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Molecular Docking Studies of Bioactive Compounds from *Psoralea corylifolia* Revealing Antibacterial Potential against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Abstract

- ***Aim:***

This study investigates the antimicrobial potential of phytochemicals from *Psoralea corylifolia* against methicillin-resistant *Staphylococcus aureus* (MRSA) and elucidates the underlying mechanisms through molecular docking studies.

- ***Methods:***

Mature seeds of *Psoralea corylifolia* were extracted using ethanol and chloroform via Soxhlet extraction. Qualitative analysis was performed to identify phytochemicals. Antibacterial activity was assessed using the agar well diffusion method against MRSA isolates, comparing results to gentamycin. GC-MS analysis characterized secondary metabolites, while molecular docking simulations with AutoDock Vina examined the binding affinity of (+)-Bakuchiol to the *S. aureus* receptor 1TSJ.

- ***Results:***

Ethanol extraction proved superior, yielding a diverse range of phytochemicals, including flavonoids and alkaloids, while chloroform extraction was less effective. The ethanol extract exhibited significant antibacterial activity, with maximum zones of inhibition observed against *S.aureus* isolates. GC-MS identified key compounds, including (+)-Bakuchiol, which demonstrated a binding affinity of -5.8 kcal/mol in molecular docking studies. Interaction analysis highlighted critical amino acid residues involved in binding.

- ***Conclusion:***

The findings confirm the efficacy of ethanol-extracted phytochemicals from *Psoralea corylifolia*, particularly (+)-Bakuchiol, as potential antibacterial agents against MRSA. These results support the need for further research into plant-derived compounds to combat antibiotic-resistant bacterial infections and offer insights into their mechanisms of action through molecular docking studies.

Keywords:- *Psoralea Corylifolia, MRSA, Ficusin, (+)-Bakuchiol, Isopsoralen, Molecular Docking.*

I. INTRODUCTION

Infectious bacterial diseases affect millions of people around the world and have caused sickness and death throughout history. The World Health Organization (WHO) says that about 50,000 people die from these infections every year [1] and these infections have raised concern in the healthcare field over the years, particularly those which have adopted resistance towards antimicrobial agents. The increasing antimicrobial resistance is due to genetic mutations or acquisition of antibiotic resistance determinants and stimulated by the misuse of antimicrobials in drug prescriptions and for other purposes [2]. Among the bacteria isolates *Staphylococcus aureus* is a serious human pathogen known to cause numerous bacterial infections at the level of the bloodstream, lower respiratory tract, and skin and soft tissue [3].

After the widespread use of beta-lactam antibiotics like methicillin and oxacillin, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged, becoming a significant cause of infectious diseases acquired in hospitals and communities around the world. It is now well-established through various studies that antibiotics targeting MRSA work on different mechanisms with various antibiotics. However, the excessive use of antibiotics is the primary factor contributing to the rise of bacterial resistance [4]. The reduced susceptibility of MRSA to antibiotics makes infections difficult to treat, leading to prolonged hospital stays, increased healthcare costs, and even treatment failure [5]. These challenges highlight the urgent need to explore new agents from alternative sources to effectively manage MRSA infections.

The screening of antimicrobial agents derived from plant phytochemicals has frequently been identified as a key

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starting point for discovering new antimicrobial drugs. The diverse range of bioactive compounds found in plant derivatives has encouraged researchers to explore their potential pharmaceutical applications, given their generally safe profiles [6]. *Psoralea corylifolia* L. (Fabaceae) is a plant species native to China. Its fruits have been traditionally used to treat various conditions, including gynecological bleeding, vitiligo, and psoriasis. Several chemical compounds, such as flavonoids and coumarins, have been extracted from this plant. Some of these compounds demonstrate antioxidant [7], estrogenic [8], immunomodulatory, and antitumor properties [9]. Additionally, antibacterial effects of certain constituents against *Staphylococcus aureus* and *S. epidermidis* have been documented [10].

Simply assessing antimicrobial activity is not enough; it is crucial to understand the underlying mechanisms as well. Molecular docking studies, as shown in prior research, can help with this understanding. However, there is a lack of docking data concerning the phytochemicals in this plant related to MRSA. Hence the aim of this study was to investigate the antimicrobial potential of phytochemicals from *Psoralea corylifolia* L. against MRSA and to elucidate their underlying mechanisms through molecular docking studies.

II. METHODOLOGY

➢ *Collection of Plant Samples*

The mature seeds of *Psoralea corylifolia* were collected from the Local market, Coimbatore, India. Collected material were shade dried in an open air and grinded into powder for further use.

➢ *Preparation of Extract*

The powdered seeds of *Psoralea corylifolia* were packed into a Soxhlet apparatus and extracted with ethanol and chloroform until the extraction was complete. The extracts were then filtered while hot, and the resulting solution was distilled under reduced pressure to remove the solvent completely. Afterward, the extracts were dried in a desiccator. Finally, the extracts of the seeds were stored in an airtight container for further study.

➢ *Qualitative Analysis of Phytochemicals*

The ethanol and chloroform extracts of *Psoralea corylifolia* were tested for the presence of alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids, terpenoids, and proteins following the methods described by Harborne [11] and Kokate [12].

➢ *In Vitro Antibacterial Activity of Psoralea Corylifolia extract Against ESBLs-Producing Isolates*

The agar well diffusion method was used to evaluate the in vitro antibacterial activity of all extracts of *Psoralea corylifolia* against ESBL-producing isolates according to Rajeswari *et al*., [13]. All tests were performed on Mueller-Hinton (MH) agar. Briefly, a sterile cotton swab was soaked in a bacterial suspension with a turbidity of 0.5 McFarland standards, excess suspension was squeezed out, and then the swab was used to inoculate the surface of the MH agar lightly and uniformly. Six-millimeter diameter wells were then created at equal distances on the MH agar using a sterile borer. Different concentrations of plant extracts were loaded into each well. A positive control of cefproflaxcin (5 µg) and a negative control of the respective solvent were added to each well. Finally, the plates were incubated at 37°C for 18 to 24 hours to measure the diameter of the inhibition zones.

➢ *GCMS Analysis*

To determine secondary metabolites from plant extract using GC-MS, prepare the sample by extracting the plant material with a ethanol, then filter and concentrate the extract as needed. Set up the Agilent GC-MS system with a DB-5ms column (30 m length, 0.25 mm internal diameter, 0.25 micron film thickness) and configure the carrier gas (helium, 99.999% purity) to a flow rate of 1 mL/min. Program the oven to start at 50°C for 1 minute, then increase to 300°C at 10°C/min, holding for 1 minute. Preheat the injection port to 250°C and inject 1-2 µL of the concentrated extract. Set the mass spectral scan range to 0.6 to 1091 m/z, and ensure the mass spectrometer is calibrated. Begin the analysis, allowing the GC to separate the compounds, which the mass spectrometer will identify based on their mass-to-charge ratios. After completion, analyze the chromatograms and spectra using Mass Hunter software, comparing them with the NIST20 library for identification, and quantify metabolites based on peak areas or heights if required. Document all findings, including retention times, m/z values, and identified compounds, while adhering to safety protocols throughout the process.

III. MOLECULAR DOCKING STUDY

➢ *3D Structure Analysis*

The 3D and 2D structures of the compound (+)-Bakuchiol were visualized using molecular visualization software. The crystal structure of the 1TSJ protein from *Staphylococcus aureus* was obtained from the Protein Data Bank (PDB). This structure was then analyzed to identify the active site, which is critical for understanding how the protein may interact with ligands.

➢ *Molecular Docking*

Molecular docking studies were conducted using PyRx with the AutoDock Vina 1.5.6 tool. The docking procedure began with the preparation of both the ligand and protein. The structure of (+)-Bakuchiol was optimized and minimized for energy using appropriate force fields. For the 1TSJ protein, the structure was prepared by removing water molecules and adding polar hydrogens to ensure proper docking.

The search space for the docking was defined with specific parameters, including a center located at coordinates x = 51.1356, y = 3.0581, z = 3.4783, and dimensions measuring x = 36.1913 Å, y = 45.6257 Å, and z = 32.5386 Å. The ligand was then docked into the active site of 1TSJ, and the best binding poses were analyzed based on their binding affinity scores.

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➢ *Interaction Analysis*

The interactions between the receptor (1TSJ) and the ligand (+)-Bakuchiol were thoroughly examined. Key residues involved in these binding interactions were identified and labeled in the 3D structure to provide insight into how the ligand engages with the protein. This analysis helps to elucidate the mechanism of action of (+)-Bakuchiol and its potential effects on bacterial metabolism.

IV. RESULT AND DISCUSSION

The extraction efficiency of various phytochemicals from plant materials were evaluated using ethanol and chloroform (Table 1). Results show that ethanol is superior for extracting a wide range of compounds, including alkaloids, carbohydrates, flavonoids, phenols, saponins, sterols, tannins, terpenoids, and proteins. Chloroform, in contrast, effectively extracts only carbohydrates, phenols, sterols, and terpenoids, failing to extract alkaloids, flavonoids, saponins, tannins, and proteins. Ethanol's effectiveness aligns with previous studies, such as Nabi *et al*. [7], which highlight its ability to disrupt cell membranes and release phytochemicals [14]. Both solvents extracted phenols, known for their antioxidant properties, but ethanol proved more efficient, as noted by Pandey *et al*. [15]. Sterols and terpenoids, which have pharmaceutical and pharmaceuticals applications, were extracted by both solvents; terpenoids are recognized for their antimicrobial properties [16]. Interestingly, quinones were not extracted by either solvent, and presence of proteins only in the ethanol extract indicates that polar solvents are more effective for macromolecules, as supported by Ares *et al*., [17].

Table 1 Preliminary Phytochemical Analysis of *Psoralea corylifolia Extract*

S. No	Name of the phytochemicals	Ethanol	Chloroform
1.	Alkaloids	+	-
2.	Carbohydrate	+	+
3.	Flavonoids	+	-
4.	Phenols	+	+
5.	Saponins	+	-
6.	Sterols	+	+
7.	Tannins	+	-
8.	Terpenoids	+	+
9.	Quinones	-	-
10.	Protein	+	-

The table 2 presents the antibacterial activity of various isolates *of Staphylococcus aureus* in response to ethanol and chloroform extracts, as well as the standard antibiotic gentamycin. The results indicate the zone of inhibition, measured in millimeters (mm), which reflects the effectiveness of each extract in inhibiting bacterial growth.

For the ethanol extract, isolates *S.aureus* 1, 2, 3, 4, and 5 exhibited varying degrees of inhibition. Isolate *S.aureus* 1 showed the highest zones of inhibition (10 mm, 12 mm, 14 mm, and 16 mm) across the four concentrations tested (1mg, 2mg, 3mg, and 4mg), indicating a strong response to the ethanol extract. Similarly, isolate *S.aureus* 4 also demonstrated notable inhibition (10 mm, 12 mm, 14 mm, and 16 mm). In contrast, isolate *S.aureus* 2 showed a lower response with zones of inhibition of 9 mm, 13 mm, and 15 mm with 2mg, 3mg, and 4mg respectively. Notably, isolate *S.aureus* 5 had the least effectiveness, with only a 10 mm and 12 mm inhibition observed at the higher concentrations. In 2019, Li *et al*., [18] determined the antibacterial activity of the ethanol extract of *Psoralea corylifolia* seeds against methicillin-resistant *S.aureus*. Additionally, Baig [19] also reported the antimicrobial activity of *P.corylifolia* seed extract against *S.aureus.*

While using the chloroform extract, *S.aureus* 4 showed a maximum zone of inhibition of 10 mm and 15 mm at 1 mg, 2 mg, 3 mg, and 4 mg concentrations, respectively. The other isolates, particularly *S.aureus* 1, 2, and 5, were exhibited minimal or no inhibition with chloroform, suggesting that this solvent may not be as effective. For comparison, the table 2 includes the performance of gentamycin, a standard antibiotic, which consistently exhibited a zone of inhibition ranging from 10 mm to 12 mm across different isolates. This indicates that gentamycin effectively inhibited the growth of all tested *S.aureus* isolates, serving as a benchmark for the antibacterial potency of the extracts.

Overall, the zones of inhibition with the chloroform extract were generally lower than those observed with the ethanol extract. This finding contrary with the report by Baig [20], which noted the highest inhibitory activity with water and chloroform extract than ethanol extracts of the seeds. Negative control of ethanol did not exhibit any measurable inhibitory effect across the isolates, indicating it may not possess antimicrobial properties against these strains. Overall, the data suggest that the tested compound has a dose-dependent inhibitory effect on *S. aureus*, with higher concentrations resulting in greater growth inhibition compared to standard antibiotic.

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Table 2 Antibacterial activity of seed of *P.corylifolia* against MRSA

Name of the isolates	Ethanol extract in mg Zone of inhibition in mm				Ethanol	Chloroform extract in mg Zone of inhibition in mm				Chloroform	Gentamycin (5 µg)
	1	2	3	4		1	2	3	4		
S.aureus 1	10	12	14	16	-	-	-	8	10	-	12
S.aureus 2	-	9	13	15	-	-	-	8	10	-	10
S.aureus 3	8	10	12	14	-	-	8	10	12	-	12
S.aureus 4	10	12	14	16	-	8	10	13	15	-	-
S.aureus 5	-	-	10	12	-	-	-	8	10	-	10

The diverse array of phytocompounds identified through GCMS analysis showcases their potential applications across various fields, supported by extensive research. Table 3 and Figure 1 reveal that various phytocompounds on ethanol extract of *Psoralea corylifolia*. For instance, caryophyllene oxide is noted for its anti-inflammatory and analgesic effects, as highlighted by Gushiken *et al*. [21], suggesting its role in natural pain relief therapies [22]. D-mannose has been shown to inhibit bacterial adhesion and prevent urinary tract infections [23], while isopsoralen's effectiveness in treating skin conditions like vitiligo [24] highlights its therapeutic potential additionally Liu *et al*., [25] were observed the isopsoralen from extract of *Psoralea corylifolia.*

Further, ficusin have demonstrated antioxidant and anti-inflammatory properties, suggesting potential cancer therapy applications [26]. The nutritional benefits of fatty acids like n-hexadecanoic acid and 10E, 12Z-octadecadienoic acid are emphasized in research by Prabha N & Bushra [27], which treated to rheumatoid arthritis. (+)-Bakuchiol, acetate has gained attention for its anti-aging properties, functioning as a natural antibacterial [28]. In 2022, Baig *et al*. [20] identified Bakuchiol from the seed extract of *Psoralea corylifolia*. Additionally, Mahajan *et al*. [29] reported the antibacterial activity of the Bakuchiol compound from *Psoralea corylifolia*.

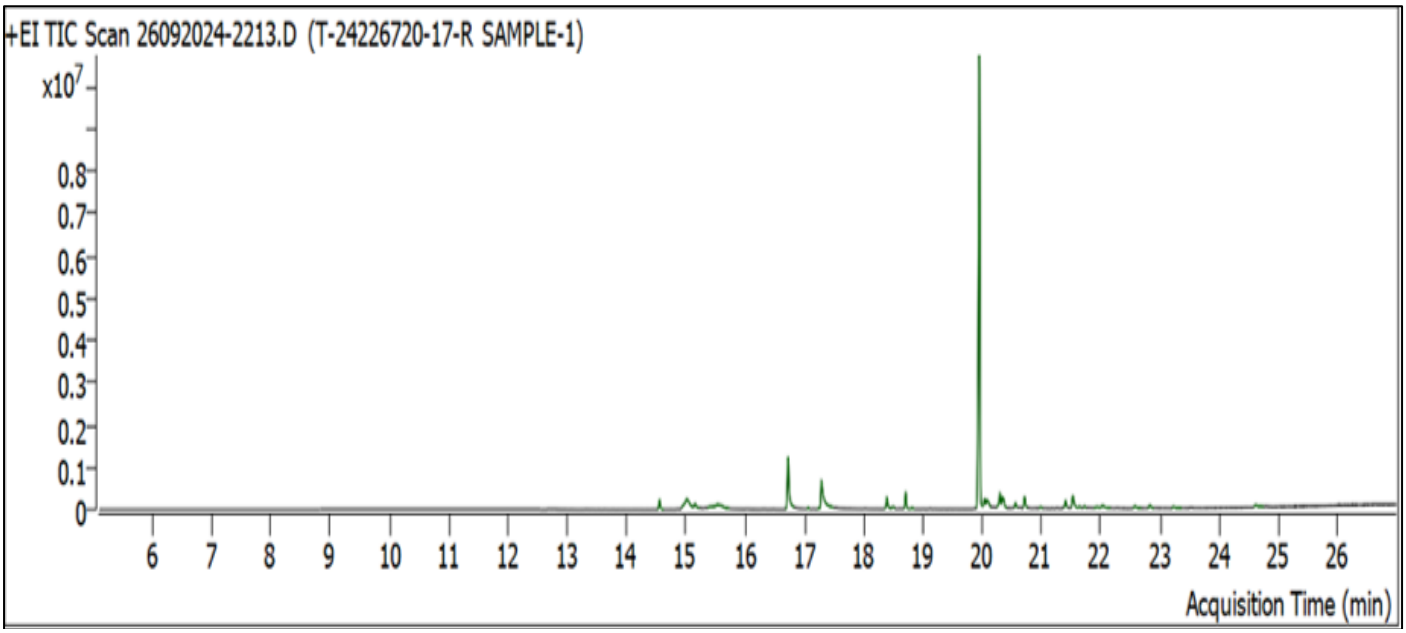

Fig 1 GCMS analysis of Ethanol Extract of *Psoralea corylifolia*

Table 3 Phytocompounds on Ethanol Extract of *Psoralea corylifolia*

RT	Compound name	CAS	Formula	Area %
14.5550	Caryophyllene oxide	1139-30-6	C15H24O	2.04
15.0138	Ethyl .alpha.-d-glucopyranoside	19467-01-7	C8H16O6	6.59
15.1522	5-Ethyl-1,3-dioxane-5-methanol, tert-butyldimethylsilylether	1000364-41-8	C13H28O3Si	1.12
15.5273	d-Mannose	3458-28-4	C6H12O6	4.98
16.7217	Isopsoralen	523-50-2	C11H6O3	15.77
17.0603	Caryophyllene oxide	1139-30-6	C15H24O	0.22
17.2388	Oxirane, [(hexadecyloxy)methyl]-	15965-99-8	C19H38O2	0.16
17.2861	Ficusin	66-97-7	C11H6O3	13.54
18.3895	n-Hexadecanoic acid	57-10-3	C16H32O2	2.45
18.5024	Phenol, 4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	1000196-38-9	C18H24O	0.49

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18.7063	Hexadecanoic acid, ethyl ester	628-97-7	C18H36O2	3.05
18.8192	Phenol, 4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl)-	1000196-38-9	C18H24O	0.26
19.9517	(+)-Bakuchiol, acetate	43010-46-4	C20H26O2	100.00
20.0354	10E,12Z-Octadecadienoic acid	2420-56-6	C18H32O2	1.55
20.0828	cis-13-Octadecenoic acid	13126-39-1	C18H34O2	2.53
20.2976	Linoleic acid ethyl ester	544-35-4	C20H36O2	3.39
20.3413	Ethyl Oleate	111-62-6	C20H38O2	3.74
20.5598	Octadecanoic acid, ethyl ester	111-61-5	C20H40O2	1.07
20.7127	2-Methyl-4,6-quinolinediamine	5443-31-2	C10H11N3	2.52
20.9822	(+)-Bakuchiol, acetate	43010-46-4	C20H26O2	0.34
21.4046	(+)-Bakuchiol, acetate	43010-46-4	C20H26O2	1.42
21.5284	(+)-Bakuchiol, acetate	43010-46-4	C20H26O2	3.46
21.6377	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	211563-96-1	C12H20O	0.26
21.7251	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	211563-96-1	C12H20O	0.29
21.9436	Cyclooctatin	139552-97-9	C20H34O3	0.48
22.0273	(9-Oxo-5,7,8,9-tetrahydro-6H-[1,2,4]triazolo[5,1-b]quinazolin-4-yl)-acetonitrile	1000275-36-1	C11H11N5O	1.26

This study provides evidence for the presence of phytochemicals in this plant that because antimicrobial activity based on various prior studies. Furthermore, understanding the mechanisms of how these phytochemicals function is essential. Therefore, this study explores the mechanism of (+)-Bakuchiol and how it acts against S. aureus through molecular docking.

The molecular docking analysis of the ligand (+)-Bakuchiol against the S.aureus receptor (1TSJ) demonstrated a binding affinity of -5.8 kcal/mol, which suggesting a robust interaction between the compound and the target protein. The 3D structures of both the ligand and the receptor, as depicted in Figures 2 and 3 provide a visual representation of their conformations, highlighting the spatial arrangement essential for effective binding.

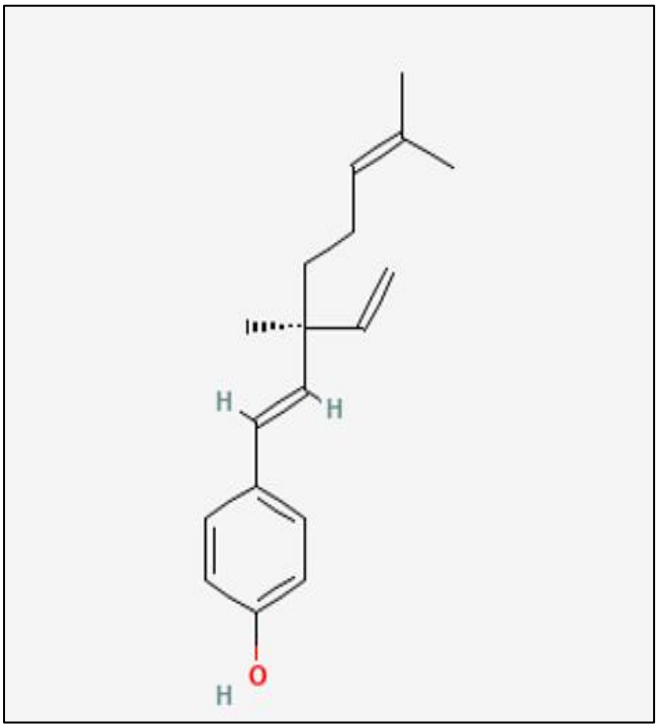

Fig 2: 3D &2D Structure of (+)-Bakuchiol Compound

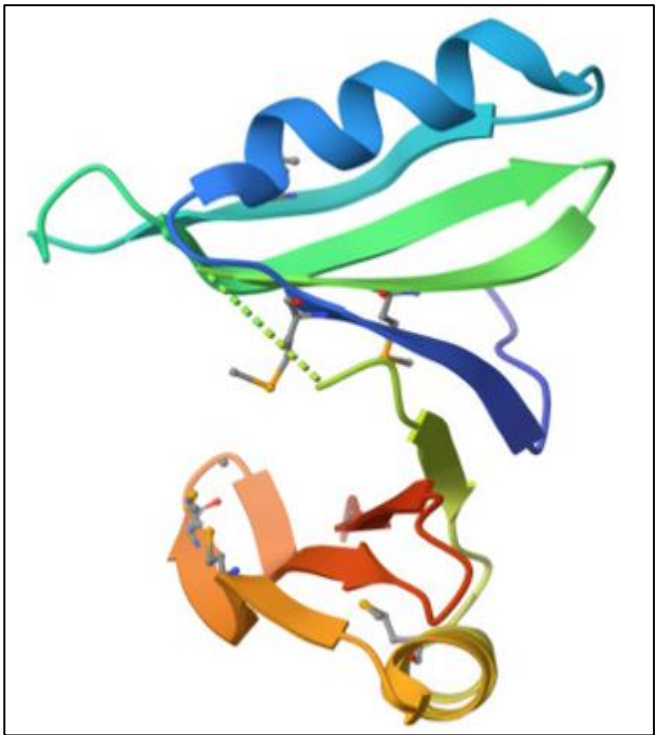
Fig 3: Protein 3D Structure of (1TSJ) Crystal Structure of Protein From S.aureus

In the docking study illustrated in Figure 4, the ligand is shown interacting with the receptor, while Figure 5 details the specific interactions at the active site, involving key amino acid residues: ILE 64, PHE 9, PHE 81, TYR 113, and PHE 116. These residues play critical roles in facilitating the binding of (+)-Bakuchiol, positioning it favorably for potential therapeutic action. The physicochemical properties of (+)-Bakuchiol, outlined in Table 3, indicate a molecular weight of 249.3 g/mol and a calculated LogP of 3.5969, which suggests moderate lipophilicity. The topological polar surface area (TPSA) of 23.1 Å² indicates the compound's potential for good bioavailability. The drug likeness score of -4.3446 further suggests that (+)-Bakuchiol may have properties conducive to pharmacological activity.

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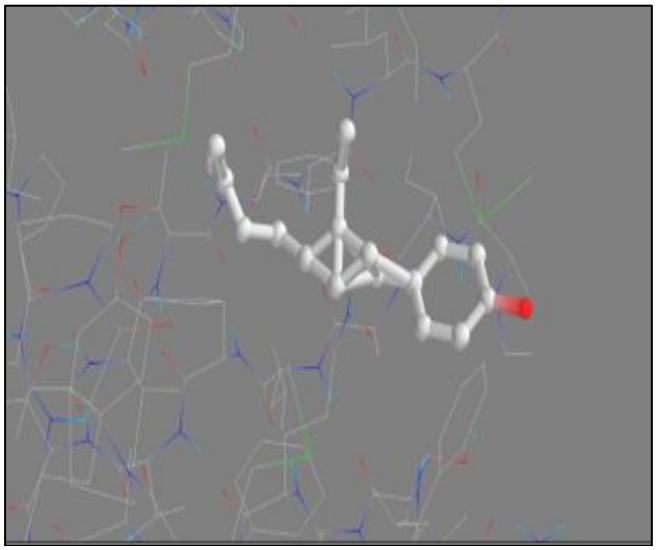
Fig 4: 3D Structure of *S. aureus* (1TSJ) with (+)-Bakuchiol Compound

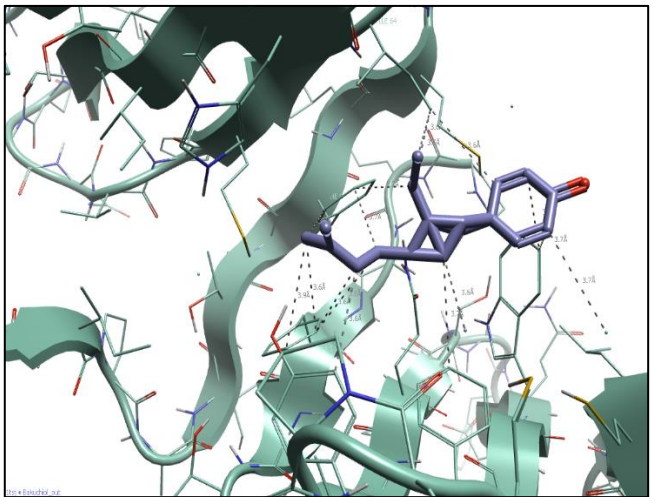
Fig 5: Interaction of Receptor (S.Aureus (1tsj)) and Ligand ((+)-Bakuchiol Compound) with Labeling

In the present study selected active site of 1TSJ is a largest cavity on its protein surface. The 1TSJ protein may play a role in bacterial metabolism by participating in redox reactions or changing small molecules. This is especially important for energy production or detoxifying harmful substances. Its function is connected to its classification in the glyoxalase/ bleomycin resistance/dioxygenase superfamily. While involved the phytochemical of (+)-Bakuchiol, the role of 1TSJ was collapsed, therefore isolate of S.aureus was inhibited.

V. CONCLUSION

In conclusion, the study demonstrates that the ethanol extract of Psoralea corylifolia exhibits significant antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA), with the compound (+)-Bakuchiol identified as a key phytochemical responsible for this effect. The qualitative analyses revealed a diverse range of bioactive compounds, while GC-MS confirmed the presence of several potential therapeutic agents. Molecular docking studies further elucidated the binding interactions between (+)-Bakuchiol and the 1TSJ protein of S. aureus, indicating its potential as a promising lead for the development of new antimicrobial agents. These findings underscore the importance of exploring plant-derived compounds to combat rising antibiotic resistance and highlight Psoralea corylifolia as a valuable resource in the search for alternative therapeutic strategies against resistant bacterial infections.

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