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RESEARCH ARTICLE

GC-MS analysis of the ethanol extract of *Hyophila involuta* (Hook.) A. Jaeger and in silico docking studies of the detected bioactive terpenoid, Squalene against target proteins of *Staphylococcus aureus*

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Abstract

Bryophytes, which include mosses, hornworts and liverworts, are early land plants that lack vascular tissues. Due to their small size and difficulties in identification, they have been neglected as a study material for a significant period of time. However, recent phytochemical studies focusing on mosses have revealed the presence of unique and biologically active substances. The objective of this research was to examine the volatile bioactive phytochemicals present in the ethanol extract of *Hyophila involuta* (Hook.) A. Jaeger using GC-MS and docking of a potential compound against the target proteins of *Staphylococcus aureus*. Through GC-MS analysis, a total of thirty-seven phytochemicals were identified, including beneficial substances like Neophytadiene, Squalene, Phytol, Lupan-3-ol acetate, Campesterol, Stigmasterol, Gamma-sitosterol, dl-alpha-tocopherol and more. Upon undergoing ADMET analysis with SwissADME, Squalene, a triterpenoid, has been identified as an orally active compound that adheres to the Lipinski rule and displays drug-like characteristics, with biological activities suited best as a ligand molecule. The docking results of Squalene against the target proteins of *S. aureus* revealed the highest docking score when interacting with the active site of Undecaprenyl diphosphate synthase (4H8E), followed by Dihydrofolate reductase (3FYV), Sortase A (1T2P) and Dehydrosqualene synthase (2ZCO) with binding energies (Kcal/mole) of -9.99, -9.04, -7.68 and -7.35 respectively. Integrating high-throughput screening methods with the docking results can expedite the validation of potential antibacterial compounds, enabling researchers to identify novel treatments more efficiently.

Keywords: Bioactivity, Terpenoids, Sortase A, Dehydrosqualene synthase, Dihydrofolate reductase, Undecaprenyl diphosphate synthase

Introduction

Bryophytes are the simplest land plants belonging to the second largest taxonomic group in the plant kingdom. They have been disregarded as a source of biologically active ingredients for a long time. Identification of significant number of bioactive substances from mosses and liverworts resulted in considerable attention to its chemical components (Asakawa et al., 2013). Bryophytes exhibits a stunning diversity of bioactive compounds such as proteins, lipids, organic acids, steroids, polyphenols, alcohols, terpenoids, aliphatic and aromatic compounds (Commiso et al., 2021), with terpenoids representing the largest group of secondary metabolites from bryophytes (Dixon, 2001; Chen et al., 2018).

The class Bryopsida (mosses) represents the largest group of bryophytes, comprises of about 14,000 species (Rachna and Vashishtha, 2015). *Hyophila involuta* under Pottiaceae family represents a light tolerant and drought resistant moss. Due to the presence of secondary metabolites like terpenoids and flavonoids, majority of the Pottiaceae members are able to thrive over strong environmental constraints like extreme temperatures and anthropogenic activities (Kalathil et al., 2022). In India, *H. involuta* is used as a traditional herbal remedy to treat cuts and burns (Motti

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et al., 2023). Its qualitative phytochemical analysis revealed the presence of amino acids, carbohydrates, fats, terpenoids, alkaloids, flavonoids, cardiac glycosides, anthraquinone, tannins, proteins, steroids and saponins (Makinde et al., 2015).

According to the World Health Organization (WHO), a significant percentage of the global population, ranging from 60 to 80%, relies on plants as their primary source of medicine. Bryophytes, the oldest land plants, have gained recognition in Europe and Asia for their therapeutic properties (Makinde et al., 2015). In particular, the *Hortus Malabaricus* provides detailed depictions and descriptions of the traditional usage of Indian bryophytes (Greeshma et al., 2016). These plants have been traditionally utilized by Chinese, Europeans, North Americans and Indians to address various health conditions such as cardiovascular issues, tonsillitis, bronchitis, skin diseases and burns (Khanam et al., 2011).

Terpenoids represent one of the largest category of secondary metabolites in bryophytes. They exhibit various pharmacological properties like antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory, hypoglycemic and anticancer activity (Adedokun et al., 2023). Squalene (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosahexane), a triterpenoid, is an intermediate in the biosynthesis of cholesterol and all steroid hormones (*Alvaro and Cardin,* 2013). It is reported to have anticancer, cardioprotective, antioxidant, antimicrobial and detoxifying properties (Azalia et al., 2018). The supplementation of Squalene in the diet of animals shows reduction in cholesterol and triglyceride levels (Kelly, 1999). Recent studies suggested its effective chemotherapeutic property against breast cancer, colon cancer, pancreatic cancer and other similar tumors (Sumi et al., 2018; Fatma, 2013).

Staphylococcus aureus (Gram positive), is a major pathogen commonly causing nosocomial infections, mainly postoperative wound infections (Kahsay et al., 2014). Antibiotic resistance of *S. aureus* to conventional antibiotics, makes it difficult to treat the infections. In order to find out potential phytocompound with novel antibacterial activity, plants have been explored. The main drivers behind the continuous use of herbal treatments are their benefits, accessibility, affordability and little toxicity. Investigating the therapeutic impact is relevant that numerous mosses have been shown to contain a variety of phytochemicals with pharmacological action (Kavitha, 2021). To identify the bioactive compounds in an extract, analytical techniques such as Gas Chromatography Mass Spectroscopy (GC-MS) can be performed (Gomathi et al., 2015; Dabhi et al., 2023; Chauhan et al., 2014).

Detecting the competent therapeutic phytocompounds using in silico methods facilitate the process of drug development by using less time and resources. The two main factors contributing to drug failure are the effectiveness and safety of the drug. Finding powerful ligand compounds with better ADMET- "drug-likeliness", allows the assessment of toxicity as well as its effectiveness before their production (Daoui et al., 2021). Molecular docking can be used to establish the interaction between a protein and ligand, and the binding affinity score can be used to validate the association (Shuvo et al., 2024).

The current research examined the percentage yield of ethanol extract of *H. involuta*, then proceeded to analyze the phytochemical constituents present in the extract using GC-MS and molecular docking of a drug like terpenoid compound- Squalene to find out the best docking score against the target proteins of *S. aureus,*.

Materials and Methods

Collection and identification of Hyophila involuta

The gametophytic plant body along with the sporophyte were collected during the Northeast and Southwest monsoon season from Pathanamthitta district of Kerala. The identification process relied on the book, MOSSES OF EASTERN INDIA AND ADJACENT REGIONS by H.C. Gangulee, focusing on morphological, anatomical and reproductive traits.

Preparation of extract

Moss samples were collected and carefully separated from any unwanted materials, washed in tap water and then rinsed with sterile distilled water. After being air dried in the shade for five days, the dried plant material was pulverized using an electric blender to produce finely powdered sample. 20 g of the powdered sample was measured, packed in a fabric bag, sealed and placed inside an extraction tube. The extraction process was carried out using a Soxhlet extractor with 96% ethanol as the solvent, maintained at 80°C for 16 hours. The resulting extract was filtered through Whatman No. 1 filter paper and stored at 4°C (Laura and Gunta, 2015).

Yield of the extract

The ethanol extract was concentrated by evaporating the solvent to obtain the dried extract. The percentage yield was determined using the provided equation.

$$\% \text{ Yield of extract (g)} = (W1 \times 100)/W2$$

where, W1 represents the weight of the remaining extract after removing the solvent and W2 indicates the weight of the powdered sample taken for extraction (Adam et al., 2019).

GC-MS analysis

After successful extraction, the ethanolic extract of *H. involuta* was subjected to GC-MS. The analysis was performed on GC-MS equipment (Shimadzu Nexis GC-2030). Experimental parameters of GC-MS system were,

column oven temperature: 70°C; injection temperature: 260°C; injection mode: split; flow control mode: linear velocity; pressure: 62.1 kPa; total flow: 14.1 ml/min; column flow: 1.01 ml/min; linear velocity: 36.8 cm/s; purge flow: 3.0 ml/min; split ratio: 10.0. Oven temperature program was 70°C hold time for 2 min, at 200°C hold time for 5 min and 280°C hold time for 15 min. The GC program has ion-source temperature at 210°C, interface temperature at 280°C, solvent cut time: 6.50 min, detector gain: 0.70 kV+0.00 kV and threshold of 0. The chemical components in the ethanol extract was identified by comparing the retention times of chromatographic peaks using NIST 20 Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Selection and preparation of ligand

Squalene ($C_{30}H_{50}$) is used as ligand. The structure of ligand was downloaded from PubChem in SDF format (PubChem CID: 638072). Using the software Open babel, the ligand in SDF format is converted to PDB, which makes it suitable for docking analysis.

Selection of target proteins

The 3D X-ray crystal structures of *Staphylococcus aureus* target proteins such as Sortase A (PDB ID: 1T2P), Dehydrosqualene synthase (PDB ID: 2ZCO), Dihydrofolate reductase (PDB ID: 3FYV) and Undecaprenyl diphosphate synthase (PDB ID: 4H8E) were availed from RCSB protein data bank in PDB format.

Binding site identifiaction

The amino acid residues in the binding pocket of the target proteins were predicted using the online software CASTp.

In silico ADME analysis

The ADME properties of Squalene were determined using the SwissADME online server (http://www.swissadme.ch). The initial step involved giving the canonical SMILE notation of Squalene [CC(=CCC/C(=C/CC/C(=C/CC/C=C(/CC/C=C(/CCC=C(C)C)\C)\C)/C)/C)C] into the server as input, which resulted in the calculation of ADME parameters. The data sheet contains information on bioavailability radar, physiochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikeness, and medicinal chemistry. Additionally, the biological targets of Squalene were predicted using the Swiss Target Prediction tool (http://www.swisstargetprediction.ch).

Bioactivity score prediction

Using online Molinspiration Cheminformatics software (http://www.molinspiration.com), the bioactivity score of Squalene was calculated by utilizing its canonical SMILES acquired from PubChem. Drug score values indicate the overall potential of a compound to be a drug candidate. Squalene was also screened for Lipinski rule using online Molinspiration.

Molecular docking

Following the preparation of the ligand and proteins with the AutoDock tool software downloaded from MGL tools, the pdbqt files for each were created. The grid box was centred and the x, y and z dimensions were set to achieve optimal docking conformations for binding to occur. The grid boxes were constructed with a size of 20 Å, and the Cartesian *xyz* coordinates for the proteins are as follows: Sortase A (PDB ID: 1T2P) (x = -19.154, y =-12.211, z =-7.486), Dehydrosqualene synthase (PDB ID: 2ZCO) (x =55.516, y =10.428, z =58.692), Dihydrofolate reductase (PDB ID: 3FYV) (x =26.500, y =14.529, z =42.873) and Undecaprenyl diphosphate synthase (PDB ID: 4H8E) (x =26.505, y =-0.097, z =6.081). The grid file was run with autogrid and saved as a (.gpf) file. Next, the docking computation was performed by the default settings of 10 runs using Lamarckian genetic process and the dock file was saved as dpf. The final docked results were acquired as a table in a (.dlg) file following the execution of autodock. The .dlg file contains information on binding residues, binding energy and inhibition constant based on their interaction between the protein and ligand molecule. Finally, the conformations exhibiting the best ligand-protein interaction were examined using Discovery Studio 2021 (Mendie and Hemalatha, 2022).

Result and Discussion

Percentage yield of ethanol extract

The effectiveness of a specific solvent in extracting the constituents of a plant was determined by calculating the percentage yield. A yield of 2.1% was obtained from the crude ethanol extract.

Analysis of major phytoconstituents by GC-MS

The GC-MS spectrum revealed the existence of numerous chemical components with variable retention times (Fig. 1). The phytochemical estimation of ethanol extract of *H. involuta* by GC-MS leads to the identification of a total of thirty seven compounds. The various compounds present in the extract with their retention time (RT) and molecular formula were represented in Table 1. The identified compound and its peak area (%) are 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-(2.5%), Ethyl 2-acetylpentanoate (0.44%), Dodecane (0.38%), 5-Hydroxymethylfurfural (13.08%), 5H-Imidazole-4-carboxylic acid, 5-amino-, ethyl ester (1.34%), Pentadecane (0.94%), Dodecanoic acid (0.44%), Hexadecane (0.67%), beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl (0.81%), Heptadecane (0.77%), Tetradecanoic acid (0.56%), Octadecane (0.66%), Neophytadiene (0.68%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.42%), n-Hexadecanoic acid (11.06%), Hexadecanoic acid, ethyl ester (3.59%), Phytol (1.48%), 9,12-Octadecadienoic

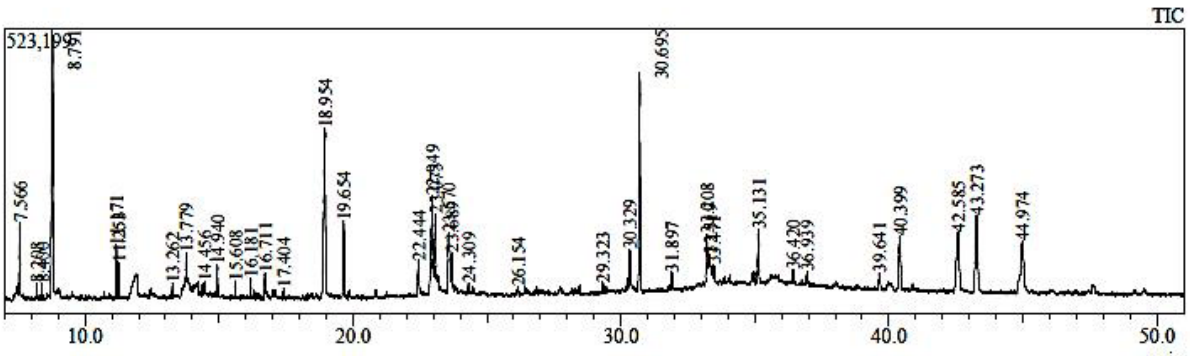

Figure 1: GC-MS chromatogram of the ethanol extract of *H. involuta*

acid (Z,Z)- (5.54%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.87%), 9,12-Octadecadienoic acid, ethyl ester (4.45%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (1.79%), Octadecanoic acid, ethyl ester (0.44%), Octanoic acid, 2-dimethylaminoethyl ester (0.54%), 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester (0.53%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (1.84%), Bis(2-ethylhexyl) phthalate (10.01%), Pentacosane (0.88%), 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (2.14%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (0.90%), Squalene (2.61%), Pentatriacontane (0.66%), 3-Methoxy-5-pentylphenol (0.60%), Lupan-3-ol, acetate (1.15%), dl-.alpha.-Tocopherol (2.54%), Campesterol (7.43%), Stigmasterol (5.09%) and gamma sitosterol (7.36%).

Among the identified compounds, terpenes are the major phytoconstituents. It includes Neophytadiene, Squalene, Phytol, Campesterol, Stigmasterol and Gamma-sitosterol. Squalene is a linear polyunsaturated triterpene with antitumor, antioxidant, antimicrobial properties and more (Gomathi et al., 2015; Ashida et al., 2022). It is an excellent emollient and moisturizer in nature, appears to be critical for minimizing the amount of free radical oxidative damage to the skin due to exposure to UV light (Lozano-Grande et al., 2018). Squalene enhances the activity of anticancer drugs and thereby inhibits the tumor growth in the skin, breast, lung and colon (Huang et al., 2009). It also helps in the stimulation of liver detoxification enzymes to remove toxins (Kelly, 1999). Neophytadiene, a sesquiterpene, have good analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant property (Gonzalez-Rivera et al., 2023). Phytol, a diterpene exhibits antianxiety, antihyperalgesic, antioxidant, antiarthritic, antitumor, anti-nociceptive, antidepressant, antiinflammatory, immune-modulating, antidiabetic, antidandruff and antimicrobial effects (Islam et al., 2018; Islam et al., 2015). Phytosterols like Campesterol, Stigmasterol and Gamma-sitosterol belongs to triterpenes (Moreau et al., 2002). Stigmasterol, commonly known as stigmasterin or wulzen anti-stiffness factor, reported to have potent anticancer, antiosteoarthritis, antiinflammatory, antidiabetic, immunomodulatory, antiparasitic, antifungal, antibacterial, antioxidant and neuroprotective activities (Bakrim et al., 2022). Campesterol has been linked with cholesterol lowering, cancer prevention (Uttu et al., 2022) and antiinflammatory properties (Nazir et al., 2023). Gamma-sitosterol reported to have antidiabetic (Nisha et al., 2013), antiinflammatory (Naikwadi et al., 2022) and anticancer properties (Sundarraj et al., 2012).

Drug likeness

The first and foremost crucial step in molecular docking is the careful selection of a suitable ligand. The chosen ligand should meet the specific criteria set by the Lipinski rule, which evaluates its drug-like properties and requires it to possess favourable pharmacokinetic characteristics. An orally active medication must satisfy the Lipinski rule. Interestingly, Squalene (Fig. 2) has been recognized as an orally active substance according to the data presented in Table 2. It possesses molecular weight below 500g/mol, no more than 10 hydrogen bond acceptors, no more than 5 hydrogen bond donors and a minimum of 10 rotatable bonds. However, it is noteworthy that the partition coefficient (Log P) value of Squalene exceeds 5. The compound violates a single parameter due to its partition coefficient value of 7.93, which exceeds the specified limit

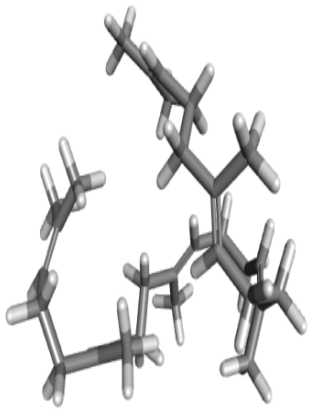

Figure 2: 3D structure of Squalene

Table 1: List of bioactive compounds detected in GC-MS analysis

Sl. No	*Name of compound*	*RT*	*Molecular formula*
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.566	$C_6H_8O_4$
2	Ethyl 2-acetylpentanoate	8.208	$C_8H_{16}O_2$
3	Dodecane	8.400	$C_{12}H_{26}$
4	5-Hydroxymethylfurfural	8.791	$C_6H_6O_3$
5	5H-Imidazole-4-carboxylic acid, 5-amino-, ethyl ester	11.171	$C_6H_9N_3O_2$
6	Pentadecane	11.253	$C_{15}H_{32}$
7	Dodecanoic acid	13.262	$C_{12}H_{24}O_2$
8	Hexadecane	13.779	$C_{16}H_{34}$
9	beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	14.456	$C_{12}H_{22}O_{11}$
10	Heptadecane	14.940	$C_{17}H_{36}$
11	Tetradecanoic acid	15.608	$C_{14}H_{28}O_2$
12	Octadecane	16.181	$C_{18}H_{38}$
13	Neophytadiene	16.711	$C_{20}H_{38}$
14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.404	$C_{20}H_{40}O$
15	n-Hexadecanoic acid	18.954	$C_{16}H_{32}O_2$
16	Hexadecanoic acid, ethyl ester	19.654	$C_{18}H_{36}O_2$
17	Phytol	22.444	$C_{20}H_{40}O$
18	9,12-Octadecadienoic acid (Z,Z)-	22.949	$C_{18}H_{32}O_2$
19	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	22.073	$C_{18}H_{30}O_2$
20	9,12-Octadecadienoic acid, ethyl ester	23.570	$C_{20}H_{34}O_2$
21	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	23.689	$C_{20}H_{34}O_2$
22	Octadecanoic acid, ethyl ester	24.309	$C_{20}H_{40}O_2$
23	Octanoic acid, 2-dimethylaminoethyl ester	26.154	$C_{12}H_{25}NO_2$
24	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	29.323	$C_{12}H_{23}NO_2$
25	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	30.329	$C_{19}H_{38}O_4$
26	Bis(2-ethylhexyl) phthalate	30.695	$C_{24}H_{38}O_4$
27	Pentacosane	31.897	$C_{25}H_{52}$
28	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	33.208	$C_{21}H_{38}$
29	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	33.319	$C_{21}H_{36}O_4$
30	Squalene	35.131	$C_{30}H_{50}$
31	Pentatriacontane	36.420	$C_{36}H_{72}$
32	3-Methoxy-5-pentylphenol	36.939	$C_{12}H_{18}O_2$
33	Lupan-3-ol, acetate	39.641	$C_{32}H_{52}O_2$
34	dl-.alpha.-Tocopherol	40.399	$C_{28}H_{48}O_2$
35	Campesterol	42.585	$C_{28}H_{48}O$
36	Stigmasterol	43.273	$C_{29}H_{48}O$
37	.gamma.-Sitosterol	44.974	$C_{29}H_{50}O$

Table 2: Lipinski's rule of five

Molecular weight	*410.72*
Partition coefficient	7.93
Hydrogen bond donors	0
Hydrogen bond acceptors	0
Number of rotatable bonds	15
Lipinski's violation	1

of 5. It is possible for an orally active compound to violate a single parameter (Lipinski, 2004).

The absorption, distribution, metabolism and excretion (ADME) characteristics of Squalene were assessed utilizing SwissADME software. Lipophilicity is quantified by the partition coefficient value (Log P), which represents the equilibrium distribution of an uncharged solute between water and an immiscible organic solvent. A higher Log P

Table 3: Physicochemical and pharmacokinetic parameters of Squalene determined by ADME

Physicochemical Properties	
Formula	*$C_{30}H_{50}$*
Molecular weight	410.72 g/mol
Number of heavy atoms	30
Number of aromatic heavy atoms	0
Fraction Csp3	0.60
Num. rotatable bonds	15
Num. H-bond acceptors	0
Num. H-bond donors	0
Molar Refractivity	143.48
Topological Polar Surface Area (TPSA)	0.00 Å^2
Pharmacokinetics	
GI Absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No

value signifies a higher degree of lipophilicity (Ranjith and Ravikumar, 2019). Squalene possesses a LogP value of 7.93.

The physicochemical and pharmacokinetic parameters of Squalene were presented in Table 3. The pharmacokinetics of Squalene revealed a low gastrointestinal (GI) absorption, inability to cross the blood-brain barrier (BBB), lack of interaction with P-glycoprotein (P-gp) and not an inhibitor of various Cytochrome P450 isoenzymes including CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The skin permeability coefficient (Log Kp) of -0.58 cm/s suggests excellent skin penetration capability of Squalene. Conversely, a more negative Log Kp value signifies low skin infusion property (Scheler et al., 2014).

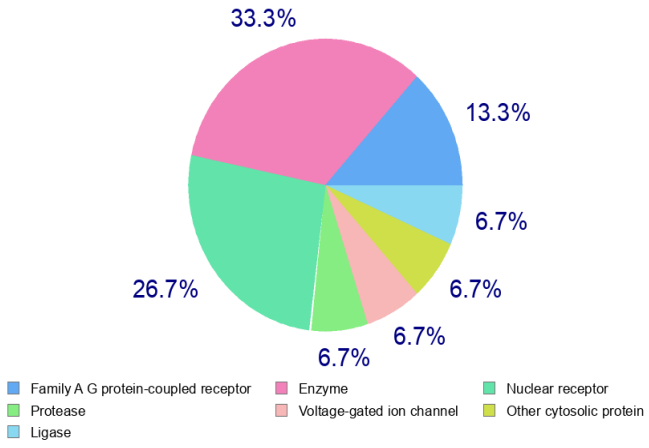

Figure 3: Biological targets of Squalene predicted using Swiss Target Prediction tool.

Table 4: Bioactivity score of Squalene produced by Molinspiration Cheminformatics software

Parameters	*Bioactivity score*
GPCR ligand	0.04
Ion channel modulator	0.01
Kinase inhibitor	-0.10
Nuclear receptor ligand	0.19
Protease inhibitor	-0.03
Enzyme inhibitor	0.16

Research carried out on animal and human subjects has explored the bioavailability of dietary Squalene. Findings indicate that around 60% of the consumed Squalene is absorbed, with the unabsorbed portion being excreted through feces. The involvement of gut microbiota in metabolizing the remaining Squalene is probable (Micera et al., 2020).

Bioactivity score

The Table 4 presents the bioactivity scores obtained from Molinspiration Cheminformatics software. In the case of organic molecules, a score greater than zero indicates activity, a score between -5.0 and 0.0 suggests moderate activity and a score lower than -5.0 for inactivity (Kuchana and Kambala, 2021). Analyzing the data, it is evident that Squalene exhibits higher activity as a G Protein-coupled receptor (GPCR) ligand, Ion channel modulator, Nuclear receptor ligand and Enzyme inhibitor. Additionally, it demonstrates moderate activity as a Kinase inhibitor and Protease inhibitor. Based on the SwissADME Target Class Prediction, Fig. 3 illustrates that Squalene has a greater probability of functioning as an Enzyme inhibitor (33.3%), Nuclear receptor ligand (26.7%) and GPCR ligand (13.3%).

Molecular docking

The AutoDock 4 software was utilized to perform the docking process, while the Discovery Studio software was employed to visualize the best confirmations of the docked complex. Squalene was effectively docked with the target proteins - Sortase A, Dihydroxysqualene synthase, Dihydrofolate reductase and Undecaprenyl diphosphate synthase (Fig. 4).

The docking score represents the evaluation of the interaction between a ligand and a protein, specifically the individual pose obtained. In the case of docking Squalene with the target proteins, Undecaprenyl diphosphate synthase (4H8E) exhibited the highest binding score, followed by Dihydrofolate reductase (3FYV), Sortase A (1T2P) and Dehydrosqualene synthase (2ZCO), with binding energies (Kcal/mole) of -9.99, -9.04, -7.68 and -7.35 respectively, as listed in Table 5 and 3D images showing the interaction are displayed in Fig. 5. It is important to note that

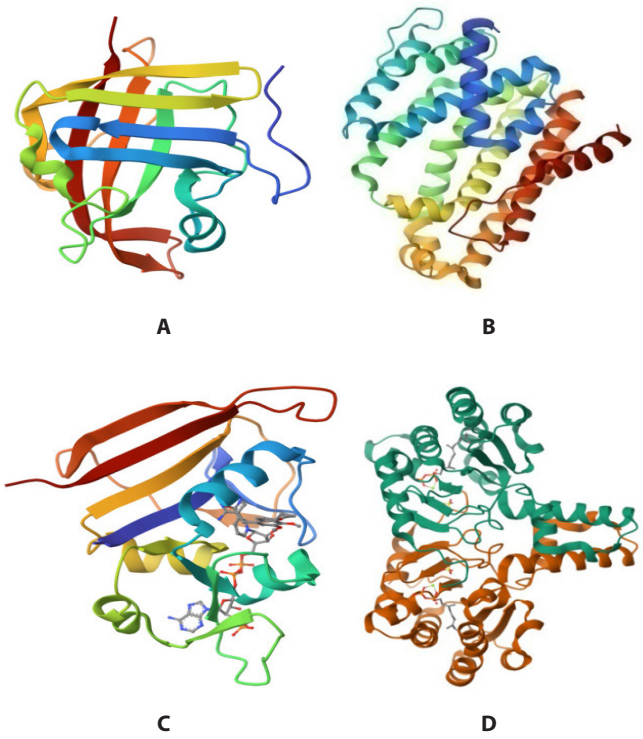

Figure 4: 3D structure of target proteins (A) Sortase A (PDB ID: 1T2P), (B) Dehydrosqualene synthase (PDB ID: 2ZCO), (C) Dihydrofolate reductase (PDB ID: 3FYV) and (D) Undecaprenyl diphosphate synthase (PDB ID: 4H8E)

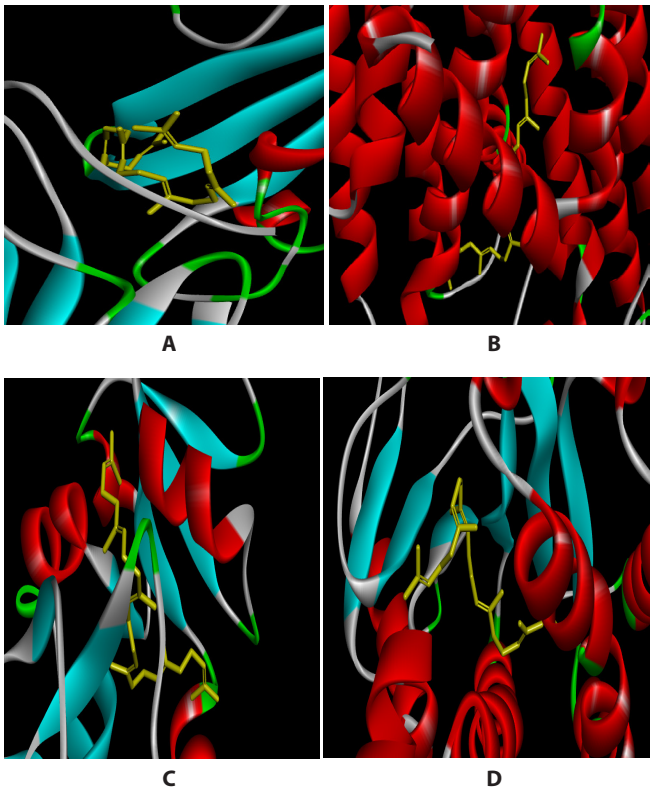

Figure 5: 3D images of binding confirmation of Squalene (yellow coloured) with target proteins A (1T2P), B (2ZCO), C (3FYV) and D (4H8E), depicted using Discovery Studio.

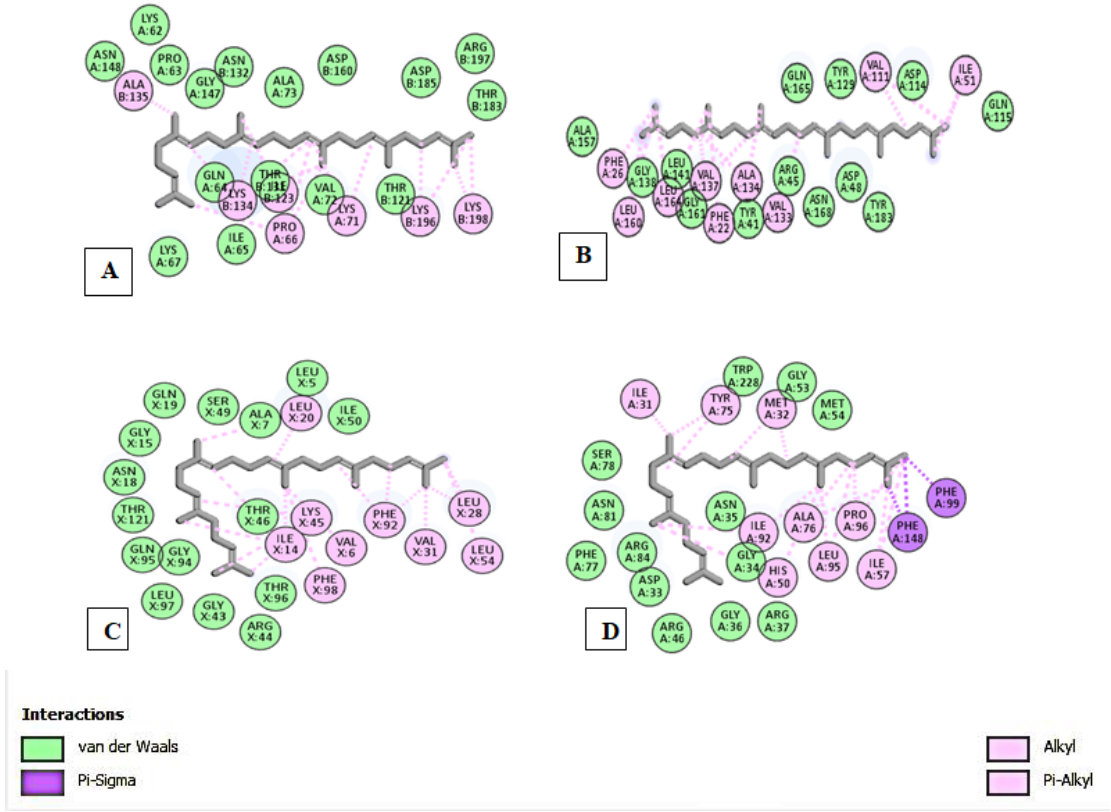

Figure 6: 2D representation of interaction between Squalene and target proteins A (1T2P), B (2ZCO), C (3FYV) and D (4H8E)

Table 5: Docking score of Squalene with the target proteins

Sl. No.	*Target protein*	*PDB ID*	*Binding energy (Kcal/mole)*
1	Sortase A	1T2P	- 7.68
2	Dehydrosqualene synthase	2ZCO	-7.35
3	Dihydrofolate reductase	3FYV	-9.04
4	Undecaprenyl diphosphate synthase	4H8E	-9.99

a lower binding energy score (more negative value) indicates a higher stability of the formed complex (Bhat et al., 2022).

It exhibited a notably high binding energy of -9.99 Kcal/mole when binding to Undecaprenyl diphosphate synthase (4H8E), interacting with amino acids such as Phe 148, Phe 99 by Pi-Sigma bond, Tyr 75, His 50 by Pi-Alkyl bond and Ile 31, Met 32, Ile 92, Ala 76, Leu 95, Pro 96, Ile 57 through Alkyl bond The docking with Dihydrofolate reductase (3FYV) displayed interactions with amino acids like Phe 92, Phe 98 by Pi-Alkyl bond and Leu 20, Ile 14, Lys 45, Val 6, Val 31, Leu 54, Leu 28 by Alkyl bond with a binding energy of -9.04 Kcal/mole. Docking with Dehydrosqualene synthase(2ZCO) revealed a binding energy of -7.35 Kcal/mole with amino acids like Phe 26, Phe 22 by Pi-Alkyl bonds and Leu 160, Leu 164, Val 137, Ala 134, Val 133, Val 111 interacted through Alkyl bonds. Finally, the docking process with Sortase A (1T2P) involved various amino acids such as Ala 135, Lys 134, Ile 123, Pro 66, Lys 71, Lys 196, Lys 198 forming Alkyl bonds resulting in -7.68 Kcal/mole as the binding score (Fig. 6).

Conclusion

Plant-derived phytochemicals are the novel sources of therapeutics. Identifying the lead compounds for drug development depends critically on the investigation and extraction of bioactive chemicals. The present study focuses on determining the volatile bioactive components in the ethanol extract of *H. involuta* followed by molecular docking of the identified pharmacologically important triterpenoid Squalene, against the target proteins of *S. aureus*. The in silico docking studies revealed the highest docking score when interacting with the active site of Undecaprenyl diphosphate synthase (-9.99 Kcal/mole) followed by Dihydrofolate reductase (-9.04 Kcal/mole), Sortase A (-7.68 Kcal/mole) and Dehydrosqualene synthase (-7.35 Kcal/mole). In conclusion, docking of phytocompounds with bacterial target proteins represents a promising strategy in the search for novel antimicrobial agents. Although the method provides insightful information on possible interactions and methods of action, it is important to recognise its limits, including the requirement for precise structural data and the challenges posed by the dynamic nature of biological systems. In order to assess the *in vitro* bioactivities of Squalene from *H. involuta*, its isolation and purification is an essential step. Despite these hurdles, advancements in computational methods, such as molecular dynamics simulations and machine learning, enhances the accuracy of docking studies. The integration of docking into the drug discovery will lead to the identification of effective phytomedicine.

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References

Adam OAO, Abadi RSM and Ayoub SMH (2019). The effect of extraction method and solvents on yield and antioxidant activity of certain sudanese medicinal plant extracts. *J. Phytopharma*. ***8*(5):** 248-252.

Adedokun KA, Imodoye SO, Bello IO and Lanihun AA (2023). Therapeutic potentials of medicinal plants and significance of computational tools in anti-cancer drug discovery In Phytochemistry. *Computational Tools and Databases in Drug Discovery* 393-455.

Alvaro LR and Cardin de Stefani (2013). Squalene: a multi-task link in the crossroads of cancer and aging. *Functional Foods in Health and Disease* **3(12):** 462-476.

Ashida S, P Nusaifa Beevi, RV Sushama Raj and Prabhakumari C **(**2022). Qualitative Detection of Triterpenes and Quantification of Betulinic Acid from Hexane Extract of *Simarouba glauca* Leaves by Gas Chromatography-Mass Spectrometry and High-Performance Thin Layer Chromatography. *Ind. J. Pharma. Sci.* **84(6**): 1380-1388.

Asakawa Y, Ludwiczuk A, Nagashima F (2013). Chemical Constituents of Bryophytes: Bio- and Chemical Diversity, Biological Activity and Chemosystematics. Progress *in the Chemistry of Organic Natural Products*, **95:** 1-796.

Azalia Lozano-Grande M, Shela Gorinstein, Eduardo Espitia-Rangel, Gloria Davila-Ortiz and Alma Leticia Martinez-Ayala (2018). Review Article: Plant Sources, Extraction Methods, and Uses of Squalene. *Int. J. Agro.* **2018(1):** 1829160.

Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, Sheikh RA, Goh KW, Ming LC and Bouyahya A (2022). Health Benefits and Pharmacological Properties of Stigmasterol. *Antioxidants* **11(10)**: 1912.

Bhat VV, Chetana PR, Dhale MA and Soman S (2022). Synthesis, characterization, in vitro biological evaluation and molecular docking studies of newly synthesized mononuclear lanthanum (III) complexes of N, N′-bis (2-aminoethyl) oxamide and phenanthroline bases. *J. Mol. Stru.* **1270:** 133903.

Chauhan A, Goyal MK and Chauhan P (2014). GC-MS Technique

and its Analytical Applications in Science and Technology. *J. Anal. Bioanal. Tech*, **5(6):** 222.

Chen F, Ludwiczuk A, Wei G, Chen X, Crandall-Stotler B and Bowman JL (2018). Terpenoid Secondary Metabolites in Bryophytes: Chemical Diversity, Biosynthesis and Biological Functions. *Critical Rev. Plant Sci.* **37(2-3)**: 210–231.

Commisso M, Guarino F, Marchi L, Muto A, Piro A and Degola F (2021). Bryo-Activities: A Review on How Bryophytes Are Contributing to the Arsenal of Natural Bioactive Compounds against Fungi. *Plants,* **10(2):** 203.

Dabhi Swati, Parkhi Vishwatej, Shukla Vinay and Patel Bhupesh (2023). LC-MS Analysis of Kigelia Pinnata (JACQ) DC. Root Bark- A Multi-Potent Medicinal Plant. *Ayushdhara,* **10(2):** 60-67.

Daoui O, Elkhattabi S, Chtita S, Elkhalabi R, Zgou H and Benjelloun AT (2021). QSAR, molecular docking and ADMET properties in silico studies of novel 4,5,6,7-tetrahydrobenzo[D]-thiazol-2-Yl derivatives derived from dimedone as potent anti-tumor agents through inhibition of C-Met receptor tyrosine kinase. *Heliyon,* ***7*****(7)**.

Dixon RA (2001). Natural Products and Plant Disease Resistance. *Nature,* **411(6839):** 843–847.

Fatma Esra Gunes (2013). Medical use of squalene as a natural antioxidant. *J. Marmara Uni. Ins. of Health Sciences,* **3(4):** 221-229.

Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K and Uma C (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. *J. Food Sci. Tech.* **52(2):** 1212-1217.

Gonzalez-Rivera ML, Barragan-Galvez JC, Gasca-Martínez D, Hidalgo-Figueroa S, Isiordia-Espinoza M and Alonso-Castro AJ (2023). In Vivo Neuropharmacological Effects of Neophytadiene. *Molecules*, **28(8):** 3457.

Greeshma GM, Bosco Lawarence, Manoj GS and Murugan K (2016). Bryophytes, the ignored medicinal herbals of the biological world - a search at Neyyar wildlife sanctuary, Trivandrum, Kerala. *Kongunadu Res. J.,* **3(1):** 22-25.

Huang ZR, Lin YK and Fang JY (2009). Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules,* **14(1):** 540–554.

Islam MT, de Alencar MVOB, da Conceição Machado K, da Conceição Machado K, de Carvalho Melo-Cavalcante AA, de Sousa DP and de Freitas RM (2015). Phytol in a pharma-medico-stance. *Chemico-Biological Interactions*, **240:** 60-73.

Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI and Atanasov AG (2018). Phytol: A review of biomedical activities. *Food and chemical toxicology*, **121:** 82-94.

Kahsay A, Mihret A, Abebe T and Andualem T (2014). Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region. *Ethiopia Archives of public Health*, **72:** 1-7.

Kalathil Sethumadhavan Abhilash and Alex Philip Alen (2022). Review on the ecological, economical, physiological phytochemical and *in-vitro* studies in the moss *Hyophila involuta* (Hook.) A. Jaeger. *Int. J. Res. Rev.* **9(7):** 313-319.

Kavitha R (2021). Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic extracts of leaf and fruit of *Trichosanthesis dioica* Roxb. *Int. J. Pharma. Sci. Res.* **12(5):** 2755-2764.

Kelly GS (1999). Squalene and its potential clinical uses. *Alternative Medicine Review* **44(11):** 29–36.

Khanam Rubina, Chaudhary BL, Khanam Shabana and Pen Kumar (2011). Antimicrobial activity of *Marchantia palmata* Nee. *Asi. J. Biochem. Pharma. Res.* **2(1):** 27-36.

Kuchana M and Kambala LB (2021). Design, synthesis and in silico prediction of drug-likeness properties of new ortho, meta and para-(2-cyano-3-(3, 5-di-tert-butyl-4-hydroxyphenyl) acrylamido) benzoic acids. *J. Appl. Pharma. Sci.* **11(8)**: 31-35

Laura Klavina and Gunta Springe (2015). Optimisation of Conditions for Extraction of Biologically Active Compounds from Common Bryophytes in Latvia. *Proceedings of the Latvian Academy of Sciences. Section B: Natural, Exact and Applied Sciences,* **69(6):** 299–306.

Lipinski CA (2004). Lead-and drug-like compounds: the rule-of-five revolution. *Drug discovery today: Technologies*, **1(4):** 337-341.

Lozano-Grande M. Azalia, Shela Gorinstein, Eduardo Espitia-Rangel, Gloria Davila-Ortiz and Alma Leticia Martinez-Ayala (2018). Review Article: Plant Sources, Extraction Methods, and Uses of Squalene. *Int. J. Agro.* **2018(1):** 1829160.

Makinde AM and Fajuyigbe EA (2015). Secondary Metabolites and Bioactivity of *Hyophila involuta* (Hook) Jaeg. *Notulae Scientia Biologicae,* **7(4)**: 456-459.

Mendie LE and Hemalatha S (2022). Molecular docking of phytochemicals targeting GFRs as therapeutic sites for cancer: an in silico study. *App. Biochem. Biotech.* **194(1)**: 215-231.

Micera M, Botto A, Geddo F, Antoniotti S, Bertea CM, Levi R and Querio G (2020). Squalene: more than a step toward sterols. *Antioxidants* **9(8):** 688.

Moreau RA, Whitaker BD and Hicks KB (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in lipid research,* **41(6)**: 457-500.

Motti R, Palma AD and de Falco B (2023). Bryophytes used in folk medicine: An ethnobotanical overview. *Horticulturae*, **9(2):** 137.

Naikwadi PH, Phatangare ND and Mane DV (2022). Active anti-inflammatory potency of γ-sitosterol from woodfordia floribunda salisb. *J Plant Sci Res.* **38(2).**

Nazir S, Chaudhary WA, Mobashar A, Anjum I, Hameed S and Azhar S (2023). Campesterol: a natural phytochemical with anti inflammatory properties as potential therapeutic agent for rheumatoid arthritis: a systematic review: campesterol: A natural phytochemical. *Pakistan J. Health Sci.* **4(5).**

Nisha Tripathi, Sunita Kumar, Rakesh Singh, CJ Singh, Prashant Singh and VK. Varshney (2013). Isolation and identification of γ-sitosterol by GC-MS from roots of Girardinia heterophylla. *Oriental J. Chem.***, 29(2)**: 705-707.

Rachna P and Vashishtha BD (2015). Bryophytes-boon to mankind-A Review *Agricultural Reviews*, **36(1)**: 77-79.

Ranjith D and Ravikumar C (2019). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Ipomoea mauritiana Jacq. *J. Pharma. Phytochem.* **8(5)**: 2063-2073.

Scheler S, Fahr A and Liu X (2014). Linear combination methods for prediction of drug skin permeation. *ADMET and DMPK,* **2(4)**: 199-220.

Shuvo MN, Halder SK, Alam N, Himel MK and Shil A (2024). Developing phytocompound-based new drugs against

multi-drug-resistant *Staphylococcus aureus Royal Society open science*, **11(7):** 231475.

Sumi ES, R Anandan, RRajesh, CN Ravishankar and Suseela Mathew (2018). Review Article: Nutraceutical and Therapeutic Applications of Squalene. *Fishery Technology,* **55:** 229 – 237.

Sundarraj S, Thangam R, Sreevani V, Kaveri K, Gunasekaran P, Achiraman S and Kannan S (2012). γ-Sitosterol from Acacia nilotica L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. *J. ethnopharma.* **141(3)**: 803–809.

Uttu AJ, Sallau MS, Ibrahim H and Iyun ORA (2022). Isolation, characterization, and docking studies of campesterol and β-sitosterol from *Strychnos innocua* (Delile) root bark. *J. Taibah Uni. Med. Sci.* **18(3):** 566–578.