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Research Article

GCMS Analysis and Molecular Docking Studies of Bioactive Compounds in Leaf Extract of *Syzgium samarangense*

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Abstract

The study focuses on the results of docking studies conducted on Staphylococcus aureus, a pathogenic bacterium responsible for causing various infections in humans. The study utilized GC-MS analysis of methanol extract of Syzygium samarangense leaf to identify potential inhibitors of specific enzymes in the organism, which are essential for its survival and virulence. The docking studies involved the virtual screening of several molecules against 4 target proteins of Staphylococcus aureus and the results were analyzed using molecular visualization and scoring tools. The study identified several compounds that showed high binding affinities to the target proteins, indicating their potential as effective inhibitors. The findings of this study provide valuable insights into the design of novel and potent inhibitors against Staphylococcus aureus, which could help in the development of effective therapies and drugs for infections caused by this organism.

INTRODUCTION

Syzygium samarangense is a bush cherry tree that belongs to the Myrtaceae family of the Myrtales order. The plant is also known as java apple, wax apple, wax jambu or semarang rose apple. The plant is widely used in traditional medicine to cure several ailments like bronchitis, asthma, diabetes, fever, pathogenic infections, gut spasms, as well as renal diseases. *S. Samarangense* has a promising potential in the prevention of NSAIDs-induced ulcers (Mahmoud MF et al., 2021). Currently, in vitro and in vivo experiments of the plant extract have demonstrated various pharmacological activities such as antioxidant, antimicrobial, anti-HIV, analgesic, anti-inflammatory, Anti-hyperglycemic, anti-diabetic, thrombolytic, spasmolytic, anti-cytotoxic, hepatoprotective, anti-cancer, anti-helminthic, anxiolytic, protease inhibitory, and immunomodulatory effect (Tarigan C et al., 2021). *Staphylococcus aureus* (S. Aureus) is a spherical shaped, Gram-positive bacterium which belongs to the family of Micrococcaceae (Lowy FD et al., 1998). These non-motile, non-sporulating bacteria are facultative anaerobes (Hennekinne JA et al., 2012). S. Aureus could be distinguished from other Staphylococcal species with the help of biochemical tests such as coagulase, mannitol fermentation, and deoxy-ribonuclease. It displays positive results for the aforementioned tests. It is a ubiquitous bacterium and is part of the normal microbial flora of humans (Weber JT 2005). Being an opportunistic pathogen, it is capable of causing various skin and soft tissue infections, such as impetigo, cellulitis, boils, and folliculitis (Stryjewski ME et al., 2008) (Tong SYC et al., 2015). FtsA has been found to play a crucial role in bacterial cell division by anchoring FtsZ protein to the cytoplasmic membrane (Fujita J et al., 2014). In 2015, James et al proposed that FtsA protein is essential to S. Aureus and nonessential in higher organisms (James OC et al., 2015). Targeting such proteins has the advantage of solving antibiotic resistance issues as well as the possibility of minimal side effects. In addition to S. Aureus, FtsA protein was also found in other Gram-positive organisms (Mura A et al., 2017) and Gram-negative pathogens such as *Pseudomonas aeruginosa* (Paradis-Bleau C et al., 2005) and Neis-seria gonorrhoeae (Zou Y et al.,

2017). Hence, molecules inhibiting FtsA protein could be very much helpful in tackling a broad range of pathogens.

MATERIALS AND METHODS

Plant collection and extraction

The fresh and healthy leaves were collected from Palakkad district in Kerala. The leaves were washed thoroughly with fresh water and dried using a hot air oven. The leaves were dried for a period of 3hrs at 50°C. the dried leaves were grinded into fine powder using an electric mixer. About 75g of the plant leaf powder was taken and soaked in about 300ml of the desired solvent (methanol and water) and incubated in an orbital incubator for 16 hrs. Later the material was taken and filtered onto a beaker using whatman filter paper no.1. The crude extract is evaporated using a hotplate. When completely dried, the yield of extracts obtained was calculated.

Gas chromatography- mass spectrometry analysis

A Shimadzu GC-MS (model QP2010) operating in El mode at 70e V and a Restek-5MS column (30 meters × 0.25 mm, 0.25 um) performed GC-MS analysis of *Syzygium samarangense* methanolic extract. The carrier gas was helium with a flow rate of 1 ml/min and an injection volume of 1µl. The oven temperature programs were held at 50°C for 1 minute, then increased by 10°C/min to 250°C and held for 3 minutes, then increased by 10°C/min to 280°C and held for 5 minutes. Spray temperature 250°C; interface temperature 250°C, ion source temperature 250°C, scan range 35-500 m/z, and solvent delay 2 min.

Identification of component

The GC–MS data was interpreted with the aid of NIST library. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The percentage of each component for both the extracts was calculated separately for the relative peak area of each component in the chromatogram. The retention time, peak area, structure and molecular weight of the compounds were identified from both plant and the endophyte extracts and represented.

MOLECULAR DOCKING

Accession of target protein

The three-dimensional structure of 4 different target proteins with PDB ID:- 1JIJ, 3G75, 3WQU and 1N67 was downloaded from the RCSB protein Data Bank.

Ligand selection

From the total of 31 compounds obtained SWISS ADME was checked and found 12 potential ligands that could be used for medicinal application and have less toxicity and more absorption into the GI tract. Based on this the chemical structure of 1-Propoxypropan-2-one, 3-Nitrophthalic acid, Tocopherols and Trans Squalene was obtained from PubChem compound database. It was prepared by ChemBioDraw and MOL SDF format of this ligand was converted to PDBQT file using PyRx tool to generate atomic coordinates.

Preparation of the protein receptors

The protein structure, a prerequisite in the docking studies was downloaded from the Protein Data Bank (PDB, http://www.rcsb.org.pdb). The PDB ID 3WQU file representing *S. aureus* FtsA complexed with ATP at 2.80 Å resolutions, the PDB ID 1JIJ representing *S. aureus* TyrRS in complex with SB-239629 at 3.20 Å resolutions, the PDB ID 1N67 representing *S. aureus* Clumping Factor A at 1.90 Å resolutions and the PDB ID 3G75 representing *S. aureus* Gyrase B co-complexed with 4-methyl-5-[3-(methylsulfanyl)-1H-pyrazol-5-yl]-2-thiophen-2-yl-1,3-thiazole inhibitor at 2.3 Å resolutions. At a higher resolution it served as a good receptor file. The structures were prepared by the removing of all heteroatom coordinates and water molecules, added with hydrogens, Kollman charges and missing C-terminal oxygen. It was ensured that no residues carry the non-integral charges which are a prerequisite for the receptor file in the software AutoDock 4.2.

Preparation of ligands

The structure of the ligands in SDF was obtained from PubChem, converted to PDB coordinates using Open Babel and energy minimized. Cheminformatics analysis including drug likeliness based on Lipinski's Rule of Five and ADME analysis was done with Swiss-ADME server. Addition of hydrogens, add Gasteiger charges to the ligands were done using AutoDock (ADT).

Molecular docking studies

Virtual screening software PyRx V 0.8 was used for molecular docking. PyRx uses AutoDock Vina as the docking engine. Both the targets and ligands were converted to pdbqt format before docking. The grid centre for docking was set with x = -11.22, y = 14.99 and z = 85.43 co-ordinates with grid box size of x = 25.0, y = 25.0 and z = 25.0 for Target 1JIJ. The grid centre for docking was set with x = 28.06, y = 48.65 and z = 63.31 co-ordinates with grid box size of x = 62.39, y = 92.31 and z = 65.71 for Target 1N67. The grid centre for docking was set with x = 50.35, y = -3.98 and z = 17.98 co-ordinates with grid box size of x = 25.0, y = 25.0 and z = 25.0 for Target 3G75. The grid centre for docking was set with x = 3.08, y = 31.39 and z = -22.20 co-ordinates with grid box size of x = 25.0, y = 25.0 and z = 25.0 for Target 3WQU. The Binding energy for best poses were tabulated and the interaction details were visualized with Biovia Discovery Studio Client software. The details of ligand- receptor interactions in 2D and 3D formats were generated for all ligands which showed hydrogen bonding interactions.

Analysis of target active binding sites

The active sites are the coordinates of the ligands in the original target protein grids, and these active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3D LigandSite virtual tools.

Molecular docking analysis

A computational ligand-target docking approach was used to analyze structural complexes of the ACE (target) with 4 ligand in order to understand the structural basis of this protein target specificity. Initially, protein-ligand attraction was investigated for hydrophobic/ hydrophilic properties of these complexes by Platinum software web server. Finally, docking was carried out by PyRx, AutoDock Vina option based on scoring functions. The energy of interaction of the ligands with the Staphylococcus proteins is assigned "grid point." At each step of the simulation, the energy of interaction of ligands and proteins was evaluated using atomic affinity potentials computed on a grid. The remaining parameters were set as default.

RESULT AND DISCUSSION

GC-MS

The result of GCMS analysis of methanol extract of *Syzygum samarangense* showed the presence of 31 compounds. These names of these compounds were obtained from the pubchem library **(Table 1).** The peaks indicate the concentration or abundance of each compound present in the extract. The position of the peak gives the time of elution which is different for each compound. The given figure **(Figure 1)** shows the GC-MS graph obtained for the methanol extract.

Molecular docking

Docking was done on 4 targets with their respective PDB

Table 1. Compounds identified from the GCMS analysis of methanol extract.

Peak	Name	Pubchem ID	Molecular formula	Molecular weight	Area%
1	**Trimethyl-D9-Acetic Acid**	23423104	$C_5H_{10}O_2$	111.19	0.4
2	(1R,3aR,7S,8aS)-Decahydro-1-methyl-4-methylene-7-(1-methylethenyl)azulene]	102156162	$C_{15}H_{24}$	204.35	0.3
3	Alpha selinene	10856614	$C_{15}H_{24}$	204.35	0.34
4	NEOPHYTADIENE	10446	$C_{20}H_{38}$	278.5	1.58
5	**6-Bromo-4-(2-phenylmorpholin-4-yl)quinoline**	51130775	$C_{19}H_{17}BrN_2O$	369.3	0.24
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5366244	$C_{20}H_{40}O$	296.5	0.51
7	n-Hexadecanoic acid	985	$C_{16}H_{32}O_2$	256.42	4.5
8	Phytol	5280435	$C_{20}H_{40}O$	296.5	0.28
9	Cyclopropanebutanoic acid	554084	$C_{25}H_{42}O_2$	374.6	0.31
10	9-Octadecenal	5283381	$C_{18}H_{34}O$	266.5	0.26
11	1,4- Methano-1h-indene	565738	$C_{15}H_{24}$	204.35	0.29
12	3-Nitrophthalic acid	69043	$C_8H_5NO_6$	211.13	0.32
13	Disparlure	205983	$C_{19}H_{38}O$	282.5	0.32
14	(1H-Benzoimidazol-2-yl)-diphenyl-methanol	254366	$C_{20}H_{16}N_2O$	300.4	1.36
15	D:A-Friedooleanan-3-ol, (3.alpha.)-	348029	$C_{30}H_{52}O$	428.7	1.42
16	Friedelan-3-one	91472	$C_{30}H_{50}O$	426.7	2.33
17	Cedrane	591419	$C_{18}H_{32}0$	264.4	0.39
18	Benzenamine	499408	$C_{12}H_{10}N_2$	182.22	1.26
19	D:A-Friedo-2,3-secooleanane-2,3-dioic acid	565450	$C_{30}H_{54}O_4$	502.8	19.15
20	Friedelan-3-one	91472	$C_{30}H_{50}O$	426.7	29.68
21	9,19-Cyclolanost-25-en-3-ol	550205	$C_{31}H_{52}O$	440.7	6.2
22	Trans squalene 2,6,10,14,18,22- Tetracosahexane	638072	$C_{30}H_{50}$	410.7	1.4
23	28-Oxours-12-en-3-yl acetate	608980	$C_{32}H_{50}O_3$	482.7	0.61
24	1-Isopropenyl-4,5-dimethylbicyclo[4,3,0] nonan-5-ylmethyl phenyl sulfoxide	565587	$C_{21}H_{30}OS$	330.5	0.28
25	Cholesta-5-en-3-ol(3 beta)	304	$C_{27}H_{46}O$	386.7	0.36
26	Cholesta-4,6-dien-3-ol	33010	$C_{34}H_{48}O_2$	488.7	0.35
27	Cholesta-3,5-diene	92835	$C_{27}H_{44}$	368.6	0.42
28	gamma- Tocopherol	92729	$C_{28}H_{48}O_2$	416.7	1
29	Longifolenbromid-I	608959	$C_{15}H_{23}Br$	283.25	1.32
30	Cholesta-4,6-dien-3-ol (3 beta)	14795191	$C_{27}H_{44}O$	384.6	1.54
31	3.beta-Myristoylolean-12-en-28-ol	609052	$C_{44}H_{76}O_3$	653.1	21.27

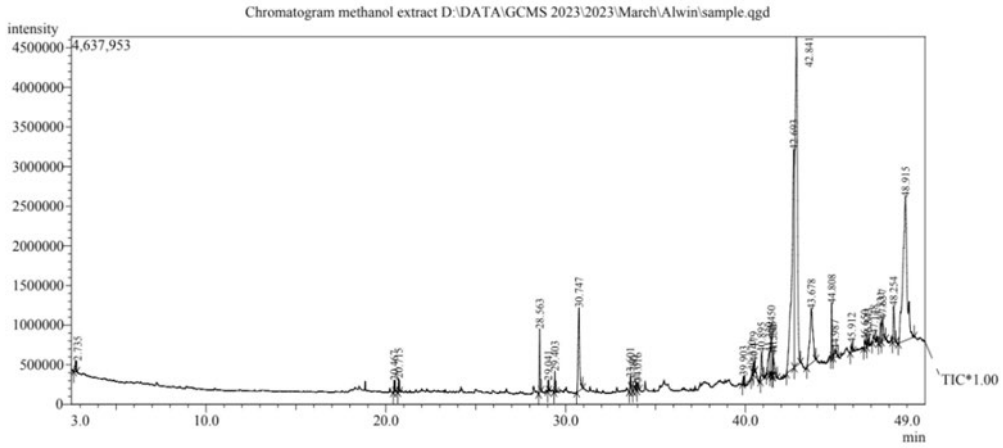

Figure 1. GC-MS graph of methanol extract of *Syzygium samarangense.*

ID; 1JIJ,1N67, 3G75, 3WQU by selecting ligand compounds based on lipophilicity, size, polarity, solubility, flexibility, saturation, GI absorption, Blood Brain Barrier permeability were analyzed.

The docking studies show that three out of four compounds bind effectively with the four targets. Trans-Squalene shows no binding to any of the targets. Whereas Tocopherol, 1-Nitrophthalic acid and 1-Propoxypropan-2-one bind effectively with the four targets when compared to the standard Tetracycline. The binding energy of Tocopherol (-7.3) and 1-Nitrophthalic acid (-7.2) shows that these compounds have appreciable inhibitory activity against the protein target (PDB ID:1JIJ) when compared to Tetracycline (-7.7). Whereas 1-Propoxypropan-2-one (-4.4) shows relatively low binding energy when compared to others. 5 H bonds were seen in Tetracycline with the target 1JIJ,1 H bond in Tocopherol, 6 H bonds in 3-Nitrophthalic acid and 3 H bonds in 1-propoxypropa-2-one. Also a number of Pi-Sigma, Pi-Alkyl, and Alkyl bonds are seen when the compounds are docked with the target.

The binding energy (B.E.) of Tocopherol (-6.1) and 1-Nitrophthalic acid (-5.9) shows that these compounds have appreciable inhibitory activity against the protein target (PDB ID:1N67) when compared to Tetracycline (-7). Whereas 1-Propoxypropan-2-one (-3.9) shows relatively low binding energy when compared to others. 7 H bonds were seen in Tetracycline with the target 1N67,1 H bond in Tocopherol, 5 H bonds in 3-Nitrophthalic acid and 3 H bonds in 1-propoxypropan-2-one. Also Pi-Anion, Pi-Cation, Pi-Alkyl, Alkyl bonds are seen when the compounds are docked with the target **(Table 2).**

The binding energy (B.E.) of Tocopherol (-5.9) and 1-Nitrophthalic acid (-5.5) shows that these compounds have great inhibitory activity against the protein target (PDB ID: 3G75) when compared to Tetracycline (-5.9). Whereas 1-Propoxypropan-2-one (-3.4) shows relatively low binding energy when compared to others. In the target 3G75, 3 H bonds are seen interacting Tetracycline and the target, 2 H bonds in Tocopherol, 6 H bonds in 3-Nitrophthalic acid and 3 H bonds in 1-Propoxypropan-2-one. Also Pi-Anion, Pi-Alkyl, Alkyl bonds are seen when the compounds are docked with the target.

The binding energy (B.E.) of Tocopherol (-6.9) and 1-Nitrophthalic acid (-7.2) shows that these compounds have superior inhibitory activity against the protein target (PDB ID: 3WQU) when compared to Tetracycline (-6.4). Whereas 1-Propoxypropan-2-one (-4.1) shows relatively low binding energy when compared to others. In the target 3WQU about 4 H bonds were formed with the ligand Tetracycline, 1 H bond with Tocopherol, 7 H bonds with 3-Nitrophthalic acid and 2 with 1-propoxypropan-2-one. Also Pi-Pi stacked, Pi-Anion and van der walls bond are seen when the compounds are docked with the target.

CONCLUSION

In conclusion, the combined approach of GC-MS analysis and molecular docking studies has provided valuable insights into the bioactive compounds present in the leaf extract of *Syzygium samarangense*. This comprehensive investigation has not only identified a diverse array of compounds with potential pharmacological significance but has also shed light on their potential interactions with specific molecular targets. The data presented here not only contributes to our understanding of the chemical composition of this plant extract but also paves the way for further research into harnessing its therapeutic potential. Continued exploration of the bioactive compounds in *Syzygium samarangense* may lead to the development of novel pharmaceuticals or natural remedies, ultimately benefiting both human health and the field of natural product-based drug discovery.

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