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Exploring the Antibacterial Potential of 2-Ethylacridine from *Salacia chinensis*: Insights into its Mechanism against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing health issue in India, leading to higher healthcare costs and increased morbidity and mortality rates. The prevalence of MRSA in both community and hospital settings has been on the rise, necessitating the exploration of alternative treatments.
Aim: This study aimed to isolate secondary metabolites from *Salacia sinensis* and determine their mode of action against MRSA through molecular docking.

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Methodology: The study involved the collection of plant samples from Tamil Nadu, India, and the extraction of phytochemicals using methanol and chloroform. GCMS analysis was conducted to identify the bioactive compounds, and molecular docking analysis was performed to assess the antibacterial activity of 2-Ethylacridine against the multi-drug resistance protein (nora) of *S.aureus*.
Results: The qualitative phytochemical screening of *S. chinensis* extracts revealed the presence of proteins, carbohydrates, flavonoids, saponins, sterols, terpenoids, and alkaloids. GCMS analysis identified bioactive compounds namely 2-Ethylacridine and Squalene. Molecular docking analysis demonstrated a high binding affinity (-6.8) of 2-Ethylacridine to the Multi-drug resistance protein (nora) of *S.aureus*.
Implications: The study provides insights into the antibacterial mechanism of 2-Ethylacridine against MRSA, suggesting its potential as a promising antibacterial agent. These findings have implications for the development of novel antibacterial agents targeting multi drug resistance proteins and addressing antibiotic-resistant strains.

Keywords: Multi-drug resistance protein (nora); MRSA; S.aureus; 2-Ethylacridine; molecular docking.

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing health issue in India. In recent years, the prevalence of MRSA has increased in both community and hospital settings, resulting in higher healthcare costs and increased morbidity and mortality rates. MRSA infections are more challenging to treat compared to non-resistant *S. aureus* infections due to their ability to resist multiple antibiotics [1].

A study by Desikan *et al*. [2] reported a high prevalence of MRSA in India, with community-associated MRSA (CA-MRSA) being the predominant strain. The study found that MRSA infections were associated with a higher risk of mortality than non-MRSA infections. Moreover, the study showed that MRSA prevalence in bloodstream infections increased significantly from 24.6% in 2008 to 42.3% in 2018.

According to another study, MRSA prevalence in Indian hospitals is also on the rise, with around 50% of hospital-acquired *S. aureus* infections being caused by MRSA. The study highlights the need to understand MRSA transmission dynamics in healthcare facilities and implement infection control measures to prevent MRSA infections.Several factors contribute to the high prevalence of MRSA in India, such as the overuse of antibiotics, inadequate sanitation, and high population density. Additionally, the scarcity of rapid diagnostic tests for MRSA makes it challenging to diagnose and treat MRSA infections effectively [3].

The current crisis of MRSA in India underscores the need for increased attention and resources to address the issue. The research to assess the effectiveness of alternative treatments, such as natural products typically medicinal plants, may also provide promising avenues in the fight against MRSA. *Salacia chinensis* L., commonly known as Saptarrangai or Saptachakra in Ayurvedic medicine, is a highly valued medicinal plant within this family.

Various parts of *S. chinensis* are rich sources of active phytoconstituents, including phenolics and flavonoids, making it a natural antioxidant [4]. The pharmacological and medicinal potentials of this plant is associated with the presence of certain bioactive molecules like salacinol, mangiferin, kotanalol, betulin-3-caffeate, morolic acid, and oleanolic acid. However, the mode of action of these chemicals against microbes has not been clearly discovered. Additionally, secondary metabolites in the *S.chinensis* plant have not been studied, particularly in terms of their mechanism of action against MRSA. Therefore, the present study aimed to isolate the secondary metabolites from the solvent extract of *S.chinensis* and determine the mode of action of the compound against MRSA through molecular docking.

2. METHODOLOGY

2.1 Sample Collection and Processing

The fresh leaves of *S.chinensis* were collected from Pichandikulam forest, Auroville, Bommayapalayam, Tamil Nadu, India. The collected plant material was identified and authenticated by the Botanical survey of India, Coimbatore, with number BSI/ SRC/5/23/2018/ Tech-2330. The collected leaves were washed with distilled water and finally dried in the shade for 2 weeks. The dried leaves were ground into fine powder to

pass a 100 mm sieve and used for further analysis.

2.2 Preparation of Solvent Extract and Determination of Preliminary Phytochemicals

Twenty gram of finely powdered *S.chinensis*was placed in the thimble of the soxhlet apparatus and then introduced into the Soxhlet tube for 8-hour extractions overheat. The extraction was performed individually using 250 mL of methanol and chloroform. The obtained extract (condensed vapor) was subsequently concentrated and dried using a rotary vacuum evaporator (Equitron, India) at 40°C under reduced pressure. The solvent was distilled off, and the dried crude residues were aseptically weighed, dissolved in respective solvents, and stored at 4°C in a sterile, labeled, airtight container until further analysis. Qualitative phytochemicals (alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids, terpenoids, and proteins) screening of methanol and chloroform extracts of *S.chinensis*was carried out using standard procedures described in the previous study [5].

2.3 Identification of phytocompounds by GCMS

Following the identification of the fraction with significant antibacterial activity through the aforementioned procedure, the compound responsible for this activity was selected for further analysis. The chosen plant extract underwent GC-MS analysis, which was conducted using a modified version of the analytical method described in the previous study [5]. Chromatograph interfaced to a mass spectrometer (GC-MS Perkin-Elmer) equipped with an Elite-1, fused silica capillary column (30 m´ 0.25 mm ID'1 m df, composed of 100% Dimethyl poly siloxane).

2.4 Molecular Docking Analysis

Protein Modeling and Visualizations: In the initial step, the gene-coded protein sequence of the multi-drug resistance protein (nora) [*Staphylococcus aureus*] (QCA26017.1) was retrieved from the NCBI Gene Database. Subsequently, the obtained protein sequence underwent automated homology modeling on the Swiss Model server (https://swissmodel.expasy.org). This process involved the conversion of the amino acid sequence into a 3D structure. The resulting modeled protein's 3D structure was then visualized using Discovery Studio software, facilitating macromolecular 3D structure visualizations.

2.5 Cheminformatics

For the cheminformatics aspect, the identification of a potential existing molecule for molecular drug docking studies was crucial. In this project, the chosen molecule was 2-Ethylacridine. To gather information on this molecule, 2-Ethylacridine was retrieved from the NCBI-PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/), with a focus on obtaining details about its 2D and 3D chemical structures.

2.6 Drug Docking Studies

The next phase involved utilizing the Mcule server (https://mcule.com/) for drug-protein docking. Molecular docking studies were performed by docking 2-Ethylacridine with the modeled multi-drug resistance protein (nora) using the Mcule server. After the docking process, the obtained results were thoroughly analyzed and interpreted to comprehend the potential interactions between the drug (2-Ethylacridine) and the target protein.

2.7 Data analysis and Interpretation

A comprehensive evaluation of the structural features and interactions revealed by the molecular docking studies was conducted. This included assessing the binding affinity and potential efficacy of 2-Ethylacridine against the multi-drug resistance protein (nora). The results were further compared with existing literature and bioinformatics databases to validate the findings.

3. RESULTS AND DISCUSSION

The results of the preliminary qualitative phytochemical screening of various extracts of *S. chinensis* indicate the presence of a diverse range of metabolites, including proteins, carbohydrates,flavonoids, saponins, sterols, terpenoids, and alkaloids. These findings align with previous studies that have demonstrated the medicinal properties associated with these chemicals [6]. Specifically, proteins and carbohydrates are important for providing the body with essential nutrients, while phenols and tannins are known for their antioxidant and anti-inflammatory properties [7]. Flavonoids and saponins have been shown to possess anti-cancer and anti-bacterial properties, while

glycosides and steroids are known for their abilities to regulate various physiological processes in the body [8]. Alkaloids have also been studied for their potential analgesic, anti-inflammatory, and anticancer effects, while terpenoids have been found to possess anti-inflammatory and anti-cancer properties, among others [9]. Therefore, the presence of these phytochemicals in *S. chinensis* highlights its potential as a valuable source of medicinal compounds.

3.1 GCMS Analysis

According to the good beneficial activity of methanol extract, it was selected for GCMS analysis. The active principles with their retention time (RT), molecular formula, molecular weight, and concentration (Peak area %) were presented in Table 1. The compounds with the major abundance were found to be Cyclotrisiloxane, hexamethyl-(1.45**%),** 2-Ethylacridine (3.59**%)** andSqualene (6.69**%).** Furthermore, the fatty acid components of Pentadecanoic acid, Hexadecanoic acid and Octadecenoic acid methyl ester were also found. Kingsley & Abraham [10] reported the antimicrobial activity of Cyclotrisiloxane, which supports the antibacterial activity of *Salacia chinensis* and 2-Ethylacridine are bacterial inhibitory substance [11]. A recent report suggested that squalene has antioxidant, moisturizing, and antimicrobial activity [12].

Table 1. GCMS analysis of *Salacia chinensis*

S.No	Retention time	Name of the Compound	Molecular formula	M. Wt. $gmol^{-1}$	Area %
1	11.842	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c] pyran-1-yl)ethanone	$C_{13}H_{18}O_2$	206.28	2.56
2	11.842	Isophthalic acid, allyl butyl ester	$C_{15}H_{18}O_4$	262.30	2.56
3	12.219	2-Phenyl-1,3-oxazol-2-ine			2.60
4	12.219	Methanone, 1,3-dithian-2-ylphenyl-	$C_{11}H_{12}OS_2$	224.342	2.60
5	12.219	1H-1,2,3-Triazole, 5-methyl-1-phenyl	$C_{10}H_9N_3O_2$	203.20	2.60
6	14.886	Hexane, 2,5-bis[(trimethylsilyl)oxy]-	$C_{12}H_{30}O_2Si_2$	262.54	1.73
7	14.886	Butane, 2,3-bis(trimethylsiloxy)-	$C_{10}H_{26}O_2Si_2$	234.48	1.73
8	17.830	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.456	3.83
9	17.830	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.5	3.83
10	19.452	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.472	5.35
11	19.452	Methyl 10-trans,12-cis-octadecadienoate	C19H34O2	294.472	5.35
12	19.529	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5	68.84
13	19.529	6-Octadecenoic acid methyl ester (z)-	$C_{19}H_{36}O_2$	296.4879	68.84
14	19.896	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.5	3.36
15	19.896	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.5	3.36
16	22.696	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6 ,7-tetrahydro-, isopropyl ester	$C_{14}H_{22}O$	179.17	1.45
17	22.696	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.4618	1.45
18	24.362	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	$C_{13}H_{22}N_2O$	222.33	3.59
19	24.362	1,2-Benzisothiazol-3-amine tbdms	$C_{13}H_{20}N_2SSi$	264.46	3.59
20	24.362	2-Ethylacridine	$C_{15}H_{13}N$	207.27	3.59
21	25.251	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (allE)-	$C_{30}H_{50}$	410.718	6.69
22	25.251	Squalene	$C_{30}H_{50}$	410.7	6.69

Although, several studies have investigated the therapeutic properties of *S.chinensis* phytochemical composition and potential [13,14,15], none have yet explored the

mechanisms underlying the activity of its bioactive compounds. Therefore, in the present study, the antibacterial activity of 2-Ethylacridine, one of the active compounds found in *S.chinensis*, was selected as a starting point for investigating the antibacterial mechanism through molecular docking analysis.

3.2 Docking Studies

Molecular drug docking studies were performed using an automated molecular drug docking server called Mcule serve. (https://mcule.com/apps/1-click-docking/). From the results obtained from drug docking studies, the binding affinities and ligand protein interactions between the modelled protein target Multi drug resistance protein (nora) [*Staphylococcus aureus*] and the selected chemical molecule (2-Ethylacridine).

>QCA26017.1 Multi drug resistant protein (nora) [*Staphylococcus aureus*]
MNKQILVLYFNIFLIFLGIGLVIPVLPVYLKDLGLTGSDLGLLVAAFALSQMIISPFGGTLADKLGKKLICIG
LILFSVSEFMFAIGQNFLILMLSRVIGGMSAGMVMPGVTGLIADISPSHQKAKNFGYMSAIINSGFILGP
GIGGFMAEVSHRMPFYFAGALGILAFIMSIALIHDPKKVSTNGFQKLEPQLLTKINWKVFITPVILTLVLS
FGLSAFETLYSLYTADKVNYSPKDISIAITGGGIFGALFQIYFFDKFMKYFSELTFIAWSLLYSVVVLILLV
FANGYWSIMLISFVVFIGFDMIRPAITNYFSNIAGERQGFAGGLNSTFTSMGNFIGPLIAGALFDVHIEA
PIYMAIGVSLAGVVIVLIEKQHRAKLKEQNM

Fig. 1. Protein FASTA Sequence (*Staphylococcus aureus*)

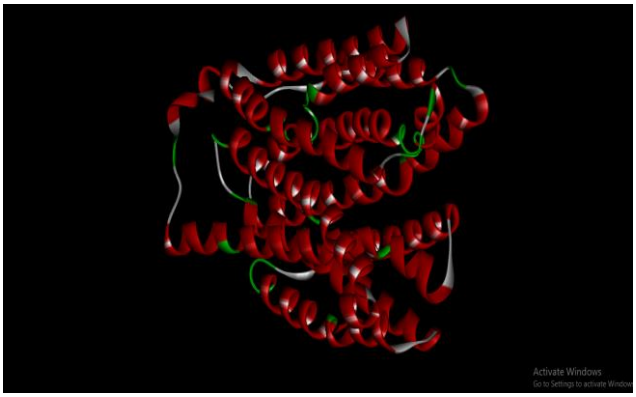

Fig.2. Protein Modelling:3 Dimensional Structure of MDR (*S.aureus*), Red: Helix, Green – Turn and White- Coils

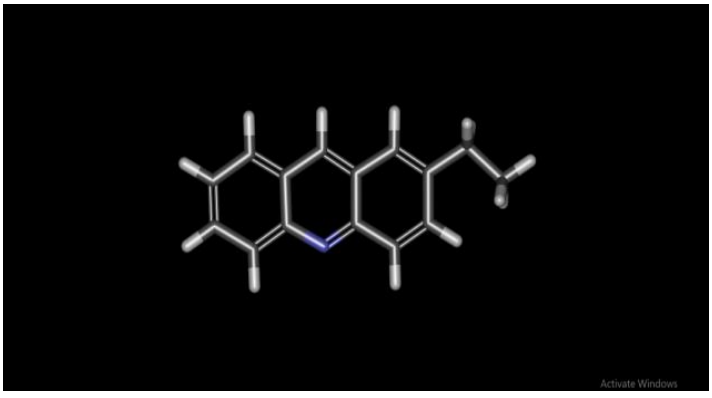

Fig.3. 3D Structure of 2- Ethylacridine: Ball and stick model OF 3D of 2-Ethylacridine – Discovery Studio software.

Docking pose	Docking score		
#1	-6.8	VISUALIZE POSE	DOWNLOAD POSE
#2	-6.8	VISUALIZE POSE	DOWNLOAD POSE
#3	-6.6	VISUALIZE POSE	DOWNLOAD POSE
#4	-6.6	VISUALIZE POSE	DOWNLOAD POSE

Fig.4. Molecular Docking – MCULE: High Binding Values -6.8 (Docking Result)

Table 2. Results of Molecular Drug Docking Patch Dock Server

	Chemical compound
Target protein	2-Ethylacridine
Multi drug Resistance protein (nora) [*Staphylococcus aureus*]	-6.8

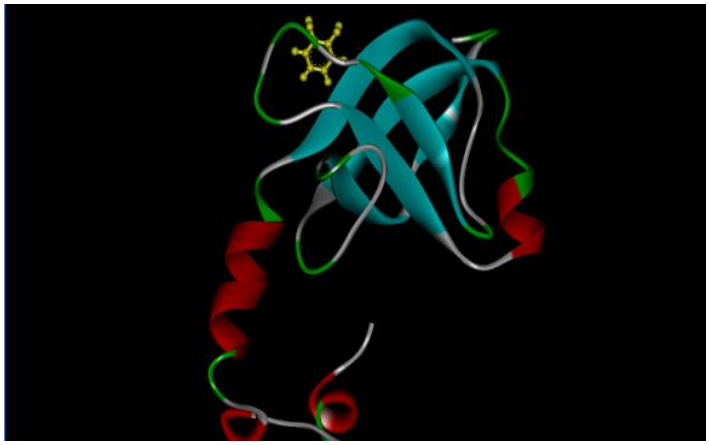

Fig.5. 3D Structure of MDR (*S.aureus*) with 2- Ethylacridine, Drug- Receptor Interaction – Active Sites

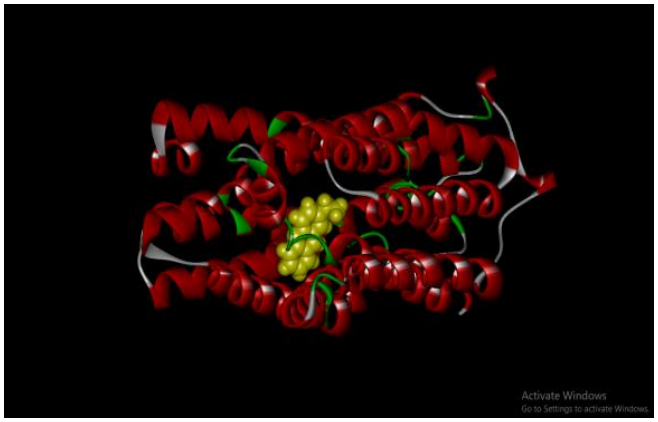

Fig.6. 3D Structure of MDR (*S.aureus*) with 2- Ethylacridine, Drug- Receptor Interaction – Active Sites with Labels

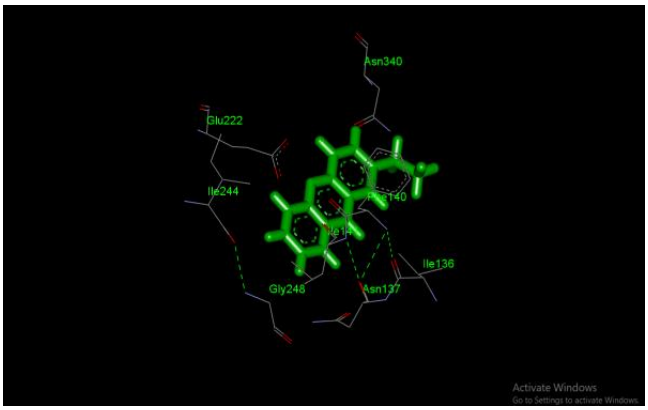

Fig.7. 3D Structure of MDR (*S.aureus*) with 2- Ethylacridine, Ligand – Receptor Binding Sites Prediction

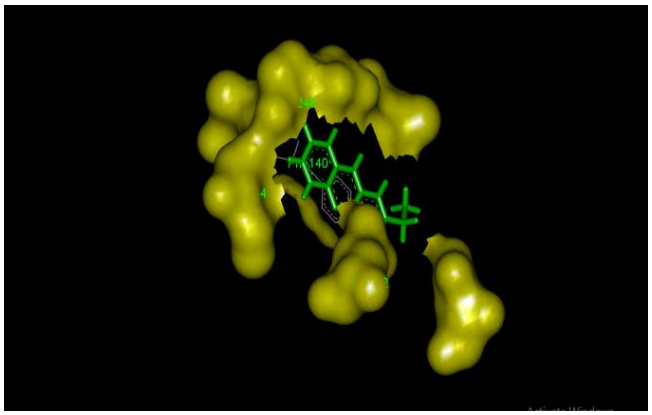

Fig.8. 3D Structure of MDR (*S.aureus*) with 2- Ethylacridine, Ligand – Receptor Binding Sites Prediction, Drug Interaction Sites with Lock and Key Model

In this present *Insilico* research investigation, the multi drug resistance protein (nora) [*Staphylococcus aureus*] (Fig: 2 and 3) and 2-Ethylacridine (Fig: 4 and 5) were docked with the help of an automated drug docking sever. The main purpose of this drug docking study is to find out whether the active site of the multi drug resistance protein (nora) [*Staphylococcus aureus*] antigen cavities fits with 2-Ethylacridine. The active sites of amino acids H – Bonds interaction with target protein are as follows: (ASN:137,SER:133,GLY:139,ASN:137,PHE:140, ILE:136,PHE:170,ASN:137,ILE:141,ASN:137,GLY:248,ILE:244,ALA:252,GLY:248,GLN:255,ASN:137)Figs. 6, 7, 8, and Table 2.

The binding of the drug is according to the Atomic contact energy (ACE) which shows a negatively high binding score of -6.8. This study provides crucial insights into the mechanism of action of 2-Ethylacridine against *S.aureus*, particularly in relation to its ability to inhibit the activity of the multi-drug resistance protein (nora). These findings may have important implications for the development of novel antibacterial agents targeting multi-drug resistance proteins, which is a significant factor contributing to the antibiotic resistance of *S.aureus*. While a previous study examined the mechanism of 2-Ethylacridine against betalactamase-producing isolates [16], this study furnishes the antibacterial mechanism of 2-Ethylacridine against MRSA as candidate for drug development to mitigate the spread of multidrug resistant strains in communities.

4. CONCLUSION

In conclusion, the phytochemical screening of *Salacia chinensis* extracts confirmed the presence of diverse metabolites, aligning with existing literature supporting their medicinal properties. This study's groundbreaking approach delves into the antibacterial mechanism of 2-Ethylacridine through molecular docking, unveiling a remarkable high binding

affinity (-6.8) to the multi drug resistance protein (nora) of *Staphylococcus aureus*. These novel findings position 2-Ethylacridine as a promising antibacterial agent, warranting further research and clinical validation to address antibiotic-resistant strains. Our research not only offers a novel perspective on combating antibiotic resistance but also underscores the vital role of collaborative efforts, benefitting the scientific community by advancing our understanding and paving the way for more effective strategies to address bacterial infections in the era of increasing antibiotic challenges.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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