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GC-MS Analysis and Molecular Docking Studies of Active Phytochemicals from Medicinal Plants against *Malassezia Globosa* LIP1 (SMG1) Enzyme

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Abstract: *Malassezia globosa* LIP1 (SMG1) lipase plays a crucial role in the pathogenicity of *M. globosa* in dandruff sufferers. In this study, GC-MS analysis of selected medicinal plants was done, and antifungal activity of these identified phytochemicals was checked by molecular docking method against *Malassezia globosa* LIP1 (SMG1) lipase using AutoDock 4.0. SwissADME tool was used to analyze the absorption, distribution, metabolism, excretion (ADME) of phytoligands. GC-MS showed various phytoconstituents in the *Glycyrrhiza glabra* extract, including glabridin, 2-propenal, 3-phenyl-eugenol, 4'-O-methylglabridin, hispaglabridin A, stigmast-5-en-3-ol, stigmasta-5,3-dien-7-one, glabrol. *Punica granatum* extract contains hydroxymethylfurfural, stigmast-5-en-3-ol, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1,2,3-benzenetriol, d-glucopyranose, 1,6-anhydro- as the major compounds. Among one hundred thirty screened compounds, twenty-one followed Lipinski's rule of five and were nontoxic in nature. Docking results reveal that among all, β-sitosterol, stigmatsa-5,3-dien-7-one, glabrol and 22,23-dibromostigmast-5-en-3-yl acetate (DBSA) showed the highest binding affinity with SMG1 i.e. -6.29 kcal/mol, -6.50 kcal/mol, -10.12 kcal/mol and -11.04 kcal/mol respectively as compared to standard inhibitor RHC 80267 (-5.83 kcal/mol). From the results, we conclude that the lead compounds may be used as potential anti-dandruff agents. Plant-based anti-dandruff products are eco-friendly and considered a safe alternative due to their less or negligible side effects.

Keywords: anti-dandruff; *Malassezia globosa*; SMG1; lipase;molecular docking; ADME; GC-MS

1. Introduction

Malassezia globosa is considered to be the main causative microbe for dandruff infection [1]. The fungus proliferates through *Malassezia globosa* LIP1 (SMG1), which is a monoacylglycerol (MAG) and diacylglycerol (DAG) lipase [2,3]. The enzyme produces saturated and unsaturated free fatty acids (FFAs) during lipid metabolism. Unsaturated FFA participates in fungal pathogenicity and induces skin inflammation, flaking, and itching [4-6]. Therefore, SMG1 can be a potential target to inhibit fungal growth. Nowadays, various antifungal agents are used for management of dandruff such as zinc pyrithione, ketoconazole, zinc pyrithione, chlorhexidine, miconazole, piroctone olamine, triclosan, selenium sulfide, miconazole, coal tar, azole compounds (fluconazole, voriconazole, itraconazole, posaconazole,

climbazole, pramiconazole), RHC80267and lipase inhibitors etc. [7]. Due to their various side effects, nontoxic target-based herbal antifungal drugs are needed to develop [8,9]. Medicinal plants such as *Cinnamomum zeylanicum*, *Glycyrrhiza glabra*, and *Punica granatum* contain various bioactive compounds that can be used for this purpose. *Cinnamomum zeylanicum* Blume (Cinnamon/Dalchini; family Lauraceae) is a small, tropical evergreen tree of south Asia and native to Sri Lanka. Its bark is extensively used and is amply mentioned in Ayurveda for various remedies [10]. Essential oils obtained from cinnamon bark reported antifungal activity against Malassezia spp [11]. It contains compounds, namely eugenol, cinnamaldehyde, β-caryophyllene, linalool, cinnamyl acetate, eugenyl acetate, (E)-ethyl cinnamate, camphor, linalool, and piperitone [12]. *Glycyrrhiza glabra*, commonly named as "Licorice/Mulethi/Yashtimadhu" in India, belongs to the family Fabaceae. Aqueous extract of *G. glabra* root is a healthy wash for falling and graying hair and is used in shampoo formulations [13,14]. The plant root contains different bioactive compounds, such as glycyrrhizin, glycirrhizic acid, glabra, glabridin, galbrene, glabrone, 4′-o-methylglabridin [15]. Licorice showed various pharmacological activities [16]. *Punica granatum* L. belonging to the family Punicaceae is well-known as pomegranate (Anar). Its fruit peels constitute approximately 60% of the total weight of the pomegranate fruit. Peel is considered an agro-waste, but it can be a potential antimicrobial source [17,18]. The dry content is composed of simple sugars (30–35%), phenolic compounds (10–20%), and polysaccharides (10–15%), while the fresh peel contains a large percentage of water (around 70–75%). The peel contains compounds including gallotannins, gallic acid, ellagic acid, ellagitannins (such as punicalagins and punicalins), Anthocyanins (such as cyanidin and delphinidin glucosides, and numerous gallagyl esters [19-21].

Nowadays, GC-MS analysis has become a key technique for the identification of secondary metabolites in medicinal plants [22]. Plant metabolites display more "drug-likeness and biological activity than fully synthetic compounds", making them superior candidates as a drug [23]. Structure-based drug design can be used to reduce the ambiguity involved and fasten up the process for drug development [24]. Very few molecular docking studies of SMG1 inhibitors of *M. globosa* have been reported. Therefore in this study, virtual screening of selected phytoligands was done to check their binding affinity for SMG1 lipase compared to standard drug RHC 80267.

2. Materials and Methods

2.1. Plant sample.

Plant materials such as rhizome of *Glycyrrhiza glabra*, the bark of *Cinnamomum zeylanicum*, and fruit peel of *Punica granatum* were collected from the local market of Rohtak city of Haryana, India. The samples were further identified with the help of the flora of Haryana and comparing the herbarium specimens available in the Department of Genetics, Maharshi Dayanand University, Rohtak, Haryana, India.

2.2. Preparation of extracts.

Plant samples were shade dried and powdered. 10 g of prepared sample were dissolved in 100 ml solvent (methanol and ethyl acetate) and rotated in incubator shaker for 48 hrs. It was then filtered, lyophilized, and stored for further use.

2.3. GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) with nonpolar column Rxi-5Sil MS (30m×0.25mm Id×0.25µm film thickness) was performed at AIRF JNU, New Delhi. The method of electron-impact ionization was applied with the following GC-MS conditions (Table 1) [25].

Table 1. Showing GC-MS analysis conditions.

GC-MS conditions	
Column Oven Temp. : 50.0 °C	Purge Flow : 3.0 mL/min
Ion Source Temp : 230.00 °C	Flow Control Mode : Linear Velocity
Injection Temp. : 260.00 °C	Linear Velocity : 40.1 cm/sec
Split Ratio : 10.0	Total Flow : 16.4 mL/min
Pressure : 69.8 kPa	Column Flow : 1.22 mL/min

2.4. ADME Analysis.

SwissADME web tool was used for ADME evaluation [26]. Canonical smiles of molecules were retrieved from PubChem, entered in SwissADME, and run to evaluate pharmacokinetics and toxicity of phytochemicals.

2.5. Molecular docking analysis.

The X-ray crystal structure of the SMG1 (PDB Id-3UUE) was retrieved from the Protein Data Bank (www.rcsb.org). SPDBV-Swiss-PdbViewer tool was used to generate model protein for docking. Ligand structures were drawn in Chemsketch, saved as mdl mol file and then converted into pdb by Open Babel 18.0. AutoDock 4 version 1.56rC3 with the Lamarckian genetic algorithm was used, and the parameter was set to 100 GA runs for each docking simulation up to 250,000 energy evaluations [27-29]. Bond orders were assigned, hydrogen atoms were added, and the water molecules that did not interact were removed. The coordinate axes of active protein whereas follow X: -3.594, Y: 6.002, and Z: 2.234. 10 runs were performed at each site, and the poses were clustered by heavy atom root-mean-square deviation (RMSD), and the value of the docking score was calculated.

2.6. Visualization.

The best docking complexes were examined by UCSF Chimera. The tool provided the visualization of the interaction between ligands and amino acids of the targeted protein.

2.7. Reference strains and chemicals.

Pathogenic reference viz. *Malassezia furfur* MTCC 1374 was used for the study. Strains were procured from IMTECH, Chandigarh (India). Glabrol, DBSA, stigmatsa-5,3-dien,7-one, β-sitosterol, and RHC-80267were purchased from Sigma (St. Louis, USA).

2.8. Antifungal activity.

Antifungal activity of the lead compound was studied by the agar well diffusion method against *Malassezia furfur* [22]. RHC-80267 was used as a positive control.

2.9. Minimum inhibitory concentration.

MIC of study samples was calculated using the micro broth dilution method with slight modifications [22]. 2 mg resazurin dye was prepared in 10 mL of distilled water. Living microbes reduced the dye, and a color change from purple to pink or colorless was observed. In the absence of living microbes, indicator color remained the same. The lowest concentration at which color change occurred was taken as MIC.

3. Results and Discussion

3.1. GC-MS analysis.

Results revealed that a total of 78 compounds were identified in the *G. glabra* ethyl acetate extract as depicted in Figure 1 & Table 2. Major phytoconstituents included glabridin with peak area (16.38%), 3-phenyl-2-propenal (8.07%), eugenol (4.64%), 4'-O-methylglabridin (4.1%), hispaglabridin a (2.67%), stigmast-5-en-3-ol (2.49%), stigmasta-3,5-dien-7-one (2.39%), glabrol (2.07%), cis-dimethyl morpholine (2.54%), guanosine (2.42%).

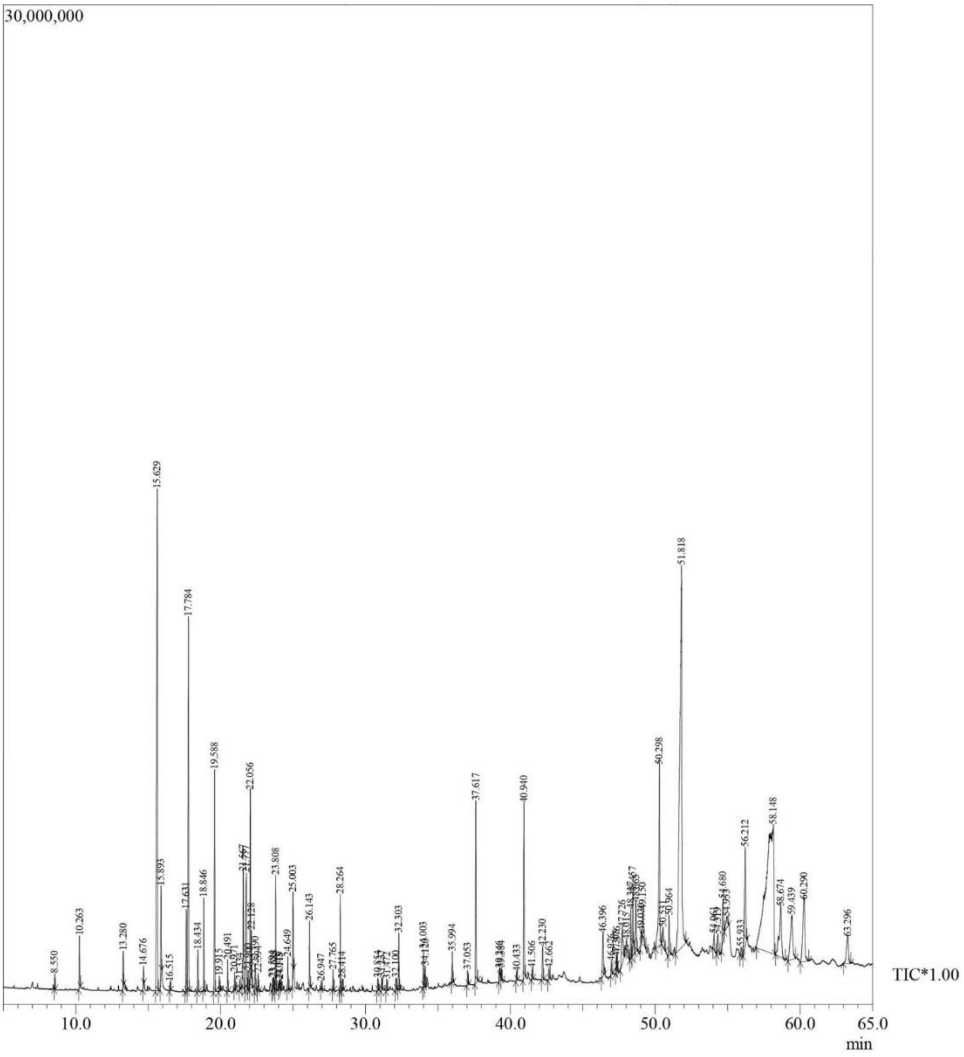

Figure 1. GC-MS spectra of ethyl acetate extract of *G. glabra* root.

Table 2. GC-MS analysis of *G. glabra* root extract.

Peak	R.Time	% Area	Compounds Name
1	8.550	0.14	Eucalyptol (1,8-cineole)
2	10.263	0.58	1,2,3-Propanetriol, 1-acetate
3	13.280	0.32	1-Dodecene

Peak	R.Time	% Area	Compounds Name
4	14.676	0.20	1,2,3-Propanetriol, diacetate
5	15.629	8.07	2-Propenal, 3-phenyl-
6	15.893	1.25	Resorcinol
7	16.515	0.10	2-Propen-1-ol, 3-phenyl-
8	17.631	0.92	α-Terpinyl acetate
9	17.784	4.64	Eugenol
10	18.434	0.42	Copaene
11	18.846	0.90	1-Tetradecene
12	19.588	2.32	β-Caryophyllene
13	19.915	0.17	2H-1-Benzopyran-2-one
14	20.491	0.34	(E,E,E)- 2,6,6,9-tetramethyl-1,4,8-Cycloundecatriene
15	20.971	0.17	γ-Muurolene
16	21.334	0.09	Decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)- naphthalene
17	21.567	1.34	α-Muurolene
18	21.777	2.22	1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene
19	21.900	0.21	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
20	22.056	1.92	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-Cis)-
21	22.128	0.47	Cis-calamenene
22	22.390	0.35	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
23	22.594	0.21	α-Calacorene
24	23.594	0.12	Caryophyllene oxide
25	23.688	0.13	Gleenol
26	23.808	1.14	1-Heptadecene
27	24.043	0.07	1-(2,4,5-triethylphenyl)- Ethanone
28	24.119	0.06	1,5,5,8-Tetramethyl-3,7-cycloundecadien-1-ol
29	24.649	0.37	Di-epi-1,10-cubenol
30	25.003	0.91	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one
31	26.143	0.73	γ- 4-Trimethyl-imidazole-5-butyric acid
32	26.947	0.08	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol
33	27.765	0.23	6-Tert-butyl-4-methylcoumarin
34	28.264	0.97	1-Heptadecene
35	28.414	0.15	Pentadecane
36	30.854	0.14	P-Octylacetophenone
37	31.137	0.13	Isolongifolen-5-one
38	31.472	0.14	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
39	32.100	0.14	Trans-sinapyl alcohol
40	32.303	0.60	1-Nonadecene
41	34.003	0.43	n-Nonadecanol-1
42	34.120	0.21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
43	35.994	0.32	n-Tetracosanol-1
44	37.053	0.17	9,12-Octadecadien-1-ol, (Z,Z)-
45	37.617	2.21	Behenic alcohol
46	39.246	0.10	5-Hydroxy-2-phenyl-4-chromanone
47	39.384	0.10	Behenic alcohol
48	40.433	0.11	9,12-Octadecadien-1-ol, (Z,Z)-
49	40.940	2.08	Behenic alcohol
50	41.506	0.13	1,2-Benzenedicarboxylic acid
51	42.230	0.46	6h-Benzofuro[3,2-C][1] benzopyran-3-ol, 6a,11a-dihydro-9
52	42.662	0.22	Oxalic acid, pentadecyl propyl ester
53	46.396	0.80	3,4-Dihydro-3-(2-hydroxy-4-methoxyphenyl)-2h-1-benzopyran-7-ol
54	46.976	0.21	Thiazolo[3,2-a]benzimidazol-3(2H)-one, 2-(4-tertbutylbenzylideno)-
55	47.308	0.13	Squalene
56	47.376	0.13	2H,6H-Benzofuro[3,2-c]pyrano[2,3-h][1]benzopyran, 6a,11a-
57	47.726	1.05	7-Hydroxy-3-(4-methoxyphenyl)-4H-1-benzopyran-4-one,
58	48.015	0.28	3-Hydroxy-4,4-dimethyl-7-oxoandrost-5-en-17
59	48.317	0.59	Di(pentamethylphenyl)ketone
60	48.457	0.66	Decamethylbenzophenone
61	48.663	0.76	17-Methoxy-4-methyl-d-homo-18-norandrosta-4,8,13,15,17
62	49.030	0.16	Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether
63	49.150	0.59	9-(4-Ethoxy-phenyl)-3,4,5,6,7,9-hexahydro-2h-xanthene-1,8-64
64	50.298	4.11	4'-O-methylglabridin
65	50.531	0.52	1H-Xanthen-1-one, 2,3,4,9-tetrahydro-9-(2-hydroxy
66	50.964	0.66	β-Stigmasta-5,22-dien-3-ol, acetate
67	51.818	16.38	Glabridin
68	54.061	0.32	β-Ergost-5-en-3-ol
69	54.319	0.42	Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether

Peak	R.Time	% Area	Compounds Name
70	54.680	0.90	Stigmasterol
71	54.953	0.47	Hispaglabridin B
72	55.933	0.18	Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether
73	56.212	2.49	β-Stigmast-5-en-3-ol
74	58.148	19.96	No name was assigned to the structure
75	58.674	2.39	Stigmasta-3,5-dien-7-one
76	59.439	2.07	Glabrol
77	60.290	2.67	Hispaglabridin A
78	63.296	1.11	N-(3,5-Dichloro-2-hydroxy-4-methylphenyl)-2- butanamide,

In the methanol extract of *P. granatum*, 37 bioactives molecules were identified with retention time and % area as shown in Figure 2 & Table 3. The main compounds were hydroxymethylfurfural (60.17%), stigmast-5-en-3-ol (6.65%), 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.11%), 1,2,3-benzenetriol (3.53%), d-glucopyranose, 1,6-anhydro- (3.51%).

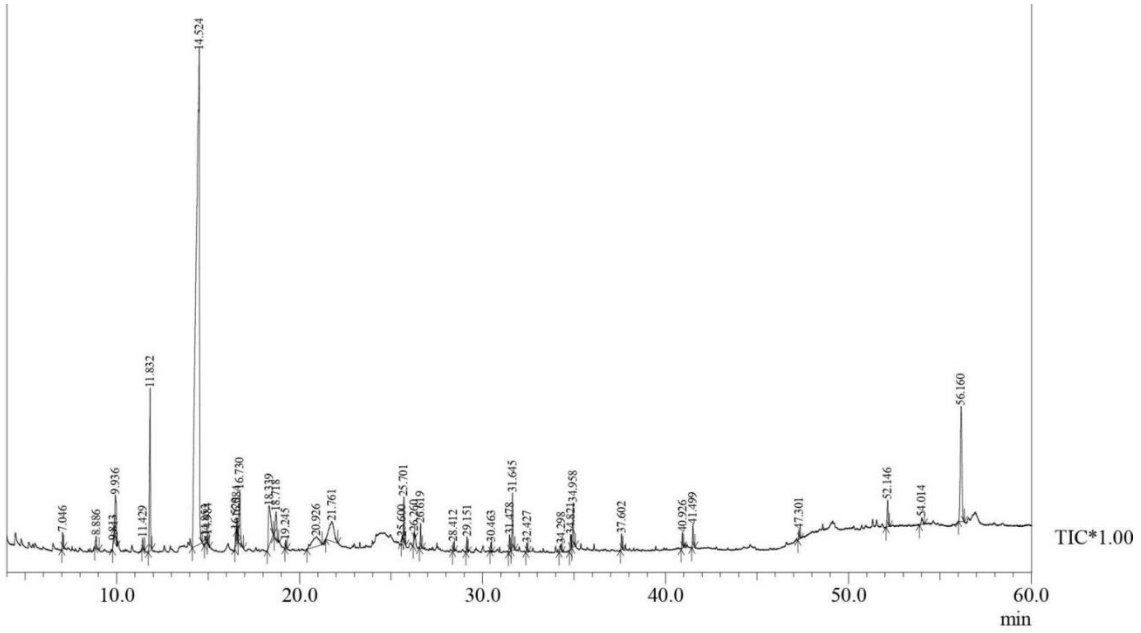

Figure 2. GC-MS spectra of methanol extract of *P. granatum* root.

Table 3. GCMS analysis of *P. granatum* peel extract.

Peak	R. Time	%Area	Compounds Name
1	7.046	0.41	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2	8.886	0.40	2-Nonene, (E)-
3	9.813	0.04	2,5-Furandicarboxaldehyde
4	9.936	1.75	Exo-norbornane-2-thiolacetate
5	11.429	0.30	2-Ethyl-5,5-dimethyl-1,3,2-dioxaborolan-4-one
6	11.832	5.11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
7	14.524	60.17	5-Hydroxymethylfurfural
8	14.853	0.24	1,2,3-Propanetriol, 1-acetate
9	14.984	0.19	2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-
10	16.520	0.20	Hydrazine, 1,1-diethyl-2-(1-methylpropyl)-
11	16.584	0.30	3-Hydroxy-2-methyl-4H-Pyran-4-one,
12	16.730	2.54	CIS-Dimethyl morpholine
13	18.339	3.53	1,2,3-Benzenetriol
14	18.718	1.51	Succinic acid, 3-hex-4-ynyl 3-methylbutyl ester
15	19.245	0.21	1,5-Pentandiol, diacetat
16	20.926	2.42	Guanosine
17	21.761	3.51	β -D-Glucopyranose, 1,6-anhydro-
18	25.600	0.11	Dodecyl isobutyl ether
19	25.701	1.03	α –Santalol
20	26.260	0.22	Heptadecane
21	26.619	0.61	2-Penten-1-ol, 2-methyl-5-(2-methyl-3-methylene-2-norbornyl)-
22	28.412	0.19	Hexadecane
23	29.151	0.36	Neophytadiene

Peak	R. Time	%Area	Compounds Name
24	30.463	0.23	Heneicosane
25	31.478	0.46	Dibutyl phthalate
26	31.645	1.76	n-Hexadecanoic acid
27	32.427	0.22	Heneicosane
28	34.298	0.32	Heneicosane
29	34.821	0.42	9,12-Octadecadienoic acid (Z,Z)-
30	34.958	1.43	Oleic Acid
31	37.602	0.42	n-Nonadecanol-1
32	40.926	0.42	n-Tetracosanol-1
33	41.499	0.69	1,2-Benzenedicarboxylic acid
34	47.301	0.26	Squalene
35	52.146	1.00	Vitamin E
36	54.014	0.34	β -Ergost-5-en-3-ol
37	56.160	6.65	β -Stigmast-5-en-3-ol

In our previous article, in *C. zeylanicum* extract, a variety of phytochemicals were reported such as cinnamaldehyde (75.58%) and various sesquiterpenes viz. β-caryophyllene, α-copaene, α-muurolene, α-humulene, β-bisabolene, cubenol, calamenene, α-cadinene, muurolol, cadine-1,4-diene [30].

The principal compound cinnamaldehyde showed antifungal activity against *Malassezia pachydermatis* [31]. Earlier studies suggested that eugenol has a role in treating skin infections and inhibiting biofilm formation in *Malassezia* spp [32,33]. β-Caryophyllene possesses antimicrobial, antifungal, antioxidant, anesthetic, and anti-inflammatory as well as exhibiting anticancerous activity [34,35]. Squalene protects human skin from lipid peroxidation caused by ultraviolet rays [36]. Metabolites, namely 4'-o-methylglabridin, glabridin, squalene, β-sitosterol, and glabrol inhibited *S. epidermidis* growth [27]. Quercetin has many health-promoting effects, including anti-inflammatory and anti-allergic effects [37,38]. The flavonoid quercetin and kaempferol reported anti-dandruff activity by inhibiting β-carbonic anhydrase of *Malassezia globosa* [39]. Essential oil components, namely α-copaene, calamenene, α-muurolene, β-bisabolene, muurolol, cubenol, humulene have shown antifungal activity [40-43]. Syringenin showed antioxidant activity [44].

3.2. ADME analysis.

Among all 21 compounds were nontoxic in nature, and they followed Lipinski's rule of five (Table 4).

Table 4. ADME analysis of phytoligands.

Compounds	MW	H-bond acceptors	H-bond donors	Log $P_{o/w}$	Violation
Cinnamaldehyde	132.16	1	0	1.65	0
Eugenol	164.2	2	1	2.37	0
Trans, β-caryophyllene	204.35	0	0	3.29	1
Squalene	410.72	0	0	6.37	1
4 '-O- Methylglabridin	338.4	4	1	3.42	0
Glabridin	324.37	4	2	2.97	0
β-Sitosterol	414.71	1	1	4.79	1
DBSA	456.74	2	0	5.19	1
Stigmatsa-5,3-dien,7-one	410.67	1	0	4.71	1
Glabrol	392.49	4	2	3.87	0
Quecertin	302.24	7	5	1.63	0
Kaempferol	286.24	6	4	1.7	0
α –Copaene	204.35	0	0	3.4	1
α -Muurolene	204.35	0	0	3.38	1
β-Bisabolene	204.35	0	0	3.67	1
Cubenol	222.37	1	1	3.24	0
Cyclohex3en1ylmethanol	112.17	1	1	1.84	0

Compounds	MW	H-bond acceptors	H-bond donors	Log $P_{o/w}$	Violation
Humulene	204.35	0	0	3.27	1
Calamenene	202.34	0	0	3.19	1
T-Muurolol	222.37	1	1	3.15	0
Syringenin	372.37	9	5	0.88	0

The molecule should not have more than 10 H-bond acceptors, not have more than 5 H-bond donors, a molecular weight (MW) less than 500 daltons, and an octanol-water partition coefficient, i.e., log P not more than 5 [45,46]. Therefore, these molecules were selected to screen out active anti-dandruff agents against lipase enzyme using *in silico* method.

3.3. Molecular docking analysis.

In the present study, active residues Ser171-Asp228-His281 is the catalytic triad of SMG1 lipase was chosen as target [47]. Structures of lead compounds and standard drug RHC80267 are depicted in Figure 3.

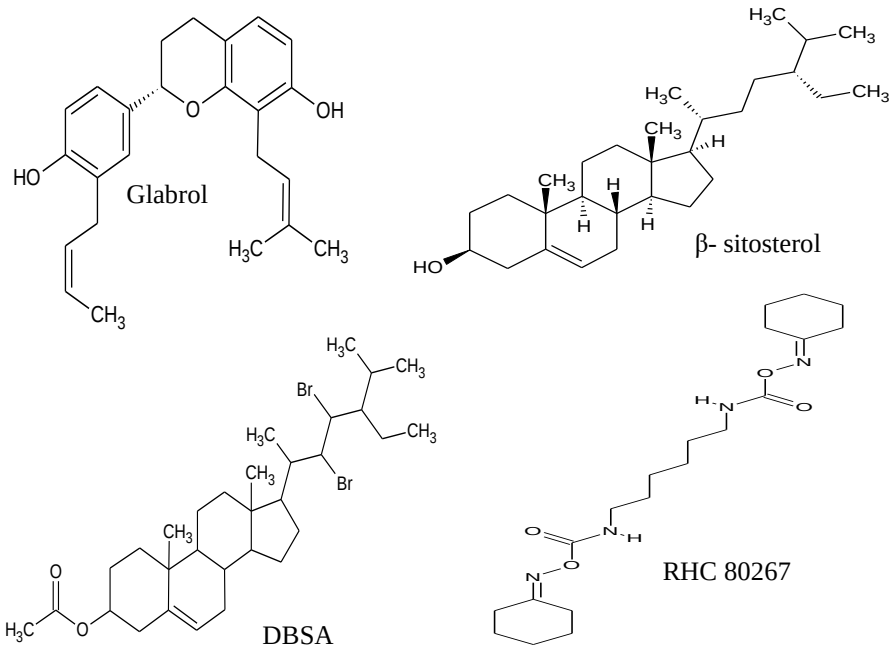

Figure 3. Structure of lead compound and RHC 80267.

Among all, 22,23-dibromostigmast-5-en-3-yl acetate (DBSA) showed the highest binding affinity followed by glabrol, stigmastsa-5,3-dien,7-one, β-sitosterol with minimum binding energy (MBE) -11.04, -10.12, 6.50, -6.29 kcal/mol, respectively as shown in Table 5. Reference drug RHC-80267 showed a lower binding affinity with MBE -5.83kcal/mol as compared to lead compounds. Interaction between the enzyme and phytoligands are shown in Table 6 and Figure 4 & 5. The protein model (LigPlot+) depicts atomic interactions, such as hydrogen bonds and amino acid interactions, between ligands and atoms in the active site pocket, as shown in Figure 6.

Table 5. Minimum binding energy of selected phytochemicals against SMG1 lipase.

S. No.	Compounds Name	MBE against SMG1 (kcal/mol)
1	Cinnamaldehyde	-4.96
2	Eugenol	-4.98
3	Trans, β-caryophyllene	-4.37

S. No.	Compounds Name	MBE against SMG1 (kcal/mol)
4	Squalene	-5.19
5	4 '-O- Methylglabridin	-4.88
6	Glabridin	-5.13
7	β-Sitosterol	-6.29
8	DBSA	-11.04
9	Stigmatsa -5,3-dien,7-one	-6.50
10	Glabrol	-10.12
11	Quecertin	-5.25
12	Kaempferol	-5.82
13	α-Copaene	-4.42
14	α-Muurolene	-4.65
15	β-Bisabolene	-2.00
16	Cubenol	-4.65
17	Cyclohex3en1ylmethanol	-4.67
18	Humulene	-4.45
19	Calamenene	-5.75
20	T-Muurolol	-4.85
21	Syringenin	-3.52
22	RHC 80267	-5.83

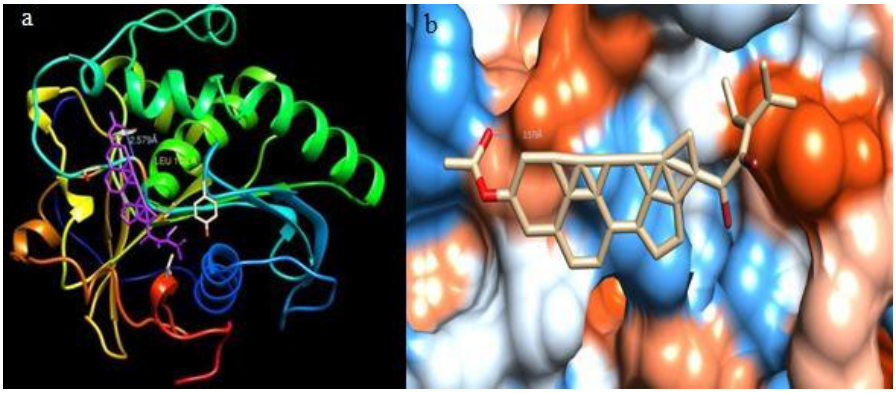

Figure 4. Binding mode of DBSA against SMG1 **(a)** solid line structure; **(b)** surface binding mode.

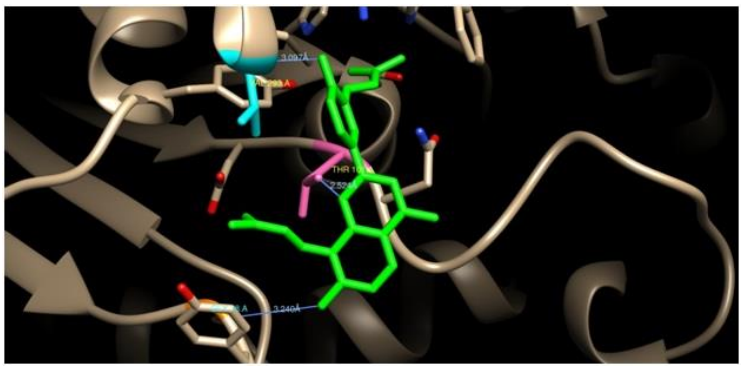

Figure 5. Binding mode of glabrol against SMG1.

Table 6. Ligands protein interaction for lead compounds.

Compounds name	MBE	No. of H-bond	Amino acid involved in H-bond	Amino acid involve in hydrophobic interaction
Glabrol	-10.12	3	Thr101, Gln282, Val 293	Tyr56, His170, Phe278, Ser171, Gly100, His281, Asn102, Ala292, Glu99, Tyr79, Gly78
DBSA	-11.04	-	-	Gly78, Thr101, Asn102, Leu103, phe104, Ser105, Lys114, Val293
Stigmastsa-5,3-dien,7-one	-6.50	-	-	Glu77, Gly78, Tyr79, Glu99, Thr101, Asn102, Leu103, Ser105 and Val293
β-Sitosterol	-6.29	-	-	Glu77, Gly78, Tyr79, Glu99, Thr101, Asn102, Leu103, Ser105 and Val293
RHC 80267	-5.83	2	Thr101, Leu103	Glu77, Gly78, Tyr79, Ala80, Glu99,, Asn102, Phe104, Lys114, Gly137, Ala141 and Val293

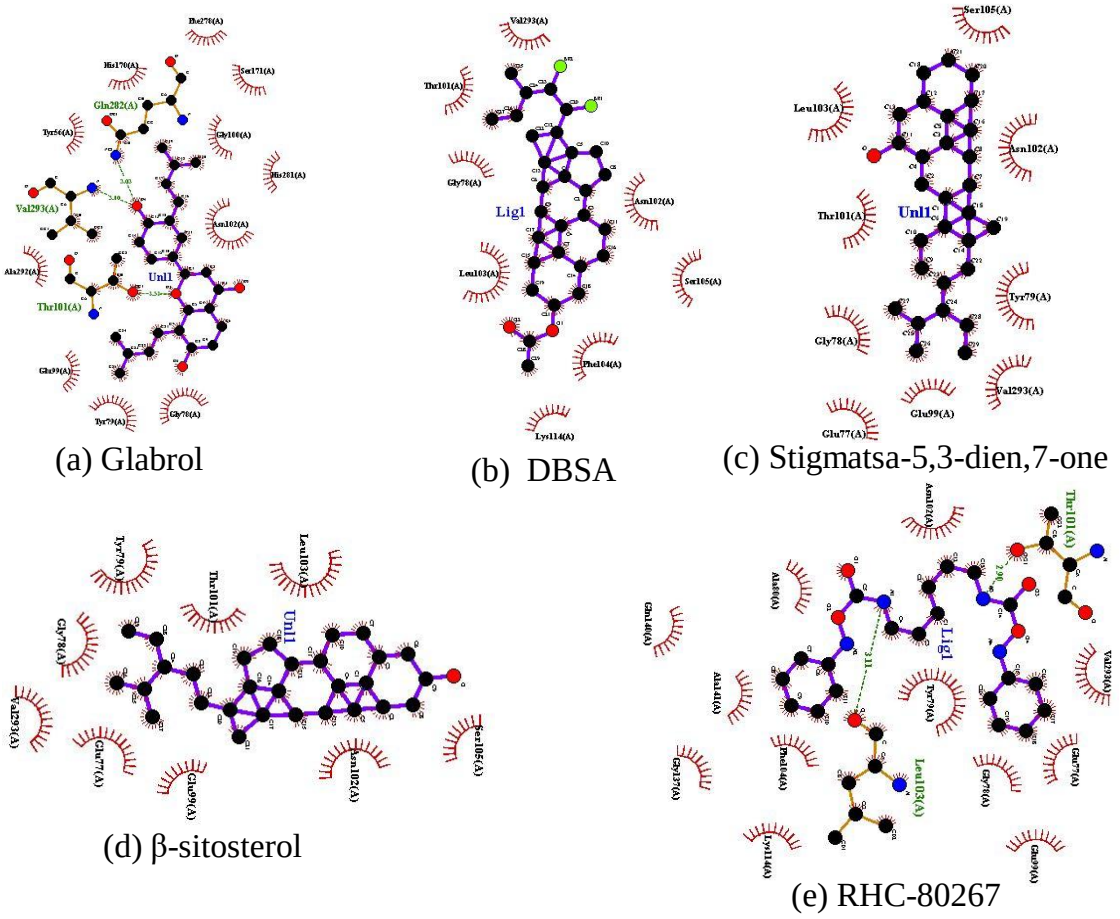

Figure 6. LigPlot+ to show molecular interaction between lead compounds **(a-d)** with the amino acid residue of SMG1 protein (3uue). Green dashed lines show H-bonds between ligand and amino acid residue. The spoked arcs represent residues making non-bonded contacts with the ligand. RHC-80267 **(e)** is a reference drug in this study.

Ligand plot (Figure 6) study clearly shows that active site of 3UUE protein formed 3 hydrogens (H) bonds with glabrol, involving Thr101, Gln282, Val293 with bond length 2.52Å, 3.03Å, and 3.10Å, respectively, whereas Tyr56, His170, Phe278, Ser171, Gly100, His281, Asn102, Ala292, Glu99, Tyr79, Gly78 participate in hydrophobic interaction; in case DBSA no any H-bond formed and Gly78, Thr101, Asn102, Leu103, phe104, Ser105, Lys114, Val293 are involved hydrophobic interaction with protein 3UUE. Stigmastsa-5,3-dien,7- one formed no H-bond with protein while Glu77, Gly78, Tyr79, Glu99, Thr101, Asn102, Leu103, Ser105, and Val293 formed hydrophobic interaction. β-Sitosterol formed hydrophobic interaction with Glu77, Gly78, Tyr79, Glu99, Thr101, Asn102, Leu103, Ser105, and Val293 with protein. RHC-80267 standard formed 2 H-bond with Thr101, Leu103, and hydrophobic interaction with Glu77, Gly78, Tyr79, Ala80, Glu99,, Asn102, Phe104, Lys114, Gly137, Ala141, and Val293, respectively.

2-(1-Oxo-1,3-dihydro-2H-isoindol-2-yl)ethyl 1-azepanecarbodithioate reported potential inhibitory activity against *M. globosa* LIP1 (SMG1) [47]. Sapondoside-A, kampferol, SNTI inhibit the phospholipase A2 isoform of humans from overcoming dandruff [48]. Compounds such as ZINC85530919, ZINC95914464 reported inhibitory activity for *M. restricta* lipase (Mrlip1) [49]. β-sitosterol and calceolarioside A inhibits Mala s1 of Malassezia [50]. β-Sitosterol does not inhibit cytochromes P450 (CYP) enzyme and is also unable to cross the blood-brain barrier (BBB). DBSA exhibited low gastrointestinal absorption, low membrane permeability but did not inhibit CYP. Stigmastsa-5,3-dien,7-one and β-sitosterol showed

moderate activity. Glabrol demonstrated high gastrointestinal absorption, high membrane permeability, although it showed inhibitory activity against CYP2C19 and CYP3A4.

3.4. Antimicrobial activity.

The antifungal potential of the lead compound such as glabrol, DBSA, stigmastsa-5,3-dien,7-one, β-sitosterol, and standard RHC-80267 was determined against *Malassezia furfur* using the agar well diffusion method. The zone of inhibition in the range of 12-20mm. The glabrol showed a maximum zone of inhibition, i.e., 20mm, followed by DBSA, β-sitosterol, stigmastsa-5,3-dien,7- one, and standard RHC-80267, i.e., 18mm, 16mm, 16mm, and 15mm respectively.

Minimum inhibitory concentration (MIC) was done by microbroth dilution method. glabrol (70µg/mL) showed lower MIC as compared to DBSA (150µg/mL), stigmastsa-5,3-dien,7- one(160µg/mL), β-sitosterol (160µg/mL), and standard RHC-80267 (100µg/mL) aginst *Malassezia furfur*.

4. Conclusions

The above results provide an insight of phytoligands from *C. zeylanicum, P. granatum,* and *G. glabra* to have an inhibitory effect on Malassezia lipase. SwisADME tool showed that these compounds possess all necessary properties of an ideal drug and follow Lipinski's rule. Therefore, the bioactive compounds including DBSA, glabrol, stigmastsa-5,3-dien,7-one, and β-sitosterol may be developed as potential anti-dandruff agents.

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Conflicts of Interest

The authors declare no conflict of interest.

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