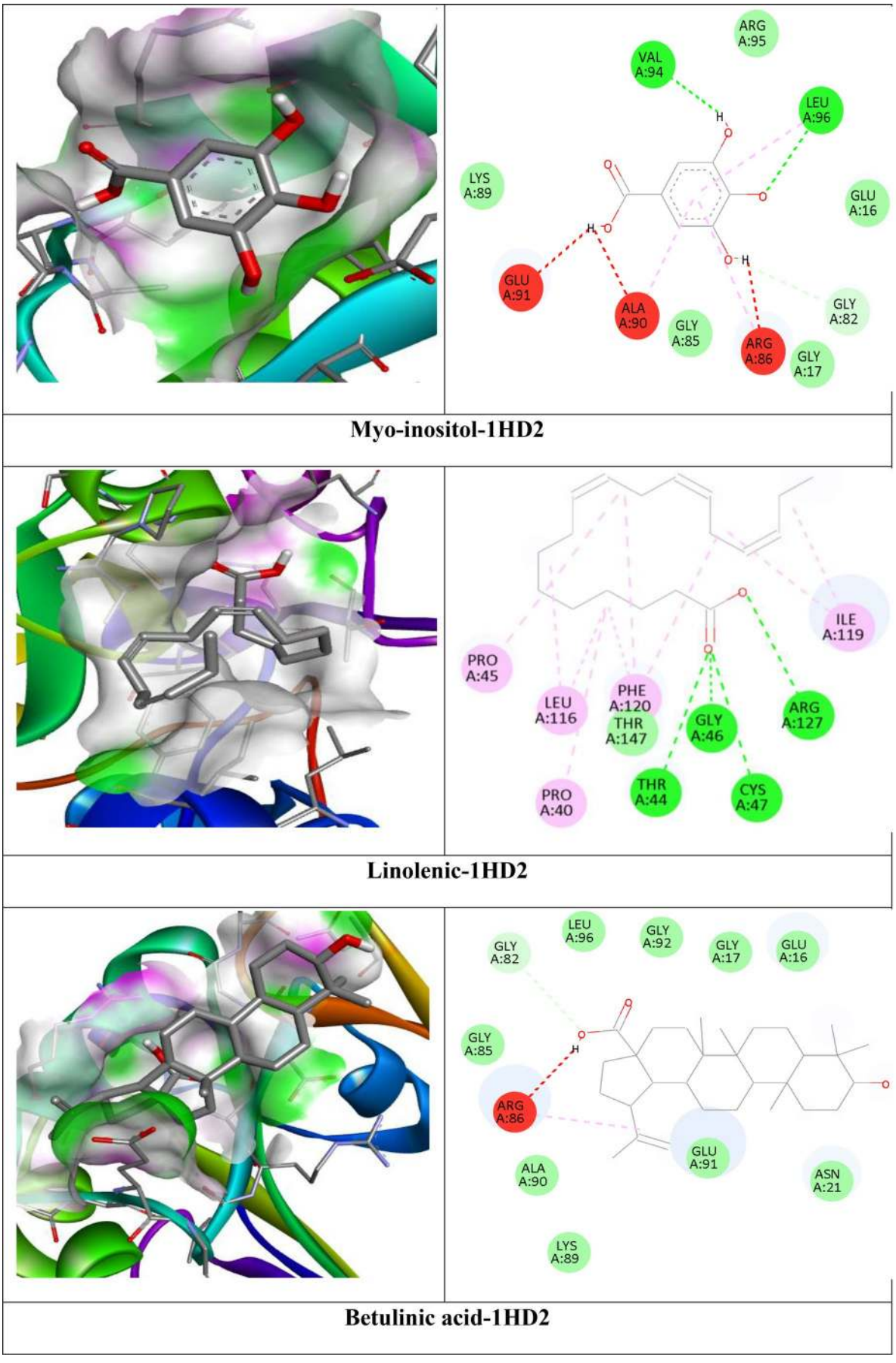


FIGURE 8 | (Continued)

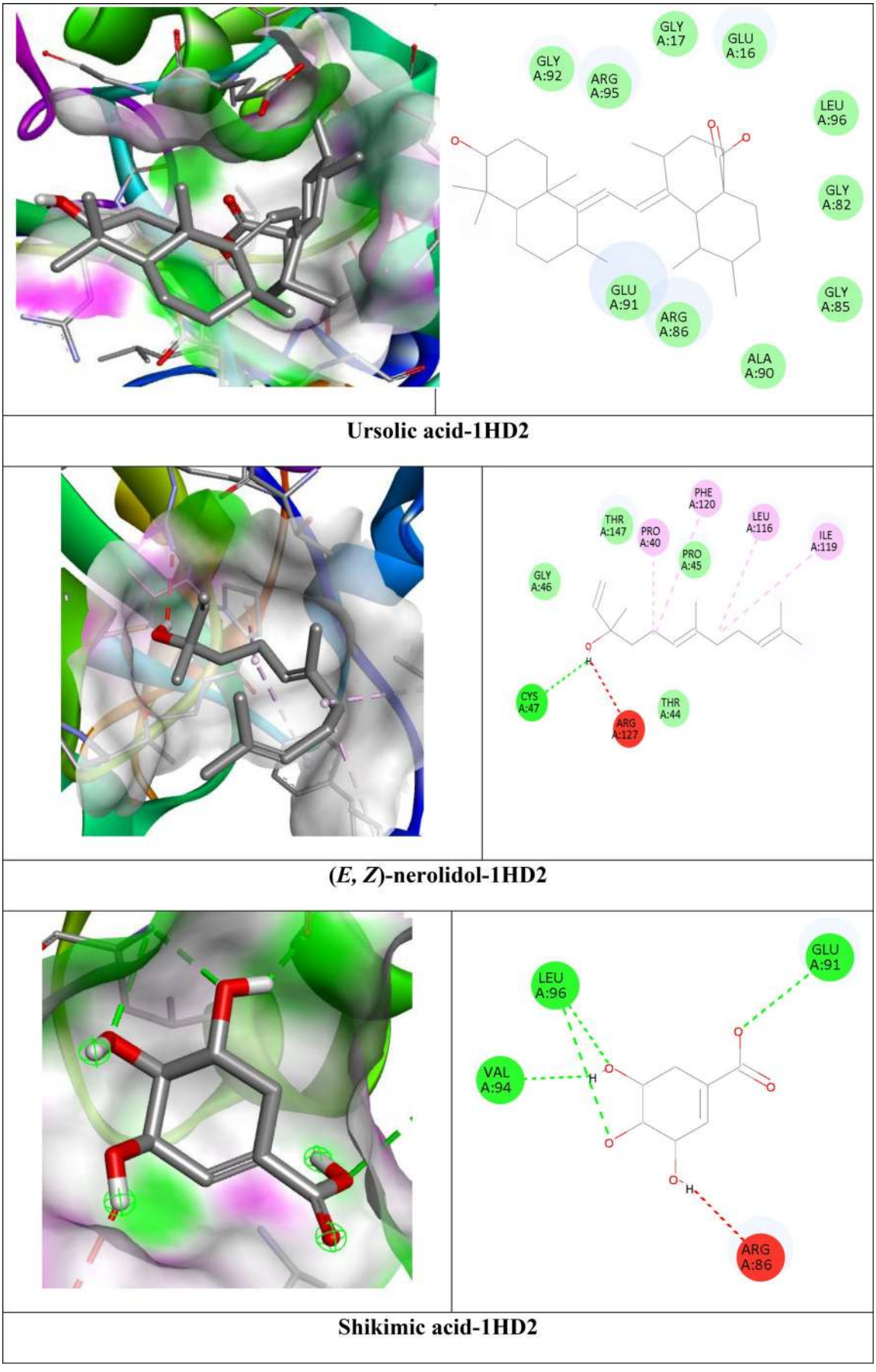


FIGURE 8 | (Continued)

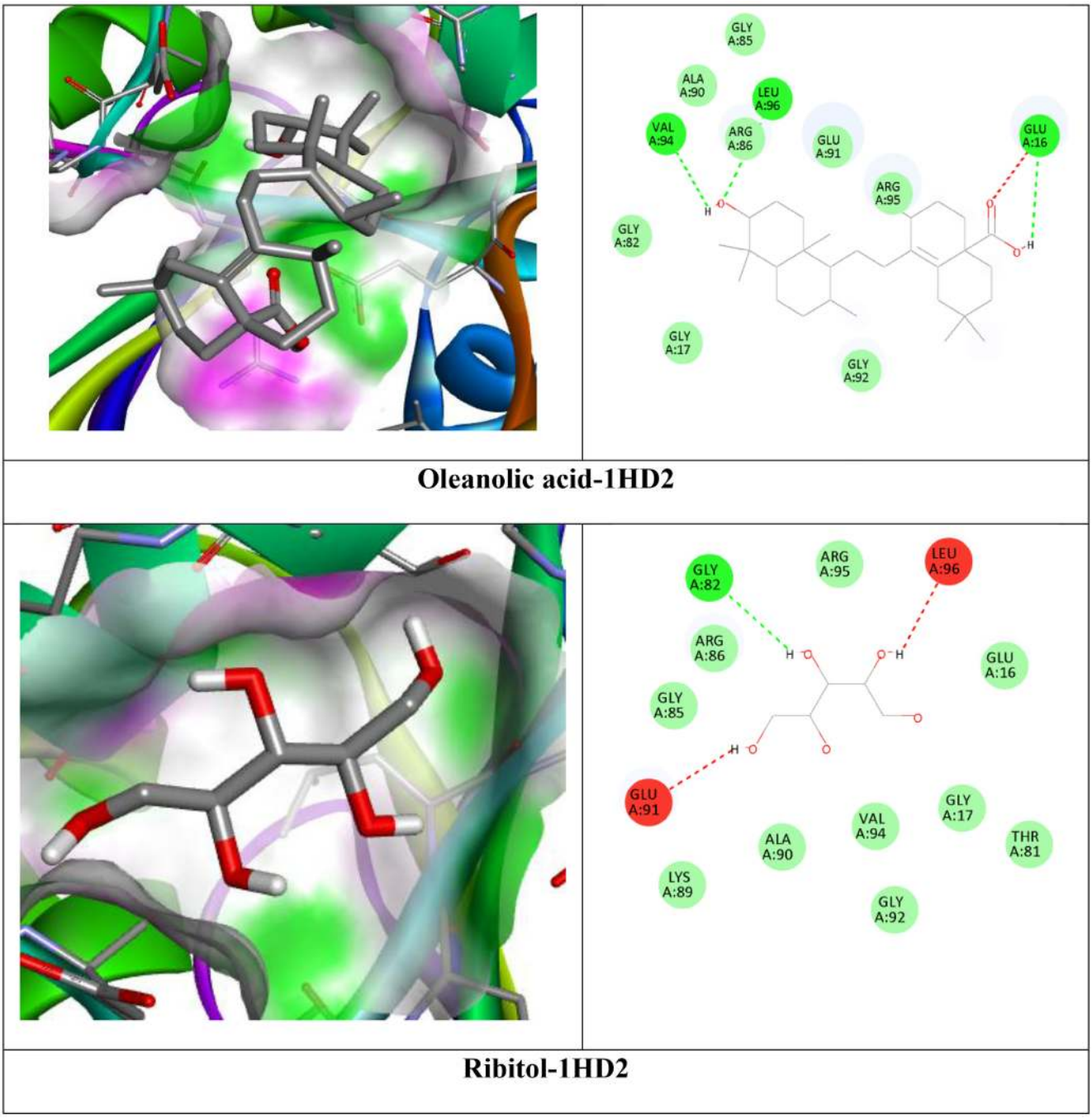


FIGURE 8 | (Continued)

a compound is considered suitable if it violates no more than one of Lipinski's rules: molecular weight (MW) < 500, topological surface area (TPSA) < 140, number of H-bond acceptors (nOHA) ≤ 5, number of H-bond donors (nOHD) ≤ 5, water partition coefficient (WLOGP) ≤ 5.88, and number of rotatable bonds (nRB) ≤ 10. Based on the current findings, most of the selected compounds follow Lipinski's, Egan's, and Verber's rules, indicating the good drug-like properties of the phytocompounds. The bioavailability score was found to be 0.55 for seven compounds of the total compounds selected, indicating higher bioactivity of the molecules, except linolenic acid, betulinic acid, ursolic acid, shikimic acid, oleanolic acid and quinic acid. The TPSA values for phytol, (*E,Z*)-nerolidol and methyl eugenol were found to be less than 30 $Å^2$, indicating good brain penetration and good lipophilicity behaviour with the consensus Log Po/w coming in the range −2.67 to 6.25 (Table 14). Eleven compounds with no P-glycoprotein (P-gp) substrate were found, suggesting good intestinal absorption. The compounds methyl eugenol, glycerol, gallic acid, (*E,Z*)-nerolidol, shikimic acid and linolenic acid exhibit high gastrointestinal absorption, whereas the rest show low gastrointestinal absorption. Methyl eugenol, (*E,Z*)-nerolidol and linolenic acid were the only compounds able to cross the blood–brain barrier (BBB); the rest of the compounds were predicted not to cross the blood–brain barrier (BBB). No compounds interacted with the cytochrome P450 (CYP) isoenzyme CYP2C19, but

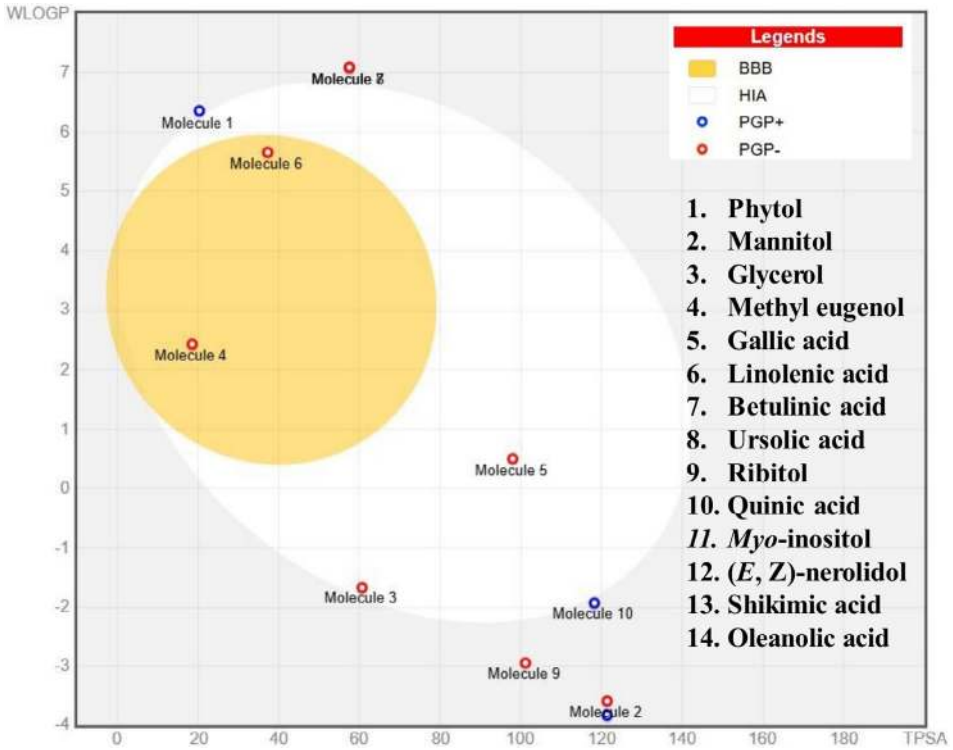


FIGURE 9 | Boiled-egg graph of the selected phytoconstituents.

three compounds interacted with CYP2C9, suggesting their efficiency while having minimal toxicity. Drug-like properties and GI absorption of selected compounds from different species of the Myrtaceae family were also represented by the boiled-egg prediction (Figure 9) and bioavailability radar graph (Figure 10). The pink region of the bioavailability radar plots indicates the compounds' drug-likeness, while the compounds in the yellow zone of the boiled-egg graph can cross the blood–brain barrier (BBB).

The toxicity parameters of selected phytocompounds were predicted using ProTox-3.0 web server (Table 15). Most of the selected compounds were predicted not to be hepatotoxic, carcinogenic, cytotoxic, immunotoxic, or mutagenic, except methyl eugenol (carcinogenic), gallic acid (carcinogenic), betulinic acid (immunotoxic) and ursolic acid (immunotoxic, hepatotoxic, carcinogenic), oleanolic acid (hepatotoxic, carcinogenic, immunotoxic). The LD_{50} values were also calculated to ensure the safety of the selected compounds, as shown in Table 15. All the compounds with $LD_{50} > 2000$ mg/kg suggest their safe use for biological administration and as potential drugs, except methyl eugenol (810 mg/kg).

4 | Conclusion

This is the first report on the study of antimicrobial, anti-inflammatory and antioxidant properties of methanolic extracts of *Melaleuca leucadendron*, *Melaleuca linnarrifolia*, *Melaleuca bracteata* and *Callistemon viminalis*, along with its phytochemical (GC–MS) profile. Several compounds of importance, viz. (*E, Z*) Nerolidol, betulinic acid, ursolic acid, ribitol, quinic acid, gallic acid, glycerol, methyl eugenol, oleanolic acid, shikimic acid and β-sitosterol were major phytochemicals identified from the extracts. The highest phenol content (Total phenol and ortho dihydric phenol content (30.26 ± 0.03 mg/g GAE & 4.82 ± 0.02 mg/g CLE)) was found in *Callistemon viminalis* extract. The strong anti-oxidant and anti-inflammatory activity was exhibited by the methanolic extract of *Melaleuca linarrifolia*. Polyphenols would be responsible for the production of various inflammatory mediators and thus lead to anti-inflammatory potential [23]. The strong activity shown by *Melaleuca linnarrifolia*, could be due to its major compound or may be due to synergic effect of various components. Interestingly, all the extracts demonstrated inhibition of bacterial strains in a dose-dependent manner against *S. typhimurium*, *E. coli*, *S. aureus* and *B. megaterium*. All studied extracts showed allelopathic activity (Against *Raphanus sativus* seeds) and the highest inhibition of ~97% and 93% were shown by *M. bracteata* and *C. viminalis* extract. Molecular docking analysis revealed strong binding affinities of the major phytochemicals towards antioxidant-related target proteins, indicating favourable interaction stability. These interactions support their potential role in modulating oxidative stress pathways and validate their antioxidant efficacy at the molecular level. The ADMET assessment demonstrated that most of the prominent phytochemicals have acceptable safety characteristics, supporting their use as safer substitutes. Nevertheless, our findings attest that the *studied plant* extract could be explored as a promising candidate for the prevention of several bacterial infections, although the responsible mechanisms behind the beneficial effects require further investigation. All these results suggest the judicious use of these plants for medicinal, nutraceutical and fragrance sectors, suggesting them a promising alternative to the synthetic ones.

TABLE 14 | In silico ADMET analysis of major constituents.

Entry	1	2	3	4	5	6	7	8	9	10	11	12	13	14
TPSA (Å^2)	20.23	121.38	60.69	18.46	97.99	30.37	57.53	57.53	101.15	118.22	121.38	20.23	97.99	57.53
Consensus Log Po/w	6.25	−2.23	−1.09	2.58	0.21	0.21	6.14	5.93	−1.76	−1.45	−2.67	4.9	−1.10	6.07
Mol wt (g/mol)	296.53	182.17	92.09	178.23	170.12	278.43	456.70	456.70	152.15	206.19	180.16	222.37	174.15	456.70
nRB	13	5	2	4	1	13	2	1	4	2	0	7	1	1
nOHA	1	6	3	2	5	2	3	3	5	6	6	1	5	3
nOND	1	6	3	0	4	1	2	2	5	5	6	1	4	2
WLOGP	6.36	−3.59	−1.67	2.43	0.50	5.66	7.09	7.09	−2.95	−1.93	−3.83	4.40	−1.52	7.23
Water solubility	Moderately soluble	Highly soluble	Highly soluble	Soluble	Soluble	Soluble	Poorly soluble	Poorly soluble	Highly soluble	Soluble	Highly soluble	Soluble	Highly soluble	Poorly soluble
GI absorption	Low	Low	High	High	High	High	Low	Low	Low	Low	Low	High	High	Low
BBB permeant	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	No
P-gp substrate	Yes	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No
CYP1A2 inhibitor	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	No	No	No	Yes	No	No	No	No	Yes	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No
Log K_p (cm/s) (Skin permeation)	−2.29	−9.61	−8.11	−5.60	−6.84	−6.84	−3.26	−3.87	−8.99	−8.90	−10.03	−4.23	−8.58	−3.77
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lipinski violation	1	1	0	0	0	0	1	1	0	0	1	0	0	1
Bioavailability score	0.55	0.55	0.55	0.55	0.56	0.56	0.85	0.85	0.55	0.56	0.55	0.55	0.56	0.85

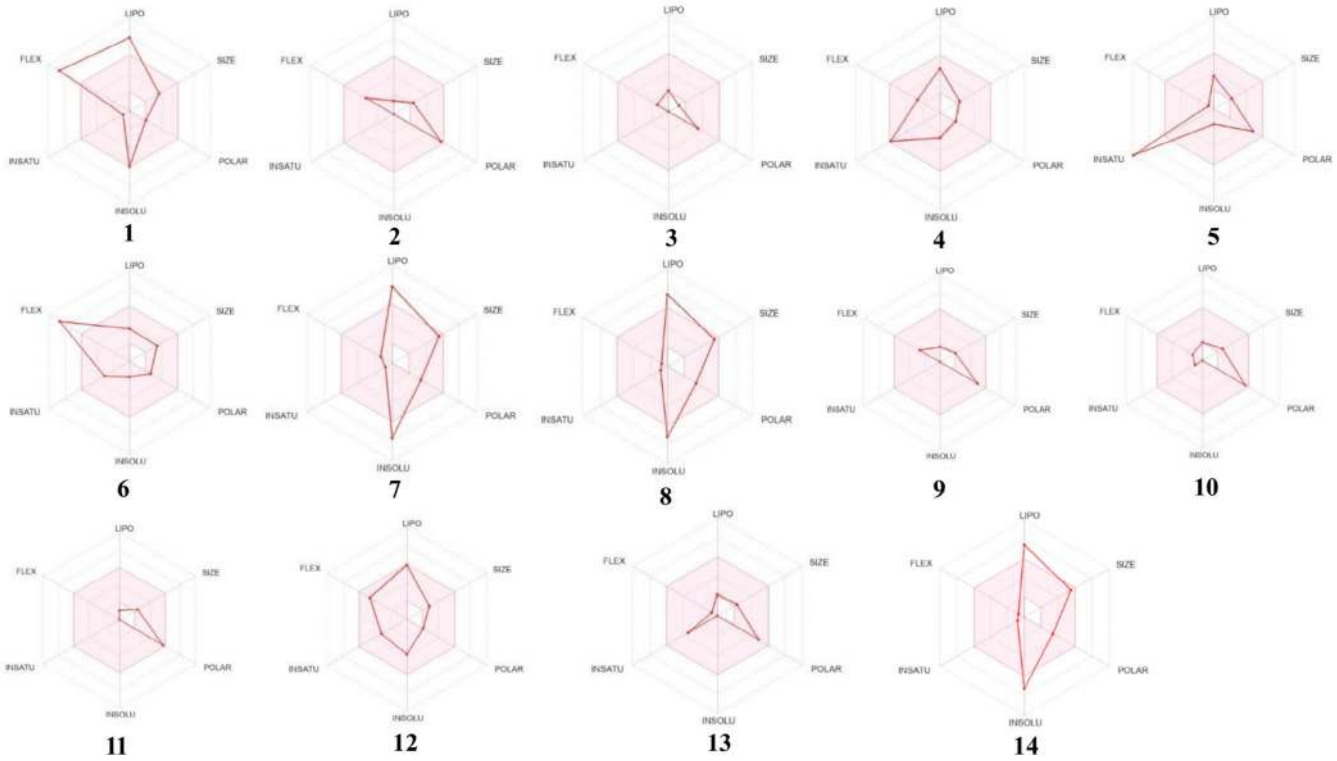


FIGURE 10 | Bioavailability radar of selected phytoconstituents (pink area showed the drug likeness properties of selected compounds) 1: Phytol, 2: Mannitol, 3: Glycerol, 4: Methyl eugenol, 5: Gallic acid, 6: Linolenic acid, 7: Betulinic acid, 8: Ursolic acid, 9: Ribitol, 10: Quinic acid, 11: *Myo*-inositol, 12: (*E,Z*)-nerolidol, 13: Shikimic acid, 14: Oleanolic acid.

TABLE 15 | Toxicological properties of selected compounds.

		Hepatotoxicity		Carcinogenicity		Cytotoxicity		Immunotoxicity		Mutagenicity		Predicted LD_{50} (mg/kg)	Toxicity class
S. no	Compounds	Pre	Pro	Pre	Pro	Pre	Pro	Pre	Pro	Pre	Pro		
1.	Phytol	In	0.79	In	0.76	In	0.85	In	0.99	In	0.97	5000	V
2.	Mannitol	In	1.0	In	0.86	In	0.87	In	0.99	In	0.95	13,500	VI
3.	Glycerol	In	0.99	In	0.86	In	0.87	In	0.99	In	0.84	4090	V
4.	Methyl eugenol	In	0.65	Ac	0.76	In	0.89	In	0.92	In	0.86	810	IV
5.	Gallic acid	In	0.61	Ac	0.56	In	0.91	In	0.99	In	0.94	2000	IV
6.	Linolenic acid	In	0.54	In	0.63	In	0.71	In	0.99	In	0.95	10,000	VI
7.	Betulinic acid	In	0.54	In	0.53	In	0.97	Ac	0.74	In	0.71	2610	V
8.	Ursolic acid	Ac	0.52	Ac	0.57	In	0.99	Ac	0.95	In	0.85	2000	IV
9.	Ribitol	In	0.98	In	0.84	In	0.87	In	0.99	In	0.87	12,500	VI
10.	Quinic acid	In	0.80	In	0.73	In	0.78	In	0.99	In	0.94	9800	VI
11.	*Myo*-inositol	In	0.87	In	0.55	In	0.84	In	0.99	In	0.60	10,000	VI
12.	(*E,Z*)-nerolidol	In	0.81	In	0.65	In	0.81	In	0.99	In	0.91	5000	V
13.	Shikimic acid	In	0.70	In	0.66	In	0.72	In	0.99	In	0.94	9000	VI
14.	Oleanolic acid	Ac	0.52	Ac	0.57	In	0.99	Ac	0.79	In	0.85	2000	IV

Note: Entry 1: Phytol, 2: Mannitol, 3: Glycerol, 4: Methyl eugenol, 5: Gallic acid, 6: Linolenic acid, 7: Betulinic acid, 8: Ursolic acid, 9: Ribitol, 10: Quinic acid, 11: *Myo*-inositol, 12: (*E, Z*)-nerolidol, 13: Shikimic acid, 14: Oleanolic acid.
Abbreviations: Ac, active; ADMET, absorption, distribution, metabolism, excretion and toxicity; BBB, blood–brain barrier; CYP, cytochrome P450; GI absorption, gastrointestinal absorption; In, inactive; nOHA, no. of H-bond acceptor; nOHD, no. of H-bond donor; nRB, no. of rotable bonds; P-gp, permeability glycoprotein; TPSA, topological polar surface area; WLOGP, water partition coefficient.

Author Contributions

Archana Joshi: writing original draft, methodology, investigation, formal analysis, data curation, conceptualisation. **Vaishnavi Rawat:** writing original draft, methodology, investigation, formal analysis. **Ravendra Kumar:** writing – review and editing, validation, supervision, conceptualisation. **Stefania Garzoli:** writing – review and editing, validation, supervision, conceptualisation. **Om Prakash:** writing – review and editing. **Anil Kumar Pant:** writing – review and editing, validation, supervision, conceptualisation. **Marcin Stocki:** writing – review and editing, validation, supervision, conceptualisation. **Valery A. Isidorov:** writing – review and editing, validation, supervision, conceptualisation. **Dharmendra Singh Rawat:** writing – review and editing, validation, supervision, conceptualisation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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