RESEARCH PAPER

Integrated Experimental and Computational Investigation of the Antioxidant and Antibacterial Potential of *Citrus maxima* (Burm.) Merr. Essential Oil

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Abstract
This study investigated the antioxidant and antibacterial potential of essential oils extracted from the peel and seed waste of *Citrus maxima* (Burm.) Merr. using integrated *in vitro* and *in silico* approaches. Antioxidant activity was evaluated through DPPH, superoxide radical, and nitric oxide scavenging assays, while antibacterial efficacy was assessed using agar well diffusion and minimum inhibitory concentration (MIC) methods against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. Both *Citrus maxima* peel essential oil (CMPEO) and seed essential oil (CMSEO) exhibited considerable antibacterial activity, with the highest inhibition observed against *E. coli* (20 mm) and the lowest against *B. subtilis* (10–11 mm) at 50 µL concentration. GC–MS analysis identified more than 150 compounds in the peel oil and over 190 compounds in the seed oil. The major constituents detected in peel oil were D-limonene (15.02%), (+)-dihydrocarveol (5.86%), cyclohexene, 1-methyl-4-(1-methylethenyl)-, trans- (3.80%), spiro[4.4]nonane-1-methylene (3.48%), and tricyclo[5.2.1.0<2,6>]decane (2.15%). In contrast, seed oil predominantly contained D-limonene (33.58%), *n*-hexadecanoic acid (20.25%), 1H-tricyclo[4.4.0.0(3,9)]decan-4-ol (15.65%), neointermedeol (13.44%), and 9-octadecenoic acid (E) (1.12%). Molecular docking studies revealed strong binding interactions between selected bioactive compounds and microbial as well as oxidative stress–related target proteins, thereby supporting the experimental observations. Overall, the findings demonstrate that *Citrus maxima* essential oils possess significant antioxidant and antibacterial properties, indicating their promising potential as natural preservatives and therapeutic agents for pharmaceutical, nutraceutical, and agricultural biorefinery applications.

Keywords: Antibacterial activity, antioxidant activity , *Citrus maxima*, essential oil, GC–MS , molecular docking, in silico analysis

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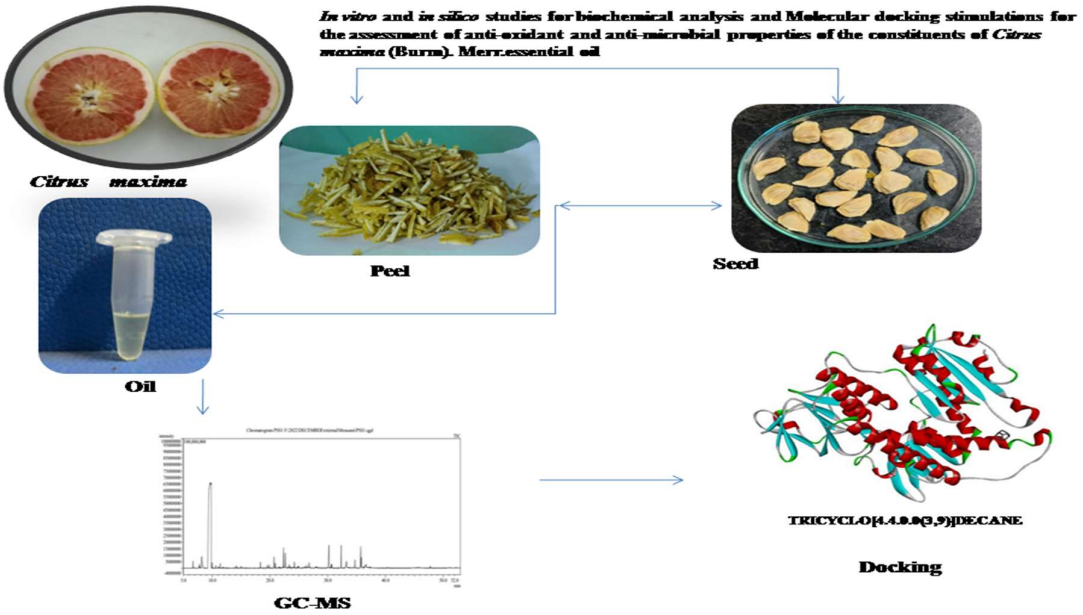

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Introduction:

India continues to rely extensively on traditional medicine due to the abundant availability of medicinal plants enriched with bioactive phytochemicals possessing significant therapeutic potential. *Citrus maxima* (Burm.) Merr. (Rutaceae), commonly known as pomelo, is an underutilized citrus fruit widely distributed from Assam to Indo-China, with Northeast India recognized as an important centre of citrus diversity (Hynniewta et al., 2014). In recent years, the growing emergence of antimicrobial resistance against synthetic drugs has stimulated increased interest in plant-derived natural products as safer and effective alternatives with antioxidant, antimicrobial, and health-promoting properties. Among various citrus species, *C. maxima* has attracted considerable scientific attention owing to its diverse pharmacological activities and industrial importance. Its essential oil is extensively utilized in fragrance, cosmetic, food, and pharmaceutical industries due to the presence of valuable bioactive constituents with potential therapeutic applications (Elkhawas et al., 2025). Furthermore, citrus peel and seed wastes represent a rich source of phytochemicals and essential oils that can be efficiently valorized for sustainable biorefinery applications. Therefore, the present study aimed to evaluate the antibacterial, antioxidant, and biochemical properties of essential oils extracted from the peel and seed waste of Meghalaya-grown *C. maxima* using combined *in vitro* and *in silico* approaches. The study further emphasizes the sustainable utilization and value addition of citrus by-products through waste valorization strategies.

Methodology

Chemicals and Reagents

All chemicals and reagents used in the present investigation were of analytical and molecular biology grade and were procured from North East Chemicals Corporation, Guwahati, Assam, India.

Collection of Plant Material

Fresh fruits of *Citrus maxima* (Burm.) Merr. were collected from Marngar Village, Nongpoh, Meghalaya, India. The plant specimen was taxonomically authenticated by a taxonomist from the Department of Botany, University of Science and Technology, Meghalaya.

Extraction of Essential Oil (CMEO)

Freshly collected fruits were thoroughly washed, tray-dried at 45 °C for 24 h, and subsequently ground into fine powder. Essential oils were extracted separately from 200 g of peel and seed powder using a Clevenger-type apparatus through hydrodistillation for 3 h following the method described by Vekiari et al. (2004). The extracted oils were collected and stored at −20 °C until further analysis.

Antioxidant Assay of CMEO

DPPH Radical Scavenging Assay

The antioxidant activity of *Citrus maxima* essential oils (CMEO) derived from peel and seed was evaluated using the DPPH radical scavenging assay. Different concentrations of CMEO (12.5–100 µL) were prepared in methanol and mixed with 2 mL of DPPH solution. The reaction mixture was incubated in the dark at room temperature for 30 min, and absorbance was recorded at 517 nm using a UV–Visible spectrophotometer. The percentage of radical scavenging activity was calculated using the following equation (Abe et al., 1998).

Scavenging activity (%)=(A−B)A×100\text{Scavenging activity (\%)}=\frac{(A-B)}{A} \times 100Scavengingactivity (%)=A(A−B)×100

where *A* represents the absorbance of the DPPH control and *B* represents the absorbance of the sample containing CMEO and DPPH solution.

Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity was determined according to the method of Robak and Gryglewski (1988). All reagents were prepared in phosphate buffer (pH 7.4). The reaction mixture consisted of 3 mL of sample solution, 1 mL of nitro blue tetrazolium (NBT, 156 µM), and 1 mL of nicotinamide adenine dinucleotide (NADH, 468 µM). The reaction was initiated by the addition of phenazine methosulfate (PMS, 60 µM). After incubation at 25 °C for 5 min, absorbance was measured at 560 nm.

Nitric Oxide (NO) Scavenging Assay

Nitric oxide scavenging activity was evaluated following the method described by Marcocci et al. (1994). Briefly, 1 mL of sodium nitroprusside solution (10 mM) prepared in phosphate buffer (pH 7.4) was mixed with 1 mL of essential oil sample. The reaction mixture was incubated at 25 °C for 150 min. Subsequently, 1 mL of Griess reagent containing 1% sulphanilamide, 2% orthophosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride was added to the mixture. Absorbance was measured at 546 nm using a UV–Visible spectrophotometer.

Total Phenolic Content (TPC)

The total phenolic content of the essential oil samples was determined using the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). Briefly, the oil sample was mixed with distilled water followed by the addition of Folin–Ciocalteu reagent. After incubation, sodium carbonate solution was added, and the reaction mixture was further incubated under dark conditions. Absorbance was measured at 650 nm, and the phenolic content was quantified using catechol as the standard reference compound.

Total Flavonoid Content (TFC)

Total flavonoid content was estimated using the aluminium chloride colorimetric assay described by Chang et al. (2002). The oil sample was initially mixed with sodium nitrite solution and incubated for a specified period. Subsequently, aluminium chloride solution was added, followed by further incubation. Sodium hydroxide was then added to the reaction mixture, and absorbance was recorded at 510 nm to determine flavonoid content.

Antimicrobial Activity

Microbial cultures were prepared by subculturing the bacterial strains in nutrient broth and incubating them at 37 °C for 24 h. The antibacterial activity of *Citrus maxima* peel essential oil (CMPEO) and seed essential oil (CMSEO) was evaluated using the agar well diffusion method against two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*. Different concentrations of the oil samples (50, 100, 150, and 200 µL/mL) were introduced into the wells, while azithromycin was used as the standard control. The inoculated plates were incubated at 37 °C for 24 h, after which the zones of inhibition were measured to determine antibacterial activity (Perez et al., 1990).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of CMPEO and CMSEO was determined using the broth dilution method following the procedure described by Wiegand et al. (2008). Serial dilutions of the oil samples were prepared in nutrient broth to obtain different concentrations. Each dilution was inoculated with a standardized bacterial suspension and incubated at 37 °C for 24 h. Bacterial growth was evaluated by observing turbidity and confirmed spectrophotometrically by measuring optical density at 600 nm. The MIC value was defined as the lowest concentration of the oil sample that completely inhibited visible bacterial growth.

Determination of Bioactive Compounds by GC–MS Analysis

GC–MS analysis of *Citrus maxima* seed essential oil (CMSEO) and peel essential oil (CMPEO), dissolved in petroleum ether, was carried out using a PerkinElmer Clarus 680 Gas Chromatography system coupled with a Clarus 600C Mass Spectrometer. Separation of compounds was achieved using an Elite-5MS capillary column (60 m × 0.25 mm internal diameter × 0.25 µm film thickness) equipped with a liquid–liquid autosampler.

The oven temperature was initially maintained at 50 °C for 1 min and then increased to 300 °C at a rate of 5 °C/min with a holding time of 2 min, followed by a final increase to 300 °C at 60 °C/min with a holding time of 10 min. The injector temperature was maintained at 280 °C, and 1 µL of sample was injected. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Electron ionization was performed at 70 eV, and the mass scan range was set between 50–600 Da. The total run time for the analysis was 53 min. Identification of bioactive compounds was performed by comparing the obtained mass spectra with those available in the NIST 2014 library using TurboMass software.

***In Silico* Studies**

Selection of Ligands

The ligands selected for the present molecular docking study were chosen based on previously reported bioactive compounds identified through literature survey and GC–MS analysis. Molecular docking against human mitochondrial glutathione reductase (PDB ID: 3SQP) was performed using PyRx software, whereas docking against *Escherichia coli* acyl-[acyl-carrier-protein]-UDP-*N*-acetylglucosamine *O*-acyltransferase (PDB ID: 6HY2) was conducted using AutoDock V4.2. The chemical structures of the selected ligands were retrieved from the PubChem database.

Ligand Preparation

Three-dimensional structures of the selected ligands were downloaded from the PubChem database in Structure Data File (SDF) format along with their respective Compound Identification Numbers (CIDs). The structures were subsequently converted into Protein Data Bank (PDB) format using PyMOL software. Prior to docking, metal ions and unwanted residues were removed to obtain optimized ligand structures suitable for docking analysis.

The crystal structures of the target proteins, mitochondrial glutathione reductase (PDB ID: 3SQP) andacyl-[acyl-carrier-protein]-UDP-*N*-acetylglucosamine*O*-acyltransferase (PDB ID: 6HY2), were retrieved from the Protein Data Bank (PDB) and prepared for molecular docking studies.

Molecular Docking Analysis

Molecular docking plays a crucial role in computer-aided drug discovery by predicting the interaction patterns and binding affinity between ligands and target proteins. Docking analysis was carried out using PyRx software integrated with AutoDock tools. AutoGrid was employed to generate grid maps for defining the active binding sites of the target proteins.For mitochondrial glutathione reductase (3SQP), a grid box dimension of 22 × 20 × 23 points with a grid spacing of 1.000 Å was used. In contrast, docking against acyl-[acyl-carrier-protein]-UDP-*N*-acetylglucosamine *O*-acyltransferase (6HY2) was performed using a grid box dimension of 40 × 40 × 40 points with a grid spacing of 0.508 Å. The docked conformations with the lowest binding energy were selected as the most stable protein–ligand complexes.Protein–ligand interactions, including hydrogen bonding, hydrophobic interactions, and bond lengths, were analyzed and visualized using PyMOL, UCSF Chimera, and Accelrys Discovery Studio Visualizer software.

***In Silico* Drug-Likeness and Pharmacokinetic Analysis**

Drug-likeness properties of the selected compounds were evaluated using the Molsoft molecular property prediction tool. The assessment was carried out based on Lipinski's Rule of Five and Pfizer guidelines, which predict oral drug suitability by considering important physicochemical parameters such as molecular weight, hydrogen bond donors, hydrogen bond acceptors, and lipophilicity (Hughes et al., 2008).

Pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the selected compounds were predicted using the SwissADME web tool. Oral bioavailability was further assessed using the bioavailability radar model, which evaluates six major physicochemical parameters including size, polarity, lipophilicity, solubility, flexibility, and saturation (Daina et al., 2017). Compounds falling within the optimal radar region were considered to possess favorable drug-like characteristics and were selected for further evaluation.

Statistical Analysis: All experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using SPSS/GraphPad Prism software. Significant differences between groups were determined using one-way ANOVA followed by Tukey's post hoc test at $p < 0.05$.

Results and Discussion :

DPPH Radical Scavenging Activity

The DPPH radical scavenging assay demonstrated a concentration-dependent antioxidant activity for both *Citrus maxima* peel essential oil (CMPEO) and seed essential oil (CMSEO). The percentage of inhibition increased progressively with increasing concentration, reaching a maximum inhibition of 69.39 ± 1.02% for CMPEO and 75.39 ± 1.06% for CMSEO at 175 µL/mL, followed by a slight decline at higher concentrations. Among the two samples, CMSEO exhibited comparatively stronger radical scavenging activity than CMPEO, indicating possible variations in the concentration and composition of bioactive phytoconstituents present in the oils.

The antioxidant potential of the essential oils was further confirmed by the characteristic reduction of DPPH radicals, evidenced by the color change from deep purple to yellow (Meryem et al., 2023). The observed antioxidant activity is comparable with previously reported studies on citrus essential oils (Abdeltaif et al., 2018). Variations in antioxidant efficiency may be associated with differences in the concentration of major phytochemicals such as D-limonene and other oxygenated terpenoids present in the essential oils (Wei & Shibamoto, 2007).

Superoxide Radical Scavenging Activity

Superoxide anion (O_2^-) is an important reactive oxygen species implicated in oxidative stress–induced cellular damage and various pathological conditions. In the present investigation, both CMPEO and CMSEO exhibited significant concentration-dependent superoxide radical scavenging activity, indicating their ability to neutralize reactive oxygen species and reduce oxidative stress.

At a concentration of 200 µL/mL, CMPEO exhibited a maximum inhibition of 65.24 ± 1.23%, whereas CMSEO showed comparatively higher scavenging activity with 69.54 ± 1.03% inhibition. Although the superoxide radical scavenging activity was lower than that observed in the DPPH assay, both oils displayed a similar dose-dependent response pattern. These findings are in agreement with recent studies on citrus peel essential oils, which reported moderate yet significant antioxidant activities due to the presence of bioactive terpenoids and phenolic constituents (Li et al., 2024).

Nitric Oxide Scavenging Activity

Nitric oxide (NO) scavenging activity of CMPEO and CMSEO increased progressively with increasing concentrations ranging from 75–200 µL/mL. At the highest tested concentration (200 µL/mL), CMPEO exhibited 64.18 ± 0.98% inhibition, while CMSEO demonstrated slightly higher scavenging activity with 67.34 ± 1.03% inhibition. Beyond this concentration, no significant increase in activity was observed.

Although the nitric oxide scavenging activity was comparatively lower than the DPPH and superoxide radical scavenging assays, the results indicate that both essential oils possess the ability to modulate nitric oxide–mediated oxidative stress. Excess nitric oxide and its reactive intermediates are known to contribute to inflammation and cellular damage; therefore, scavenging of nitric oxide radicals may play an important role in cellular protection. The present findings are consistent with earlier reports highlighting the antioxidant potential of citrus-derived phytoconstituents (Karuppagounder et al., 2013).

Total Phenolic Content

Phenolic compounds are considered major contributors to antioxidant activity and are associated with numerous biological and pharmacological functions. In the present investigation, the total phenolic content was found to be 21.48 ± 1.12 mg GAE/g oil in *Citrus maxima* peel essential oil (CMPEO) and 26.73 ± 1.35 mg GAE/g oil in seed essential oil (CMSEO), indicating comparatively higher phenolic accumulation in the seed oil. The elevated phenolic content observed in CMSEO may contribute to its stronger antioxidant activity compared to CMPEO.

The phenolic values obtained in this study were comparatively higher than several previously reported values for citrus essential oils (approximately 8.96 mg/g), suggesting enhanced antioxidant potential of the investigated samples. Phenolic compounds are known to act as effective hydrogen donors and free radical

scavengers, thereby reducing oxidative stress and cellular damage. Similar positive correlations between total phenolic content and antioxidant capacity in citrus species have been extensively documented in earlier studies (Barreca et al., 2011).

Total Flavonoid Content

Flavonoids represent an important class of bioactive phytochemicals with significant antioxidant, anti-inflammatory, and antimicrobial properties. In the present study, total flavonoid content was determined to be 12.84 ± 0.96 mg QE/g oil in CMPEO and 16.27 ± 1.08 mg QE/g oil in CMSEO, indicating relatively higher flavonoid accumulation in the seed-derived essential oil.The increased flavonoid concentration observed in CMSEO may contribute substantially to its enhanced antioxidant and antibacterial activities. Flavonoids are known to neutralize reactive oxygen species through electron donation and metal ion chelation mechanisms, thereby protecting biological systems against oxidative stress. The present findings highlight the potential role of seed-derived phytoconstituents in enhancing the biological efficacy of *C. maxima* essential oils.

Antibacterial Activity

The peel and seed essential oils of *Citrus maxima* exhibited considerable antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The highest antibacterial activity was observed against *Escherichia coli*, showing an inhibition zone of approximately 20 mm, whereas comparatively lower activity was recorded against *Bacillus subtilis* (approximately 10–11 mm) at 50 µL concentration.Similar antibacterial effects of *C. maxima* essential oil against *Bacillus licheniformis* have previously been reported by Chen et al. (2018). The present findings are also consistent with the observations of Patra et al. (2019), who reported an inhibition zone of approximately 22 mm. In addition, comparable antibacterial activity against *Staphylococcus aureus* and *E. coli* has been documented for *C. maxima* and *Citrus grandis* essential oils (Ou et al., 2015), indicating their broad-spectrum antibacterial potential.The minimum inhibitory concentration (MIC) analysis further supported these results, revealing that CMSEO exhibited a lower MIC value (150 µL/mL) than CMPEO (200 µL/mL), thereby confirming the comparatively higher antibacterial potency of the seed essential oil. The antibacterial activity observed may be attributed to the presence of bioactive terpenoids, phenolics, and oxygenated compounds capable of disrupting microbial cell membranes and inhibiting bacterial growth.

Identified bioactive compounds in GC-MS:

More than 190 components were found in the GC-MS chromatogram of *C. maxima* seed oil, and 36 significant components are presented in this research (Table 1 and Fig. 1). Based on the GC-MS spectra data, the most prevalent bioactive chemicals include D-Limonene (33.58%), n-Hexadecanoic acid (20.25%), 1h Tricyclo[4.4.0.0(3,9)]decan-4-ol (15.65%), Neointermedeol (13.44%), 9-Octadecenoic acid, (E)- (1.12%). The GC-MS chromatogram of *C. maxiama* peel oil revealed more than 150 components and this paper has reported 25 major components (**Table 2 and Fig 2)**. From the results of GC-MS spectra, the most abundant in occurrence bioactive compounds are D-Limonene (15.02%), (+)-Dihydrocarveol (5.86%), Cyclohexene, 1-methyl-4-(1-methylethenyl)-, trans- (3.80%), Spiro[4.4]nonane, 1-methylene- (3.48%), Tricyclo[5.2.1.0<2,6>]decane (2.15%). Both the petroleum extract have 7 components in common namely Alpha-Pinene, Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, D-Limonene, Caryophyllene, Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-, Nerolidyl acetate and Nootkatone.

According to the present study and review of the literature, similarities were observed among the following compounds: Both peel and seed essential oils shared several common phytoconstituents, including α-pinene, bicyclo[3.1.1]heptane-6,6-dimethyl-2-methylene, D-limonene, caryophyllene, nerolidyl acetate, and nootkatone. The occurrence of compounds such as D-limonene, trans-linalool oxide, geranyl acetate, α-pinene, β-pinene, β-myrcene, linalool, nootkatone, and 9,12-octadecadienoic acid (Z,Z) methyl ester is in agreement with previous reports on *C. maxima* essential oils (Sapkota et al., 2022; Visakh et al., 2022; Monteiro et al., 2022). Variations in the chemical composition may be associated with genotypic differences, environmental conditions, geographical origin, and ecotype variations.

D-limonene, the predominant constituent identified in both oils, is widely recognized for its therapeutic significance, including gallstone dissolution, gastroesophageal reflux management, and cancer chemopreventive properties mediated through apoptosis induction (Mukhtar et al., 2018). Dihydrocarveol has been reported to possess antibacterial, antioxidant, anti-inflammatory, anticancer, and antidiabetic activities, in addition to its industrial applications in flavor and fragrance formulations (Zhang et al., 2022). Furthermore, nootkatone has been identified as a potent insect repellent effective against mosquitoes, fleas, and ticks (Fraatz et al., 2009).

Based on their abundance and reported biological activities, D-limonene, tricyclo[4.4.0.0(3,9)]decane, nootkatone, spiro[4.4]nonane-1-methylene, and dihydrocarveol were selected for molecular docking analysis against *E. coli* acyl-[acyl-carrier-protein]-UDP-*N*-acetylglucosamine acyltransferase (PDB ID: 6HY2) and human mitochondrial glutathione reductase (PDB ID: 3SQP).

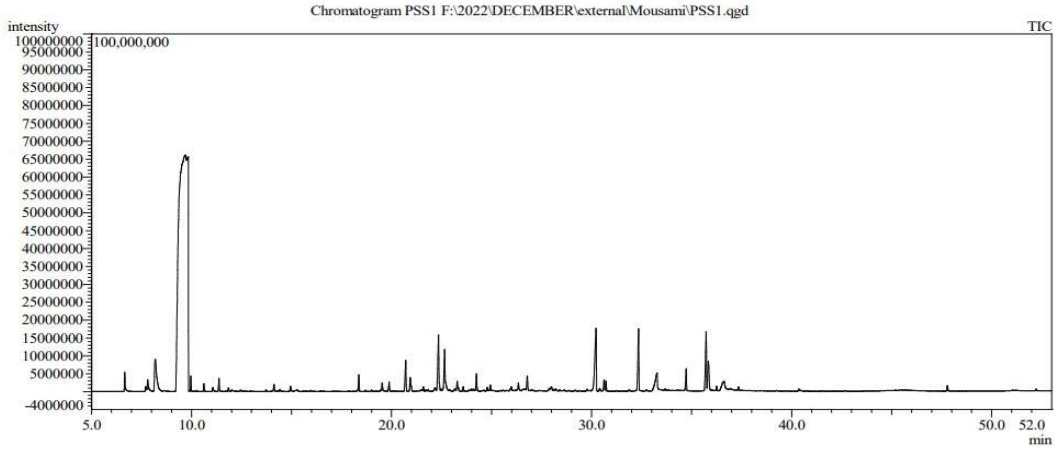

Fig.1. GC-MS spectrum of *Citrus maxima*seed oil

Table 1: Bioactive compounds of *C. maxima* seed oil extract using GC-MS analysis.

Sl. No.	Name of compound	Rt[a]	Ri[b]	Pa[c]	Ph[d] (%)	Mf[e]	Mw[f]	Pcid[g]	A/H[h]	Nature of Compound	CAS NO.
1.	Alpha.-Pinene	6.662	1022	0.50	0.86	$C_{10}H_{16}$	136.23g/mol	6654	2.85	Terpenes	80-56-8
2.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	7.711	1136	0.15	0.22	• $C_{10}H_{16}$	136.23 g/mol	18818	3.25	Monoterpene	3387-41-5
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	7.810	1110	0.48	0.53	$C_{10}H_{16}$	136.23 g/mol	14896	4.43	Terpenes	• 27-91-3
4	D-Limonene	9.564	1789	34.03	10.21	• $C_{10}H_{16}$	136.23 g/mol	440917	16.13	Terpenes	68647-72-3
5	Tricyclo[4.4.0.0(3,9)]decan-4-ol	9.681		18.09	10.51	$C_{10}H_{16}O$	152.23 g/mol	564241	8.33	Alcoholic compound	33540-03-3
6	4,7-Methano-1H-indene, octahydro-	9.751	1243.4	3.20	10.37	$C_{10}H_{16}$	136.23 g/mol	556495	1.49		40954-37-8
7	D-Limonene	9.838	1789	3.90	10.21	• $C_{10}H_{16}$	136.23 g/mol	440917	1.81	Terpenes	68647-72-3
8	Trans-Linalool oxide (furanoid)	11.062	1484	0.13	0.18	• $C_{10}H_{18}O_2$	170.25 g/mol	6432254	3.44	Terpenes	34995-77-2
9	Linalool	11.373	1544	0.31	0.59	• $C_{10}H_{18}O$	154.25 g/mol	6549	2.56	Monoterpenoid	78-70-6
10	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	14.950	1831	0.16	0.23	$C_{10}H_{16}O$	152.23 g/mol	330573	3.33	Monoterpenoid	1197-06-4
11	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-	18.363	1470	0.41	0.74	• $C_{15}H_{24}$	204.35 g/mol	89316	2.69	Sesquiterpenoid	20307-84-0
12	Geranyl acetate	19.533	1753	0.24	0.38	• $C_{12}H_{20}O_2$	196.29 g/mol	1549026	3.09	Monoterpenoid Acetate ester	68311-13-7
13	Cyclohexane, 1-ethenyl-1-methyl-	19.880	1593	0.26	0.41	• $C_{15}H_{24}$	204.35 g/mol	6918391	3.09	Sesquiterpenoid	33880-83-0

14	2,4-bis(1-methylethenyl)-, Caryophyllene	20.707	1591	0.96	1.38	• $C_{15}H_{24}$	204.35 g/mol	5281515	3.35	Sesquiterpene	87-44-5
15	.gamma.-Muurolene	22.040	1665	0.18	0.16	• $C_{15}H_{24}$	204.35 g/mol	5281520	5.40	Monocyclic sesquiterpene	6753-98-6
16	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	23.338	1713	0.10	0.17	• $C_{15}H_{24}$	204.35 g/mol	288227	2.80	Eromophylene	4630-07-3
17	Androsta-1,4-dien-3-one, 17-hydroxy-17-methyl-,	23.495		0.13	0.22	• $C_{20}H_{28}O_2$	300.4 g/mol	6300	2.94	Androgen	• 2-63-9
18	Nerolidyl acetate	24.244	2271	0.52	0.79	• $C_{17}H_{28}O_2$	264.4 g/mol	5363426	3.20	Sesquiterpenoids	2306-78-7
19	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-	24.779	2206	0.13	0.18	• $C_{15}H_{24}O$	220.35 g/mol	922331	3.49	Tricyclic sesquiterpene	• 750-60-3
20	Caryophyllene oxide	24.938	1570.	0.19	0.27	• $C_{15}H_{24}O$	220.35 g/mol	1742210	3.39	Sesquiterpenoid oxide	1139-30-6.
21	Isospathulenol	25.986	2206	0.14	0.20	• $C_{15}H_{24}O$	220.35 g/mol	14038848	3.32	Sesquiterpenoids	88395-46-4
22	Dodecanoic acid, TMS derivative	26.341	1651	0.27	0.37	• $C_{15}H_{32}O_2Si$	272.50 g/mol	521640	3.52	Saturated fatty acid	55520-95-1.
23	Neointermedeol	26.662	1660	0.16	0.12	• $C_{15}H_{26}O$	222.37 g/mol	11877394	6.48	Terpenoids	5945-72-2
24	Neointermedeol	26.715	1660	0.52	0.68	• $C_{15}H_{26}O$	222.37 g/mol	11877394	3.71	Terpenoids	5945-72-2
25	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-1	27.882		0.14	0.13	$C_{20}H_{32}$	272.5 g/mol	5377896	0.31	Terpene	• 898-13-1
26	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	27.991	2343	0.21	0.21	• $C_{15}H_{26}O$	222.37 g/mol	445070	5.06	Sesquiterpenoid	• 06-28-5
27	1-Heptatriacotanol	28.212	3942	0.11	0.11	• $C_{37}H_{76}O$	537 g/mol	537071	4.88	Alcoholic compound	105794-58-9
28	Nootkatone	30.223	2527	3.09	2.81	• $C_{15}H_{22}O$	218.33 g/mol	1268142	5.33	Sesquiterpenoid	4674-50-4
29	2-Pentadecanone, 6,10,14-trimethyl-	30.625	2131	0.32	0.52	• $C_{18}H_{36}O$	268.5 g/mol	10408	3.02	Sesquiterpenoid	• 02-69-2

30	Hexadecanoic acid, methyl ester	32.349	2202	2.01	2.78	• C$_{17}$H$_{34}$O$_2$	270.5 g/mol	8181	3.50	Ester	• 12-39-0
31	N-Hexadecanoic acid	33.273	2039.1	1.59	0.81	• C$_{19}$H$_{40}$O$_2$Si •	328.6 g/mol	521638	9.51	Saturated fatty acid	55520-89-3
32	Palmitic Acid, TMS derivative	34.726	2930	0.61	1.00	• C$_{16}$H$_{32}$O$_2$	256.42 g/mol	985	2.96	Saturated fatty acid	57-10-3
33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	35.726	2476	1.89	2.63	• C$_{19}$H$_{34}$O$_2$	294.5 g/mol	5284421	3.47	Ester	• 12-63-0
34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	36.559	2476	0.47	0.38	• C$_{19}$H$_{34}$O$_2$	294.5 g/mol	5284421	5.95	Ester	• 12-63-0
35	9-Octadecenoic acid, (E)-	36.628		0.51	0.41	• C$_{18}$H$_{34}$O$_2$	282.5 g/mol	637517	5.97	Ester	• 12-79-8
36	Hexadecanoic acid, butyl ester	37.344	2419	0.10	0.16	• C$_{20}$H$_{40}$O$_2$	312.5 g/mol	8090	3.09	Ester	111-06-8.

[a] Retention time; [b] Kovats Retention Indices were calculated from our analysis with respect to a series of n-alkenes; [c] Percentage of peak area; [d] Percentage of height [e] Molecular formula; [f] Molecular weight; [g]Pubchem CID; [h] Area/Height; CAS No- Chemical abstract service number

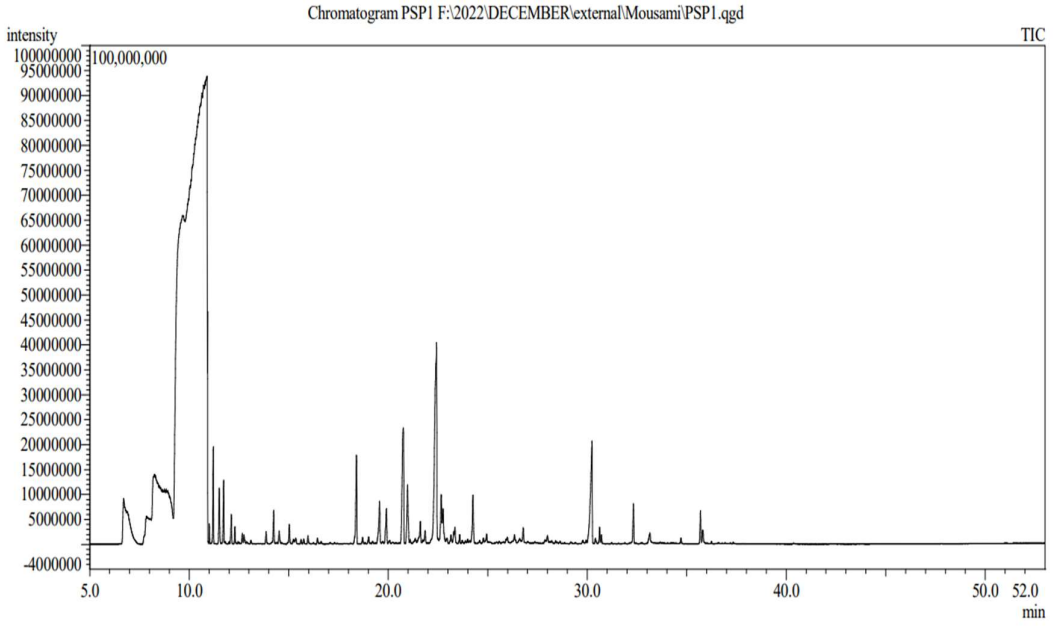

Fig.2. GC-MS spectrum of *Citrus maxima* peel oil.

Table 2: Bioactive compounds of *C. maximus* peel oil extract using GC-MS analysis.

Sl. No	Name of compound	Rt[a]	Ri[b]	Pa[c]	Ph[d] (%)	Mf[e]	Mw[f]	Pcid[g]	A/H[h]	Nature of Compound	CAS NO.
1.	Alpha.-Pinene	6.701	1022	0.74	0.48	$C_{10}H_{16}$	136.23g/mol	6654	7.91	Terpenes	80-56-8
2.	Alpha.-Pinene	6.894	1022	0.80	0.34	$C_{10}H_{16}$	136.23g/mol	6654	12.12	Terpenes	80-56-8
3.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	7.845	1110	0.45	0.29	$C_{10}H_{16}$	136.23g/mol	440967	8.04	Monoterpene	18172-67-3
4.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,	8.190	1110	0.62	0.71	$C_{10}H_{16}$	136.23 g/mol	14896	4.47	Terpenes	• 127-91-3
5.	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	8.242	920.7	0.42	0.72	$C_{10}H_{16}$	136.23 g/mol	86182191	2.96	Terpene	• 16626-39-4
6.	beta.-Pinene	8.269	1110	0.42	0.73	$C_{10}H_{16}$	136.23 g/mol	14896	2.95	Terpene	• 127-91-3 •
7.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,	8.313	1110	0.83	0.72	$C_{10}H_{16}$	136.23 g/mol	14896	5.90	Terpenes	• 127-91-3
8.	beta.-Myrcene	8.913	1159	0.75	0.56	$C_{10}H_{16}$	136.23 g/mol	31253	6.84	Monoterpene	• 123-35-3
9.	beta.-Myrcene	9.020	1159	0.90	0.50	$C_{10}H_{16}$	136.23 g/mol	31253	9.19	Monoterpene	• 123-35-3
10.	D-Limonene	9.662	1789	15.02	3.45	• $C_{10}H_{16}$	136.23 g/mol	440917	22.30	Terpenes	68647-72-3
11	D-Limonene	9.966	1789	7.60	3.62	• $C_{10}H_{16}$	136.23 g/mol	440917	10.74	Terpenes	68647-72-3
12	Spiro[4.4]nonane, 1-methylene-	10.035	1336	3.48	3.76	• $C_{10}H_{16}$	136.23 g/mol	565056	4.74	*Sesquiterpene*	19144-06-0
13	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-, (-)-	10.089	1569	2.00	3.82	$C_{15}H_{24}$	204.35 g/mol	94164	2.68	Alcoholic compound	512-61-8
14	Spiro[4.4]nonane, 1-methylene-	10.153	1336	2.98	3.96	• $C_{10}H_{16}$	136.23 g/mol	565056	3.85	*Sesquiterpene*	19144-06-0
15	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, trans-	10.402	1350	3.80	4.38	• $C_{10}H_{18}O_2$ •	170.25 g/mol	854498	4.43	Terpenes	• 42370-41-2
16	m-Mentha-4,8-diene, (1S,3S)-(+)-	10.430	993	1.81	4.44	• $C_{10}H_{16}$	136.23 g/mol	91746608	2.09	Terpenes	• 5208-51-5
17	(+)-Dihydrocarveol,	10.719		5.86	4.82	• $C_{10}H_{18}O$ •	154.25 g/mol	89755	6.23	Terpene	• 22567-21-1
17	Tricyclo[5.2.1.0<2,6>]decane	10.894	1457	2.15	4.91	• $C_{10}H_{12}O_4$ •	196.20 g/mol	5944	2.24	monoterpenoid	• 56-25-7
18	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	11.209	1544	0.45	1.02	• $C_{10}H_{18}O$	154.25 g/mol	6549	2.25	Monoterpenoid	78-70-6

19	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methyletheny	18.403	1470	0.65	0.93	• $C_{15}H_{24}$	204.35 g/mol	89316	3.58	Sesquiterpenoid	20307-84-0
20	Caryophyllene	20.762	1591	1.34	1.22	• $C_{15}H_{24}$	204.35 g/mol	5281515	5.61	Sesquiterpene	• 87-44-5
21	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	20.970	1580	0.54	2.11	• $C_{15}H_{24}$	204.35 g/mol	87529	7.35	Terpene	18252-44-3
22	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	20.970	1580	0.54	2.11	• $C_{15}H_{24}$	204.35 g/mol	87529	7.35	Terpene	18252-44-3
23	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	22.662	1767	0.43	0.53	$C_{15}H_{24}$	204.35 g/mol	92313	4.29	Sesquiterpenes	39029-41-9
24	Nerolidyl acetate	24.257	2271	0.43	0.51	• $C_{17}H_{28}O_2$	264.4 g/mol	5363426	4.26	Sesquiterpenoids	2306-78-7
25	Nootkatone	30.238	2527	1.32	1.08	• $C_{15}H_{22}O$	218.33 g/mol	12681 42	6.25	Sesquiterpenoid	4674-50-4

Molecular Docking:

The orientation of the binding between the two proteins; Acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine acyltransferase from *Escherichia coli* (PDB ID: 6HY2), and Human Mitochondrial Glutathione Reductase (PDB ID: 2SQP) with the ligand were studied using in silico molecular docking shown in figure 1-10. For each ligand, the most convenient conformation i.e., the ones with lowest docked energies were taken into account (Yohannes et al., 2023) .

From *E. coli* Acyl-[Acyl-carrier-protein]-UDP-N-Acetylglucosamine O-Acyltransferase (6HY2) docking; D-limonene, Tricyclo[4.4.0.0(3,9)]decane, Nootkatone, Spiro[4.4]nonane, 1-methylene, and Dihydrocarveol were found to have the following binding energies respectively as their ligands -3.73 kcal/mol, -3.99 kcal/mol,-5.25kcal/mol ,-4.12kcal/mol ,and – 4.46kcal /mol as depicted in the table 3. Nootkatone had the strongest binding affinity for the protein with -5.25kcal /mol. Interaction analysis showed that Nootkatone forms two hydrogen bonds with the protein through residues ILE:199 and GLU:200, along with interactions with MET:170 and ILE:186 (figure 3a). The fact that there are hydrogen bonds indicates a strong interaction between these two compounds (Riaz et al., 2023)(Saleem et al., 2023).

Similarly, based on docking studies with human glutathione reductase (2SQP), different affinities of binding for the compounds were revealed: Nootkatone (-4.6 kcal/mol) was the most effective, D-Limonene (-4.2 kcal/mol), Dihydrocarveol (-4.0 kcal/mol), Spiro[4.4]nonane (-3.9 kcal/mol), and Tricyclo[4.4.0.0(3,9)]decane (-3.1 kcal/mol). The interacting residues turned out to be PHE:78 and HIS:75 (figure 3b). Visualization tools including Chimera and Discovery Studio 2020 Client were used for demonstrating protein-ligand interactions comprising hydrogen bonds as well as generating two-dimensional and three-dimensional graphic representations in order to facilitate understanding of docking process.

Consequently, the molecular docking study and in vitro antibacterial, DPPH radical scavenging activities indicate the peels of *C. maxima* as potentially useful antibacterial agents, supporting the plant's traditional applications. Additionally, the study's findings suggest that essential oils used as antibacterial ingredients.

Table 3: The ligands and proteins used for assessing the antioxidant and antimicrobial activity of CMEO.

Ligand	**Binding energy (Kcal/mol)**		**Molecular Weight**	**PubChem Id**
	3SQP	**6HY2**		
D-Limonene (C10H16)	-4.2	-3.73	136.23	440917
Tricyclo [4.4.0.0(3, 9)] decane (C10H16)	-3.1	-3.99	136.23	565074
Nootkatone (C15H22O)	-4.6	-5.25	218.33	1268142

Spiro[4.4]nonane, 1-methylene (C10H16)	-3.9	-4.12	136.23	565056
Dihydrocarveol (C10H18O)	-4.0	-4.46	154.25	89755

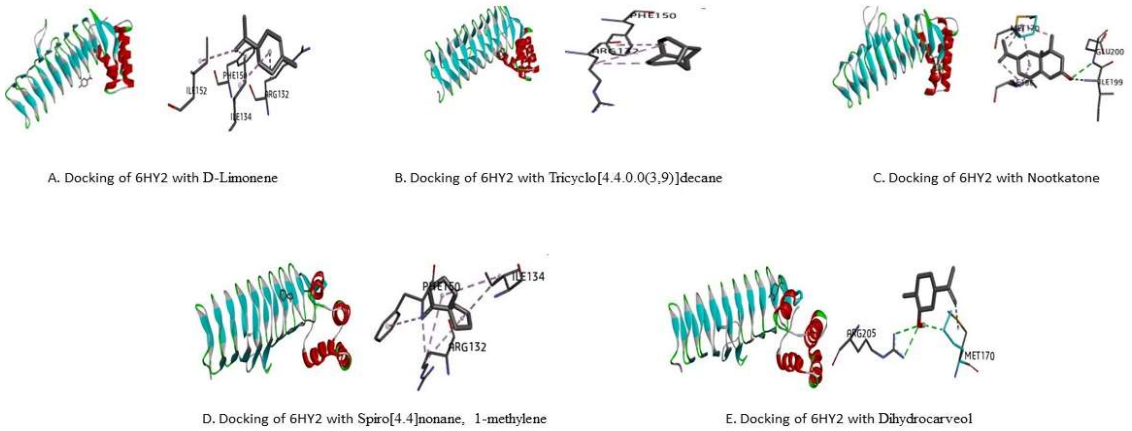

Figure: 3a, Molecular docking of 6HY2 with the five compounds, showing 3D model of the interaction and H-bonding interaction.

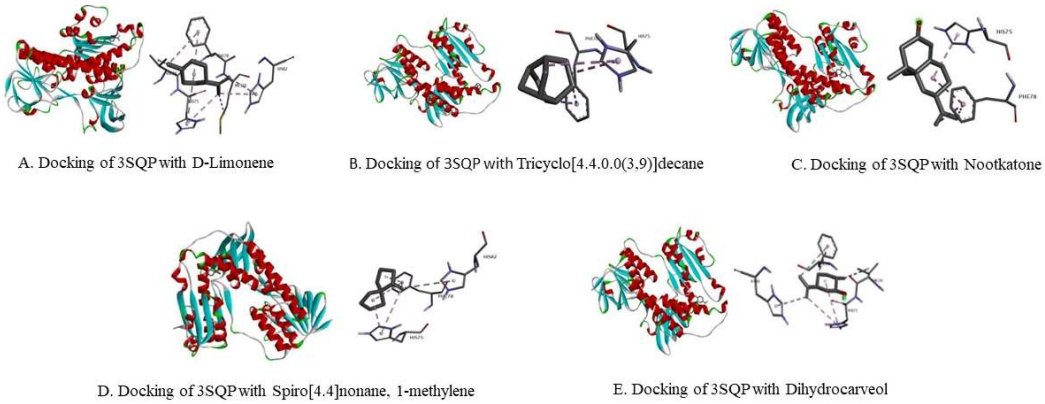

Figure: 3b, Molecular docking of 3SQP with five compounds, showing 3D interaction and H-bonding interaction.

Drug-Likeness and Pharmacokinetics study:
All five compounds were subjected to Lipinski's rule of five in order to be considered for drug-likeness. As shown in Table 6, all five of the compounds have favourably satisfied to the Lipinski criterion, indicating that they are drug-like molecules. Partition coefficient, or LogP, is an essential physiochemical characteristic in the creation of new drugs
(Baccari et al., 2023).

Table 4: Physiochemical & Drug likeness properties of the compounds

	Pharmacokinetics			Drug Likeness				Water Solubility		
Compound	**GI absorption**	**CYP enzyme inhibitors**	**BBB**	**Molecular Weight (g/mol)**	**HBA**	**HBD**	**log P**	**LogS (ESOL)**	**LogS (ALI)**	**LogS (SILICOS-IT)**
D-Limonene	Low	Only CYP2C9 inhibitor	Yes	136.13	0	0	4.53	Soluble	Moderately soluble	Soluble

Tricyclo[4.4.0.0(3,9)]decane	Low	Only CYP2C9 inhibitor	Yes	136.13	0	0	3.32	Soluble	Soluble	Soluble
Nootkatone	High	CYP2C19 & CYP2C9 inhibitors	Yes	218.17	1	0	3.90	Soluble	Soluble	Soluble
Spiro[4.4]nonane, 1-methylene-	Low	Only CYP2C9 inhibitor	Yes	136.23	0	0	2.76	Soluble	Soluble	Soluble
Dihydrocarveol	High	No	Yes	154.14	1	1	3.41	Soluble	Soluble	Soluble

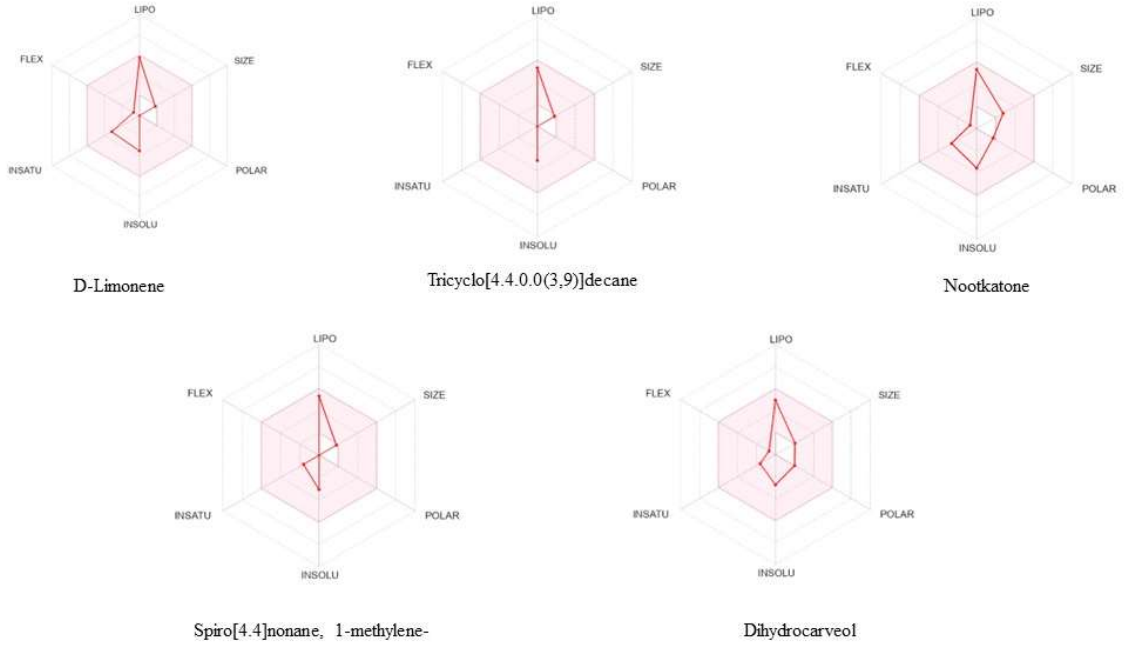

Fig.4. Bioavailability radar chart of the compounds.

The ADMET property such as GI absorption, CYP enzyme inhibitor, LogKp, LogS(ESOL), LogS(ALI) and LogS(Silicos-IT) were presented in the table 4. According to this, Nootkatone and Dihydrocarveol have shown high Gasto intestinal absorption increasing the chances of drug reaching the systemic circulation, potentially increasing the therapeutic effect. The inhibition of Cytochrome CPY2C9 have been shown by all compound except for Dihydrocarveol, which doesn't inhibit any of the Cytochrome P450 (CYP). All the compounds have shown to have Blood brain barrier (BBB) permeation property that have the potential to treat diseases such as CNS, such as Alzheimer's, Parkinson's and epilepsy (Jacob & Alexander, 2014). All the selected compounds have shown water solubility (Table 4), which is advantageous for oral drug delivery due to enhanced absorption and distribution, leading to better bioavailability (Figure 4) and suitability for systemic therapeutic application. These compounds can readily dissolve in gastrointestinal (GI) fluids, facilitating efficient absorption.

This result indicates that most of the compounds are likely to be drug-like molecules and could potentially serve as lead molecules. However, further research is necessary to confirm their suitability.

Conclusion:

In conclusion, the present study demonstrates that *Citrus maxima* essential oils possess significant

antioxidant and antibacterial activities, highlighting their potential as valuable natural bioactive agents. The combined *in vitro* and *in silico* investigations, supported by GC–MS profiling and molecular docking analyses, provided important insights into the phytochemical composition and possible molecular mechanisms underlying their biological activities. The presence of major bioactive constituents such as D-limonene, dihydrocarveol, and nootkatone appears to contribute substantially to the observed antioxidant and antimicrobial effects. Among the identified compounds, nootkatone exhibited promising binding affinity toward the selected target proteins, suggesting its potential as a lead molecule for future therapeutic applications. Furthermore, the study emphasizes the importance of valorizing *Citrus maxima* peel and seed waste as sustainable sources of biologically active compounds for pharmaceutical, nutraceutical, and agricultural applications. Overall, *Citrus maxima* essential oils represent promising natural alternatives for combating oxidative stress and microbial infections, thereby offering potential benefits for human health and sustainable biorefinery development.

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