

In silico and In vitro Antibacterial Activity of Essential oil of *Eucalyptus globulus* Labill

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Abstract

Eucalyptus globulus Labill. Traditionally used as disinfectant, fumigant, insect repellent, it is also used to cure asthma, bronchitis, arthritis, boils etc. The current research is focused on to study the *in vitro* and *in silico* antibacterial activity of *Eucalyptus globulus* essential oil. Essential oil of *Eucalyptus globulus* was extracted by hydro-distillation method and characterized by GC-MS. *In vitro* antibacterial activity was done by using agar well diffusion and broth microdilution method. Further the antibacterial activity was predicted *in silico* by using Autodock vina. Essential oil extraction yield of *Eucalyptus globulus* leaves was found to be $2.13 \pm 0.115\%$. Total seventeen mono- and sesqui-terpenoids were identified in essential oil, of which eucalyptol (60.327), D-limonene (6.964), terpinyl-acetate (3.564) and gamma gurjunene (2.505%) were the major constituents. Essential oil of *Eucalyptus globulus* showed significant antibacterial activity against Clinical strains of *Escherichia coli*, *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus* with minimum inhibitory concentration values of 0.125, 0.125 and 0.3125% respectively. In molecular docking study, γ -gurjunene showed good binding energy with penicillin binding protein (3VSL) and MD simulation confirmed the stability of protein ligand complex. Essential oil of *Eucalyptus globulus* has good antibacterial activity against clinical strains of gram positive and gram negative bacteria. Essential oil of *Eucalyptus globulus* can be used as antibacterial agent with addition of *in vitro* and *in vivo* toxicity.

1. Introduction

Since ancient times, plants have been used as a source of medicine. As of now, approximately half of all medicines are derived from plants or their derived products, fueling interest in the plant world (Tuorkey 2015). Bacterial resistance to commonly used antibiotics has reached alarming rates throughout the world, implying that numerous of the treatment options available for severe infections seem to become ineffective, resulting in prolonged illness, longer hospital stays, and increased mortality (WHO, 2016). *Eucalyptus* is a non-native evergreen plant in India that is now widely cultivated for a variety of purposes including medicinal, phytochemical, pharmaceutical, timber wood, wooden product, mosquito repellent, antioxidant, antibacterial properties, and essential oils. (Salem et al. 2014)

There are approximately 700 species of *Eucalyptus* genus out of which 300 species contains essential oil in their leaves and these oils are used in the cosmetics, pharmaceutical, food industries and many more (Marzoug et al. 2011). The plant oil has allelopathic properties and protects eucalyptus leaves from attack by harmful insects, acting as a natural pesticide (Batish et al. 2008). According to their economic values, various species are cultivated in subtropical and tropical regions (SASTRI 1963) *Eucalyptus globulus* is well-known for its high concentration of bioactive compounds such as phenolic acids, hydrolysable tannins and flavonoids (Chinnarasu et al. 2015). Multiple studies have demonstrated that *Eucalyptus globulus* seems to have antibacterial, astringent, anti-inflammatory, antiseptic and anticancer properties (Takahashi et al. 2004). Traditionally peoples are using essential oil of *Eucalyptus globulus* as anaesthetic, disinfectant, fumigant, insect repellent, asthma, bronchi, arthritis, boils, vermifuge, expectorant, abscess, astringent, diaphoretic, deodorant, makeup and soap industries (Bajaj 2012). In the study by (Bachir and Benali 2012) demonstrated that essential oil of the leaves of *Eucalyptus globulus* has antimicrobial activity against gram-negative bacteria (*Escherichia coli*) as well as gram-positive bacteria (*Staphylococcus aureus*). Similarly, (Kumar et al. 2012) in their study showed good activity of *Eucalyptus globulus* oil for larvae and pupae of housefly. In addition,

significant mortality of both, larvae and pupae were observed for contact and fumigation toxicity. However, the results obtained in the fumigation assay were more potent compared to contact toxicity assay. A study (Boukhatem et al. 2020) showed that essential oil of *E. globulus* acts as an effective and potent inhibitor of food spoilage fungi in a real Orange juice, and might act as a potential natural source of preservative for the food industry.

One of the most serious threats to successful infectious disease treatment is the spread of drug-resistant microbial pathogens. Multiple drug resistance is becoming threat to the modern world and will become deadly in the coming years. To cure the spread of diseases, we need to combine the allopathy with the traditional knowledge to enhance the activity of the modern drugs. Current study is focused on *in vitro* and *in silico* antibacterial activity of essential of *Eucalyptus globulus*.

2. Materials and Method

2.1 Chemicals and solvents

Antibiotics such as cloxacillin, vancomycin and ciprofloxacin were bought from the HiMedia Laboratories Pvt. Limited, Mumbai, India. Solvents like n-hexane and dimethyl sulfoxide (DMSO) were bought from the Loba Chemie Pvt. Limited, Mumbai, India. All chemicals used in this study were of analytic grade.

2.2 Micro-organisms and Growth media

Clinical strains of Bacteria (*Staphylococcus aureus*, *Escherichia coli*, and Methicillin-resistant *Staphylococcus aureus*). Bacterial strains were maintained on nutrient agar (NA) media, at temperature 35 ± 2 °C. Growth media were bought from the from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

2.3 Collection and identification of plant sample

The Leaf samples of *Eucalyptus globulus* Labill. Was collected from Khuda Lahora (Elevation: 367.49 m, Latitude: 30.76 °N, Longitude: 76.77 °E) region of Chandigarh, India in the month of June 2022. The plant sample was identified at the Department of Forestry products, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, H.P, India (voucher no. UHF-13790). The name of the plant was verified from The Plant List (www.theplantlist.com) also from World Flora Online (www.worldfloraonline.org).

2.4 Essential oil extraction from leaves of *Eucalyptus globulus* Labill.

The leaves of *E. globulus* were washed under running tap water and followed by distilled water. Extraction of essential (Rolta et al. 2020b) oil was done by using hydro distillation method by using Clevenger apparatus (oil lighter than water)(Salaria et al. 2022b). Essential oil was stored in amber tubes at 4°C. The extraction yield percentage (v/w) of essential oil from leaves of *E. globulus* was calculated by using formula described in previous study by (Salaria et al. 2022c; Salaria et al. 2022b).

2.5 Characterization of essential oil of *E. globulus* essential oil by GC-MS

GC–MS of *E. globulus* essential oil was done on by using Gas chromatography-mass spectrometry (GC-MS) of Clarus 600 Gas chromatograph with Clarus 600 C mass spectrometer (Perkin-Elmer, Massachusetts, USA). The GC was connected to an Elite 5MS hair column, 30 m x 250 µm The temperature program Oven: Initial temperature 50°C for 2min. Ramp 10c/min to 230°C hold for 10min inject Auto = 220°C 50–230 °c solvent Delay = 4min, transfer Temp = 200°C source temp = 220°C, at 5°C/min then isothermally at 220°C detected Helium was used as the carrier gas at a flow rate of 4.0 ml/min 1µl sample was injected in the split mode. A split ratio of 0:1 and ionization voltage of 70eV were maintained. Scan: 90-400Da. Phytoconstituents of essential oil were identified in relation to the baseline in terms of their retention time (Rt). The phytoconstituents (mono- and sesqui-terpenoids) were identified based on matching unknown peaks with MS-data bank (NIST electronic Library).

2.6 Antibacterial activity of *E. globulus* essential oil

The antibacterial potential of essential oil of *E. globulus* was done by using qualitative (C 1990) and quantitative methods (Humphries et al. 2021) CLSI, 2021 M100, (Salaria et al. 2022c); and (Rolta et al., 2020a,b). DMSO (10%, v/v) was used as a negative control in both trials, whereas antibiotics such as cloxacillin, vancomycin and ciprofloxacin were used as a positive control. Both experiments were carried out in triplicate and the results were recorded as a mean standard deviation.

2.7 Molecular docking analysis of phytocompounds of *E. globulus*

Molecular docking of phytocompounds of *E. globulus* essential oil was done by using AutoDock vina software (<http://vina.scripps.edu/>).

2.7.1 Protein preparation

Penicillin-binding proteins (PBPs) are responsible for antibacterial action in bacteria and for mediation of antibiotic resistance to beta-lactams. The 3D structure of the penicillin 3 binding protein (PDB ID: 3VSL; Yoshida et al., 2012) was obtained from the protein data bank ([HTTPS://www.rcsb.org/](https://www.rcsb.org/)). The Auto-Dock 1.5.6 tool was used to prepare the protein. Earlier research has served to predict the active site of the target protein (Salaria et al. 2022b) and (Rolta et al. 2022).

2.7.2 Ligand preparation

The 3D structure of phytocompounds of *E. globulus* essential oil and antibiotics were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Energy of the all phytocompounds and antibiotics were minimized by using Chem3D 15.0 software. All the ligands were prepared by using Auto-Dock 1.5.6 tool.

2.7.3 Molecular docking

A total of seventeen phytocompounds of *E. globulus* essential oil were docked with Penicillin Binding protein 3, PDB ID: 3VSL by using AutoDock vina tool (Rolta et al. 2022; Trott and Olson 2010). The phytocompounds with highest binding energy were selected for MD simulation. 2D and 3D interactions of protein ligand complexes were analyzed through discovery studio.

2.8 Drug likeness, ADME/Toxicity prediction of phytocompounds of *E. globulus*

Drug likeness prediction of phytocompounds of *E. globulus* was predicted by using molinspiration cheminformatics online web server (<https://www.molinspiration.com/>). Toxicity of all the phytocompounds were predicted by using PROTOX II web server (https://tox-new.charite.de/protox_II/).

2.9 Molecular Dynamics simulation

MD simulation of Gamma-Gurjunene in complex with 3VSL was done by using GROMACS 2021.2 (simulation in duplicate) (Abraham et al. 2015) software which was installed in ubuntu 18.04 LTS, to study the stability of protein–ligand complexes over the period of 100 ns.

The target protein (3VSL) was processed and the topology file was prepared using pdb2gmx and CHARMM36-Jul2021. The ligand topology file was prepared using the swissparam online server (<https://www.swissparam.ch/>). MD method is earlier described by (Fadare et al. 2021) ([CSL STYLE ERROR: reference with no printed form.]).

The free energy of binding calculations (interaction free energy between the study proteins and complexed ligands) were done using the stand alone program, gmx_MMPBSA by Valdes (Assaggaf et al. 2022) based on the MMPBSA.py (from AMBER) functionalities.

3. Results and Discussion

3.1 Extraction yield of *E. globulus* essential oil

The extraction yield of *E. globulus* essential oil was 2.13 ± 0.115 . In contrast to our study Singh et al., 2016 reported the extraction yield of *E. globulus* essential oil by four different methods hydro distillation (2.0%), solvent extraction (2.1%), ultrasonication (2.6%) and super critical carbon dioxide (3.6%) respectively. (Derwich et al. 2009) reported the extraction yield of *E. globulus* essential oil 1.21%. (Assaggaf et al. 2022) reported the extraction yield of *E. globulus* essential oil 2.2%.

3.2 Phytochemical characterization of *E. globulus* essential oil

Characterization of *E. globulus* essential oil was performed by GCMS technique. Total seventeen mono-/sesqui-terpenoid compounds were identified in the essential oil. These constituents were identified by comparing the constituent's spectra with NIST mass spectral library (<https://chemdata.nist.gov/>), the results are summarized in Table 1 and Fig. 1. Of the seventeen phytocompounds, eucalyptol (60.372%), D-limonene (6.964%), Terpinyl acetate (3.564%), γ -Gurjunene (2.509%), pentanoic acid (1.108%) and 17-pentatriaconten (1.074%), remaining 11 phytocompounds have area percentage less than 1%. In contrast to our (Assaggaf et al. 2022) reported the higher percentage of eucalyptol (90.14%) followed by α -pinene (3.85%), β -pinene (0.62%), γ -terpinene (2.39%), α -phellandrene (0.96%), β -myrcene (0.58%), camphene (0.48%) and β -ocimene (0.28%), they have reported total 8 phytocompounds. (Almas et al. 2021) reported the oxygenated phytocompounds eucalyptol with area percentage 51.62% followed by terpinen-4-ol 2.74% and deoxygenated phytocompounds α -pinene (23.62%), p-cymene (10%) and β -myrcene (8.74%) in essential oil of *E. globulus* from Tanzania. Essential oil of *E. globulus*

contains twelve phytocompounds compounds with different concentrations out of twelve phytocompounds 1.8- Cineole is major phytocompounds with area percentage 22.35%, followed by limonene, solanol, p-pinene, transverbenol, terpine-4-ol, aristilene, terpinyl acetate, isosativene, sabinene, α -myrcene with area percentage 7.01, 6.05, 4.02, 3.10, 2.35, 2.10, 1.85, 1.49, 1.15 and 1.10%, respectively.

Table 1
Phytocompounds identified in essential oil of *E. globulus* by GC-MS

S. No.	Name of Phytocompounds	Retention Time (RT)	Area (%)	Molecular weight g/mol (M.W.)	CAS No.
1.	Alpha- phellandrene	3.519	1.671	136.23	99-83-2
2.	D-limonene	3.694	6.964	136.23	5989-27-5
3.	Eucalyptol	3.744	60.372	154.25	470-82-6
4.	4-Carvomenthenol	5.15	0.462	154	562-74-3
5.	Ocimenol	5.314	1.376	154.29	5986-38-9
6.	Terpinyl Acetate	7.385	3.654	196.29	80-26-2
7.	Piperidin-4-One Hydrochloride	8.05	0.654	135.59	41977-39-9
8.	Caryophyllene Oxide	9.10	0.194	220.35	1139-30-6
9.	Pentanoic Acid	10.496	1.108	306	166273-38-7
10.	Gamma-Gurjunene	12.022	2.509	204.35	22567-17-5
11.	Phthalic Acid	14.6	0.098	166.13	88-99-3
12.	17-Pentatriaconten	14.064	1.074	490.9	6971-40-0
13.	11-TRICOSENE	15.39	0.933	322.6	52078-56-5
14.	Cyclohexane	17.79	0.222	168.62	74663-66-4
15.	Butanoic Acid	19.76	0.079	117.15	32723-74-3
16.	Propanoic Acid	21.0	0.046	160.17	59247-16-4
17.	Alanine ethylamide	24.16	0.038	116.16	71773-95-0

3.1 *In vitro* antibacterial activity of *E. globulus* essential oil

Antibacterial activity of essential oil was done by qualitative and quantitative method against clinical strains of *Staphylococcus aureus*, *Escherichia coli*, and Methicillin-resistant *Staphylococcus aureus*. Qualitative assay was done by agar well diffusion method and qualitative assay was done by Broth microdilution method.

In qualitative assay there is no antibacterial activity of 1 and 2% essential oil against all the selected bacterial strains, where as positive control cloxacillin showed the effect against gram positive bacteria *S. aureus* and MRSA as shown in supplementary Fig. S1.

3.2 Quantitative antibacterial activity of *E. globulus* essential oil

Minimum inhibitory concentration (MIC) of *E. globulus* essential oil and antibiotics (cloxacillin, vancomycin and ciprofloxacin) was determined by broth microdilution method. Essential oil showed 0.125% MIC against *E. coli* and MRSA, 0.3125% against *S. aureus*. Whereas antibiotics cloxacillin showed 62.5 µg/mL against *E. coli* and MRSA, 3.90 µg/mL against *S. aureus*, vancomycin showed 0.244 µg/mL against *E. coli* and MRSA, 3.90 µg/mL against *S. aureus*, ciprofloxacin showed 1.95, 7.81, 0.244 µg/mL MIC against *E. coli*, MRSA and *S. aureus* respectively. MIC data is summarized in Table 2 and Fig. 2.

Table 2
Minimum Inhibitory concentration of *E. globulus* essential oil and Antibiotics

Bacterial strains	Minimum inhibitory concentration			
	MIC %	MIC µg/ml		
	Essential oil	Cloxacillin	Vancomycin	Ciprofloxacin
<i>E. coli</i>	0.125	3.90	0.244	1.95
MRSA	0.125	3.90	0.244	7.81
<i>S. aureus</i>	0.3125	62.5	3.90	0.244

In contract to our study (Clavijo-Romero et al. 2019) reported the antibacterial activity of oil emulsions *Eucalyptus essential* against *E. coli*, *S. aureus* and *P. aeruginosa* by death kinetics in this study they found that essential oil inhibit the bacterial growth less than 1 min. Previous studies showed that *E. globulus* essential oil has good antibacterial and antifungal activity. Essential oil is effective against *E. coli*, *P. mirabilis*, *S. typhimurium*, *L. monocytogenes*, *B. subtilis* and *S. aureus* with zone of inhibition 16.9 ± 3.2 to 21.1 ± 0.1 mm and MIC range 1 to 0.5%. Essential oil is also active against fungal strains *Candida albicans*, *Trichophyton rubrun* and *Aspergillus Niger* with zone of inhibition 12.9 ± 0.1 to 16.1 ± 3.2 and MIC range 4 – 2% (Bachir and Benali 2012) reported the antifungal activity of *E. globulus* essential oil, (Salem et al. 2014) also reported the antibacterial activity of *E. globulus* against *B. subtilis* and *S. aureus* and plant pathogen *Agrobacterium tumefaciens*. Studies also showed that essential oil of *E. globulus* is only effective against Grams positive bacteria's *S. aureus*, MRSA, *B. cereus*, *Enterococcus faecalis*, *Alicyclobacillus acidoterrestris* etc (Takahashi et al., 2004). Bachir and Benali, 2012) reported the antibacterial activity of essential oil against *E. coli* and *S. aureus*. Apart from antibacterial activities essential oil of also has antioxidant anti-inflammatory activities (Boukhatem et al. 2020; Chinnarasu et al. 2015). Similarly, (Rolta et al. 2020a; Salaria et al. 2022c) also reported the antimicrobial activity of essential oil.

3.3 Molecular Docking of *E. globulus* Phytocompounds with PBP

Molecular docking of *E. globulus* phytocompounds with PBP (3VSL) was done by autoDock vina software. Among all the seventeen phytocompounds Phthalic Acid, Gamma-Gurjunene, Caryophyllene Oxide and Terpinyl Acetate showed the best binding energy – 6.3, -6.0, -5.8, and – 5.4 kcal/mol respectively. Phthalic Acid showed hydrophobic interactions with Lys 395, Asn 450, Ser 449, His 447, Tyr 430, Thr 603, Gly 626 and hydrogen bonding with Ser 448, Ser 392, Thr 619, Thr 621, Gamma-Gurjunene showed hydrophobic interactions with Leu 663, Thr 619, Pro 626, Ser 634, Thr 621, Tyr 636, Pro 660, Thr 603, Val 606, Caryophyllene Oxide showed the hydrophobic interactions with Thr 527, Gln 387, Val 624, Gln 626, Asn 633, Asn 655, Thr 654, Phe 388, Asp 348 and hydrogen bonding with Asn 349 and Terpinyl Acetate showed the hydrophobic interactions Gly 620, Thr 635, Thr 619, Thr 603, Pro 660, Val 606, Gln 656, Val 658, Tyr 636, Leu 663 and hydrogen bonding with Thr 621, Ser 634. Whereas antibiotics cloxacillin showed binding energy – 8.0 kcal/mol and hydrophobic interactions with Tyr 430, Ser 429, His 447, Pro 661, Asp 601, Thr 621, Pro 600, Lys 618, Lys 395, Asn 450, Phthalic Acid, Gamma-Gurjunene showed the comparative binding energy to antibiotics, Phthalic Acid are toxic to human and Gamma-Gurjunene is the best phytocompounds from *E. globulus* essential oil. Binding energy, hydrogen bonding and hydrophobic interactions are summarized in Table 3 and Fig. 3. Furthermore, all the phytocompounds were screened for *in silico* drug likeness and toxicity to identify much safer phytocompounds.

Table 3

Binding energy, H-bonds and interacting amino acids of *E. globulus* essential oil Phytocompounds

Phytocompounds/ Antibiotics	Binding energy (kcal/mol)	Hydrogen bonding	Hydrophobic interactions
Alpha-phellandrene	-4.5	SER 392, THR 621, THR 619	THR 603, VAL 606, THR 619, THR 620 THR 621, SER 634, TYR 636, GLY 620, PRO 660, LEU 663
D-limonene	-4.7	-	THR 603, LEU 603, VAL 606, THR 619, GLY 620, THR 621, THR 635, TYR 636, SER 636, PRO 660,
Eucalyptol	-4.8	TYR 636	THR 603, LEU 603, VAL 606, THR 619, GLY 620, THR 621, SER 634, THR 635, PRO 660, GLY 665, SER 607, GLY 666, THR 664,
4-Carvomenthenol	-5.2	THR 619, THR 603	TYR 636, GLY 665, SER 607, THR 664, GLY 666, VAL 606, LEU 603, THR 621, PRO 660
Ocimenol	-4.3	LEU 663	VAL 606, THR 603, PRO 661, TRP 602, SER 634, THR 621, TYR 636, PRO 660, GLY 665, THR 664
Terpinyl Acetate	-5.4	THR 621, SER 634	GLY 620, THR 635, THR 619, THR 603, PRO 660, VAL 606, GLN 656, VAL 658, TYR 636, LEU 663
Piperidin-4-One Hydrochloride	-4.1	LEU 663, THR 619, SER 634	TYR 636, GLY 620, THR 635, THR 621, PRO 666, THR 604
Caryophyllene Oxide	-5.8	ASN 349	THR 527, GLN 387, VAL 624, GLN 626, ASN 633, ASN 655, THR 654, PHE 388, ASP 348
Phthalic Acid	-6.3	SER 448, SER 392, THR 619, THR 621	LYS 395, ASN 450, SER 449, HIS 447, TYR 430, THR 603, GLY 626
Pentanoic Acid	-4.2	THR 619, GLY 620, THR 621, SER 634	LEU 663, TYR 636, THR 635, THR 603, PRO 666
Gamma-Gurjunene	-6.0	-	LEU 663, THR 619, PRO 626, SER 634, THR 621, TYR 636, PRO 660, THR 603, VAL 606
17-Pentatriaconten	-4.4	-	THR 601, TYR 605, VAL 606, LYS 600, LEU 603, PRO 660, PRO 661, THR 619, THR 621, SER 392, SER 448, HIS 447, SER 429, GLY 626, ASN 450, TYR 430
11-TRICOSENE	-4.5	-	GLY 602, TYR 605, ASP 601, HIS 447, TYR 436, SER 429, THR 619, THR 621, LYS 600, THR 603, PRO 660, VAL 606, TYR 636, LEU 663, SER 634
Cyclohexane	-3.1	-	THR 603, HIS 447, ASP 601, TYR 430
Propanoic Acid	-3.9	SER 634, THR 619, THR 621, GLY 620	THR 635, TYR 636, LEU 663, PRO 660

Phytocompounds/ Antibiotics	Binding energy (kcal/mol)	Hydrogen bonding	Hydrophobic interactions
Alanine Ethylamide	-4.6	TYR 636, LEU 663, THR 619	GLY 604, THR 603, VAL 606, THR 621, SER 607, THR 664, GLY 665, GLY 666, VAL 658, PRO 660, SER 634
Cloxacillin	-8.0	-	TYR 430, SER 429, HIS 447, PRO 661, ASP 601, THR 621, PRO 600, LYS 618, LYS 395, ASN 450

In contrast to our study (Rolta et al. 2022) reported the antibacterial activity of phytocompounds of *R. emodi* with penicillin binding protein 3VSL, they have found emodin showed the best binding energy as compare to other phytocompounds. Similarly, (Batish et al. 2008) Eucalyptus (family Myrtaceae), an Australian native, represented by around 700 species is a genus of tall, evergreen and magnificent trees cultivated world over for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematocidal. The present paper discusses this environmentally benign pest control using eucalyptus oils against bacteria, fungi, insects, nematodes, weeds and mites. The use of eucalyptus oil as a natural pesticide is of immense significance in view of the environmental and toxicological implications of the indiscriminate use of synthetic pesticides and overcoming/reducing the problem of increasing pest resistance. (Batish et al. 2008; Salaria et al. 2022b) also reported the best binding energy of thymol active phytocompound of *Thymus serpyllum* essential oil with 3VSL.

3.4 *In silico* drug likeness and toxicity of *E. globulus* phytocompounds

All the identified seventeen phytocompounds were screened for drug likeness activity by Molinspiration and ProtoxII online web servers. Among all the seventeen phytocompounds fifteen phytocompounds follows drug likeness rules, only 17-Pentatriaconten and 11-Tricosene showed the one violation. Drug likeness data is summarized in Table 4.

Table 4
Drug likeness of *E. globulus* phytochemicals

Phytochemicals	MiLog P	TPSA (Å ²)	Number of Atoms	Number of Nitrogen and Oxygen	Number of -OH and -NHn	Number of violations	Number of Rotations	M.W.
Alpha-phellandrene	3.79	0.00	10	0	0	0	1	136.24
D-limonene	3.62	0.00	10	0	0	0	1	136.24
Eucalyptol	2.72	9.23	11	1	0	0	0	154.25
4-Carvomenthenol	2.60	20.23	11	1	1	0	1	154.25
Ocimenol	2.98	20.23	11	1	1	0	4	154.25
Terpinyl Acetate	3.30	26.30	14	2	0	0	3	196.29
Piperidin-4-One Hydrochloride	-0.68	9.10	7	2	1	0	0	135.59
Caryophyllene Oxide	4.14	12.53	16	1	0	0	0	220.36
Phthalic Acid	1.03	74.60	12	4	2	0	2	166.13
Pentanoic Acid	1.50	37.30	7	2	1	0	3	102.13
Gamma-Gurjunene	5.00	0	15	0	0	0	1	204.36
17-Pentatriaconten	10.38	0	35	0	0	1	31	490.94
11-TRICOSENE	9.45	0	22	0	0	1	18	308.59
Cyclohexan	3.21	0	6	0	0	0	0	4.16
Butanoic Acid	1.00	37.30	6	2	1	0	2	8.11
Propanoic Acid	0.44	37.30	5	2	1	0	1	74.08
Alanine Ethylamid	-1.38	55.12	7	3	3	0	1	102.14
miLogP: Molinspiration LogP (to measure lipophilicity); TPSA: Topological polar surface area; MW: Molecular Weight								

Toxicity data showed that hepatotoxicity Terpinyl Acetate is active, immunotoxicity of caryophyllene Oxide is active, carcinogenicity of cyclohexan is active. Toxicity data is summarized in Table 5. Among all the above parameters molecular docking, drug likeness and toxicity showed that Gamma-Gurjunene is the best phytochemicals with LD₅₀ 5000 mg/kg class five.

Table 5
Toxicity prediction of phytocompounds *E. globulus* phytocompounds using
Protox II server

Phytocompounds	Protox-II					
	1	2	3	4	5	6
Alpha- phellandrene	5700 (Class 6)	In	In	In	In	In
D-limonene	4400 (Class 5)	In	In	In	In	In
Eucalyptol	2850 (Class 5)	In	In	In	In	In
4-Carvomenthenol	1016 (Class 4)	In	In	In	In	In
Ocimenol	5000 (Class 5)	In	In	In	In	In
Terpinyl Acetate	4800 (Class 5)	A	In	In	In	In
Piperidin-4-One Hydrochloride	338 (Class 4)	In	In	In	In	In
Caryophyllene Oxide	5000 (Class 5)	In	In	A	In	In
Phthalic Acid	2530 (Class 5)	In	In	In	In	In
Pentanoic Acid	134 (Class 3)	In	In	In	In	In
Gamma-Gurjunene	5000 (Class 5)	In	In	In	In	In
17-Pentatriaconten	2760 (Class 5)	In	In	In	In	In
11-TRICOSENE	2760 (Class 5)	In	In	In	In	In
Cyclohexan	178 (Class 5)	In	A	In	In	In
Butanoic Acid	91 (Class 5)	In	In	In	In	In
Propanoic Acid	300 (Class 3)	In	In	In	In	In
Alanine Ethylamide	1980 (Class 4)	In	In	In	In	In

1-LD, (mg/kg), 2-Hepatotoxicity, 3-Carcinogenicity, 4-Immunotoxicity, 5-Mutagenicity, 6-Cytotoxicity, A-Active and In- Inactive.

3.5 Molecular Dynamic simulation (MD) of Protein-Ligands complexes

MD simulation (100 ns) was done to study the stability of the protein ligand complex. Based on molecular docking and *in silico* drug likeness and toxicity study, the best protein ligand complex (Gamma-gurjunene + 3VSL) was selected for MD simulation. Root mean square deviation (RMSD) of protein ligand is stable at the beginning of the simulation, it showed little fluctuation at 30–40 ns and got stabilized at 40 ns and remained stable up to 100 ns time period (Fig. 4A). In the root mean square fluctuation (RMSF) plot, we found that Gamma-gurjunene is showing the stronger interaction with many amino acid residues across different regions of the protein. The root-mean-square fluctuation (RMSF) measures the average deviation of a particle (e.g. a protein residue) over time from a reference position (typically the time-averaged position of the particle). Thus,

RMSF analyzes the portions of structure that are fluctuating from their mean structure the most (or least). The residues that fluctuated the most, recording higher values (nm) are in the regions 55–200 which characterizes a domain in the protein and then 250–280, 300, 350 and then 400–520 which also characterizes another domain in the protein (Fig. 4B).

Solvent-accessible range for gamma-gurjunene and 3VSL complex is 320–340 nm² (Fig. 5A). Radius of gyration plot showed the compactness of gamma-gurjunene-3VSL complex, which confirms the stability of the protein-ligand complexes (Fig. 5B).

The binding free energy and energy components of the complex was calculated using the MM-PBSA binding free energy method in order to verify the stability of the complex in terms of energetics. The various components of the interaction energy and the sum total is as shown in Fig. 6. The overall binding energy (Delta G Total) is -5.23 kcal/mol, the negative value implies that the binding is feasible and the complex is stable. Similar to our study (Kaur et al. 2022; Rolta et al. 2022; Salaria et al. 2022a) reported the MD simulation of Phytocompounds with target proteins of 3VSL.

4. Conclusion

The study reports on the extraction yield, phytochemical characterization and antibacterial activity of Eucalyptus globulus essential oil. The extraction yield of oil was found to be 2.13 ± 0.115 . GCMS analysis revealed 17 phytocompounds with eucalyptol being the major component (60.372%). The essential oil exhibited antibacterial activity against Staphylococcus aureus, Escherichia coli and Methicillin resistance Staphylococcus aureus with MIC ranging from 0.125–0.3125%. Molecular docking analysis showed that Pthalic acid, Gamma- Gurjunene, Caryophyllene oxide and Teriny; acetate exhibited the best binding energy with PBP.

Declarations

Conflict of interest:

Authors have no Conflicts of Interest

Funding:

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Authors contributions:

All the experiments were performed by Dr. Deeksha Salaria and Rajan Rolta, Molecular docking and MD simulation was done by Dr. Olatomide A. Fadare, Prof. Sanjay Jachak Contributed in GCMS analysis of Essential oil, Dr. Ajay Prakash done the data analysis, all the experiments were done under the supervision of Prof. Bikash Medhi. All the authors read the manuscript and approved for submission.

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Figures

Qualitative Report

File:	C:\TURBOMASS\NPGCMS1.PRO\Data\Eucalyptus globulus oil1.raw	
Acquired:	25-May-20 12:27:35 PM	Printed: 25-May-20 02:50 PM
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GC/MS Method:	GC: Eucal glo June22.mth MS: Eucal glo june22.EXP	Page 1 of 3
Sample ID:	ecal glo1	Vial Number: 1

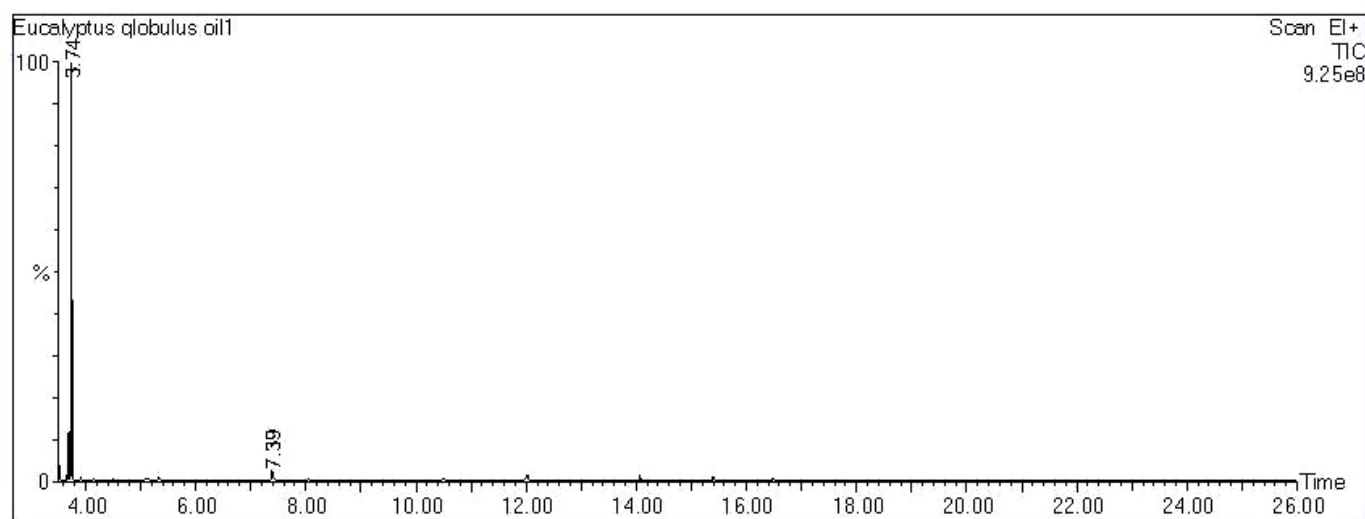


Figure 1

GCMS spectra of *E. globulus* essential oil: X-axis showing Retention time (RT) and Y-axis showing percentage (%)

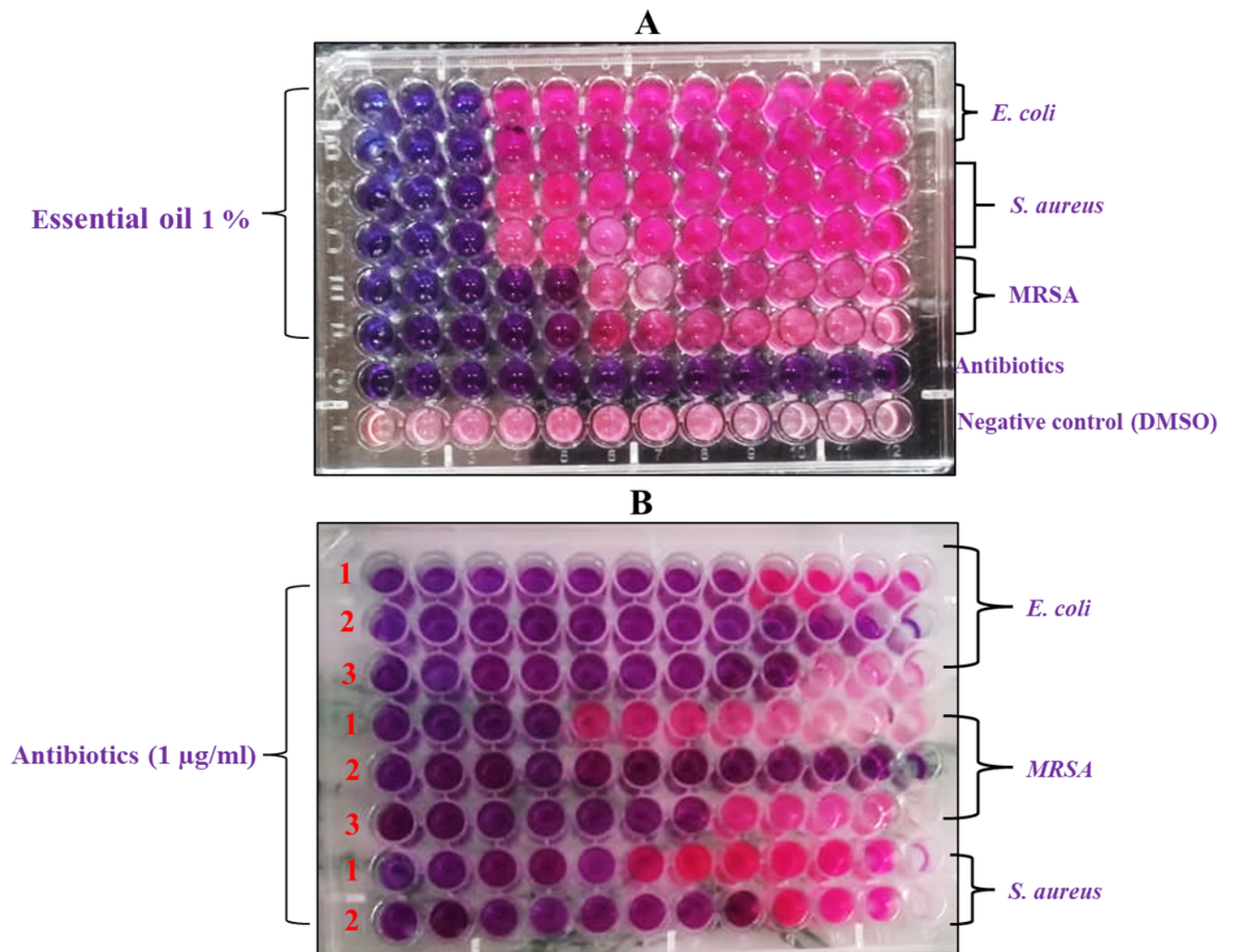


Figure 2

Minimum inhibitory concentration essential oil and antibiotics: (A) Essential oil of *E. globulus* and (B) Antibiotics (1- Cloxacillin, 2-Vancomycin and Ciprofloxacin), purple color indicates dead color and pink color indicated viable cells.

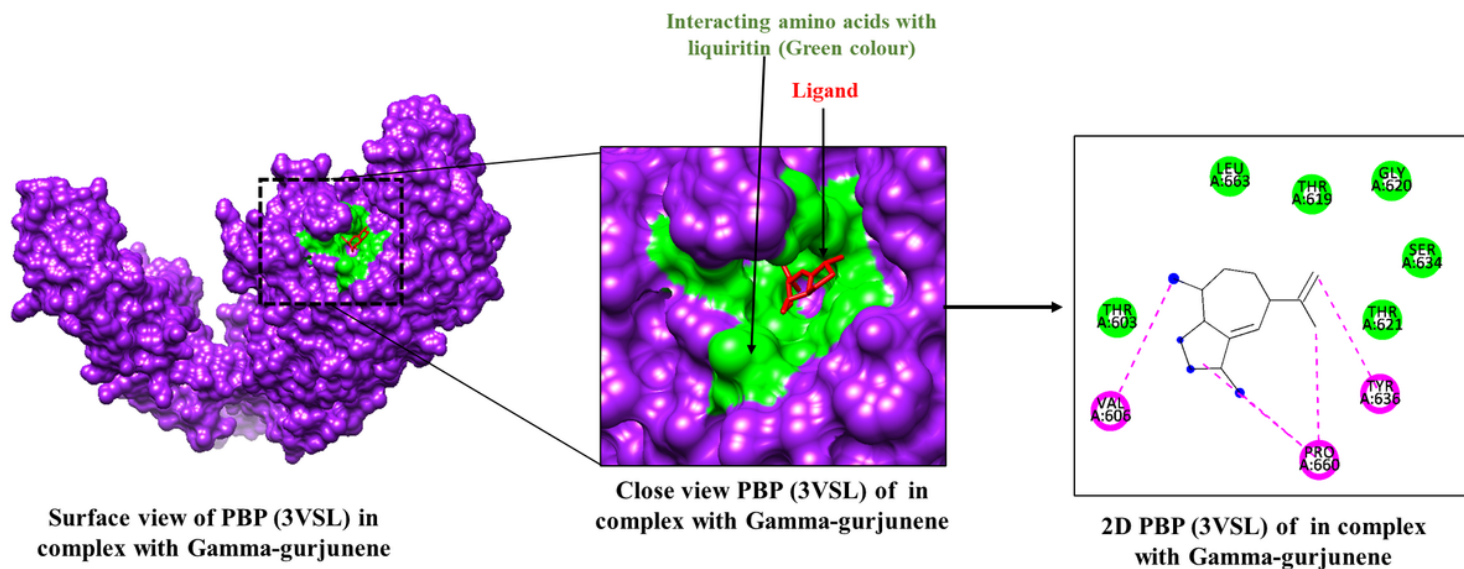


Figure 3

Interactions of Gamma-Gurjunene with PBP (3VSL):Close view of 3VSL in complex with Gamma-Gurjunene, purple colour is showing target protein, green colour is showing hydrophobic interactions and red colour ligand

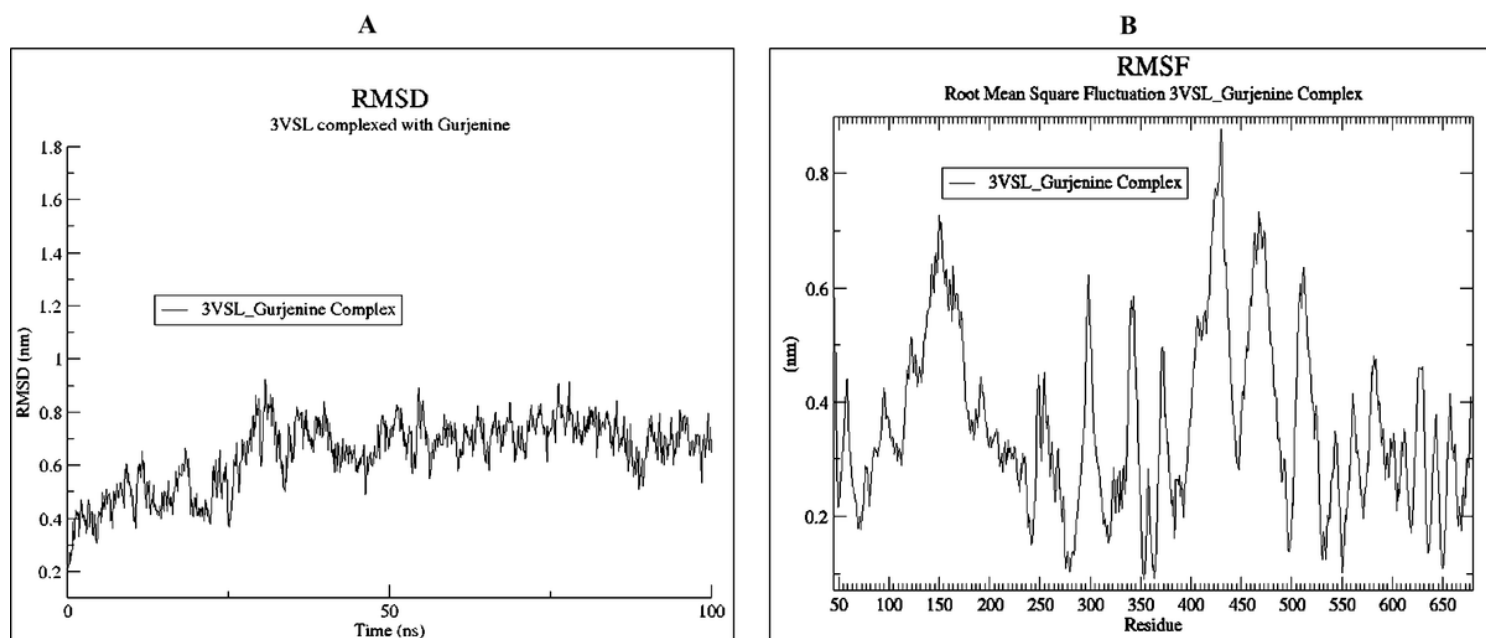


Figure 4

RMSD and RMSF of gamma-gurjunene in complex with 3VSL:(A) RMSD and (B) RMSF

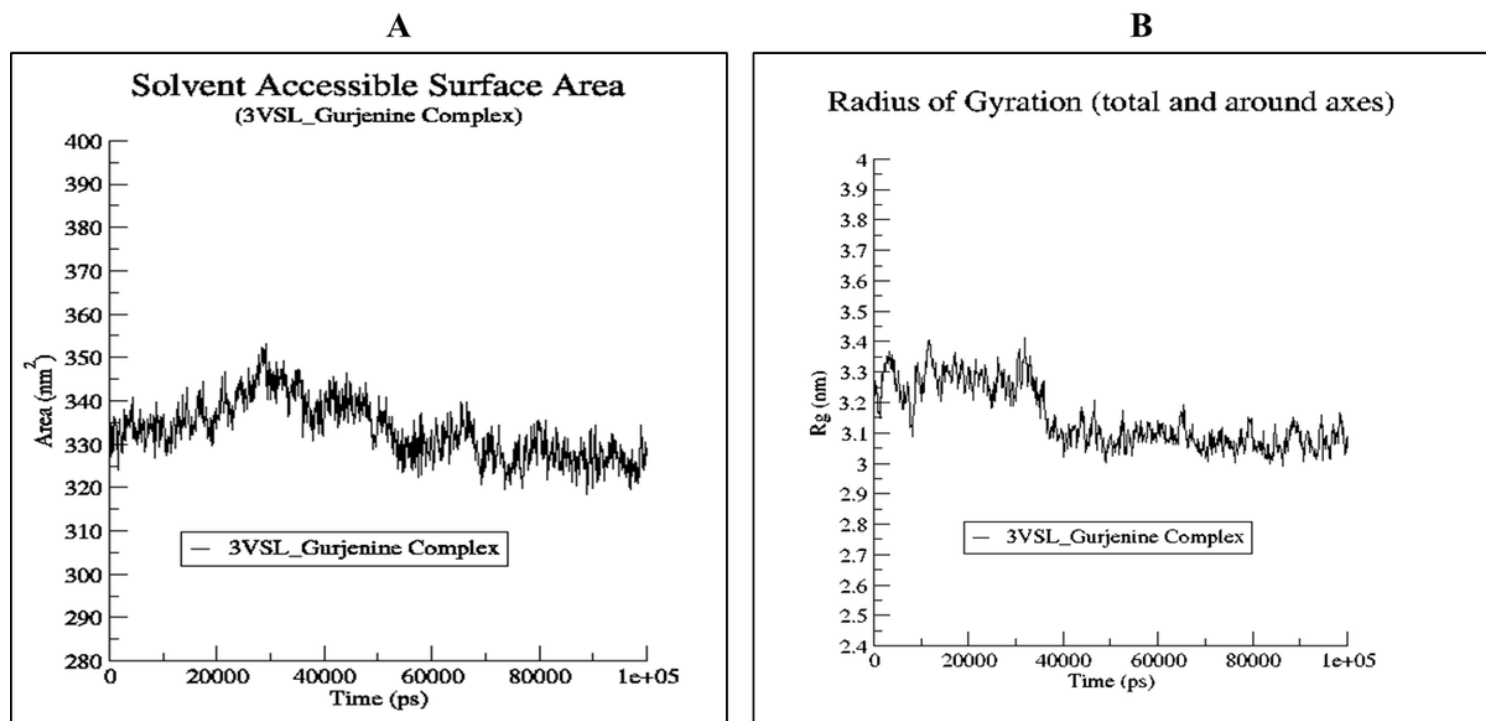


Figure 5

SASA and Radius of gyration gamma-gurjunene in complex with 3VSL:(A) SASA and (B) Radius of gyration

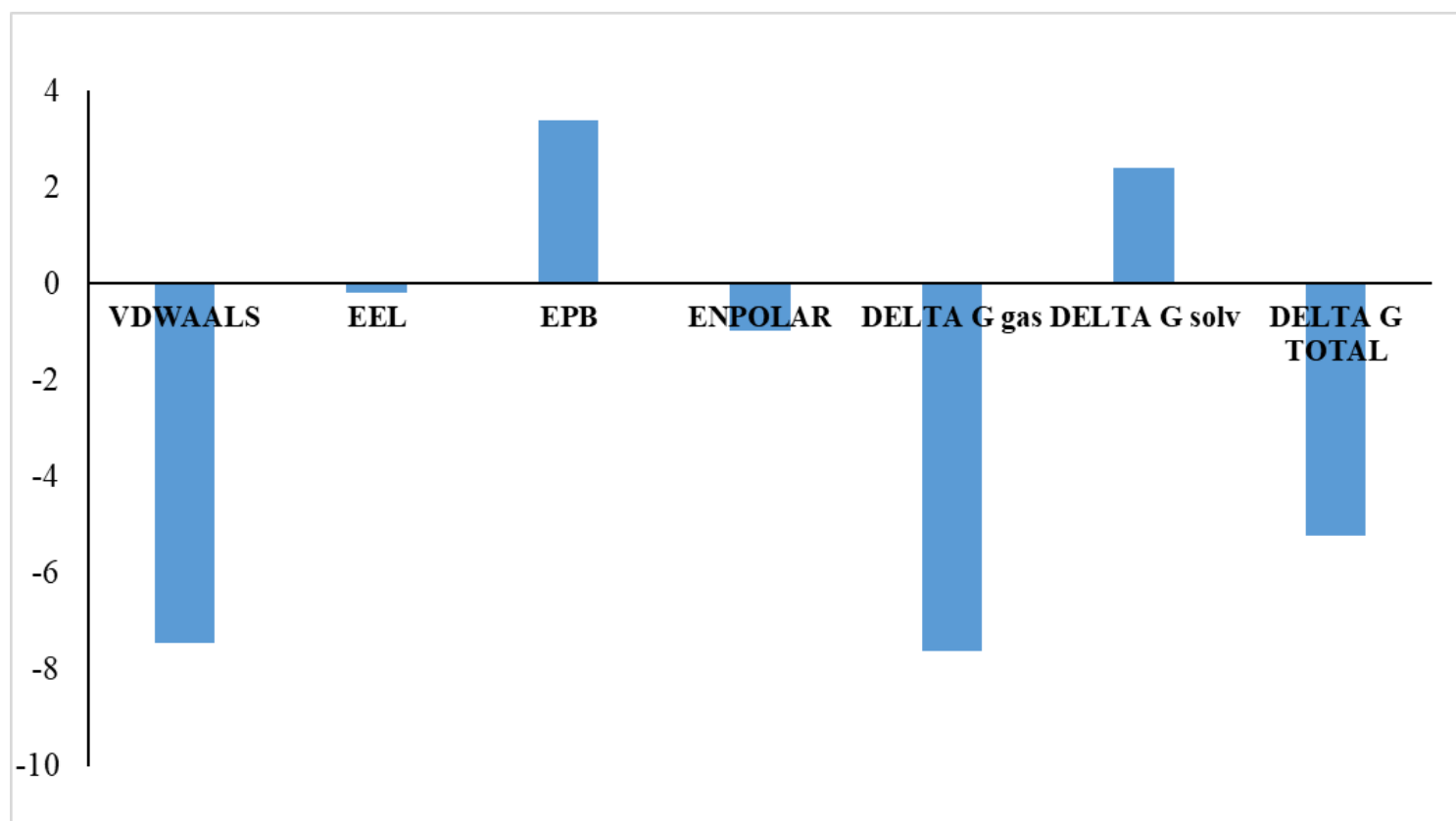


Figure 6

