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Research Article

ANTI-METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* POTENTIAL OF PHYTOCHEMICALS IN *TERMINALIA CATAPPA* AND THEIR PROPOSED *IN SILICO* MECHANISM OF ACTION

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ABSTRACT

Objective: The objective of this study was to investigate the antibacterial potential of leaves of this *Terminalia catappa* and identify the mechanism of action for those phytochemicals present in this leaves.

Methods: Phytochemicals were extracted using maceration and the extracts were analyzed using gas chromatography–mass spectrometry (GC-MS) to identify the chemical structure. Antibacterial potential was evaluated using agar well diffusion. The phytochemicals were subjected to *in silico* protein–ligand docking study to identify the mechanism of action.

Results: *In vitro* antibacterial study demonstrated that the ethanol extract of the leaves has significant antibacterial activity against *Staphylococcus aureus* (SA) and methicillin-resistant SA (MRSA) with a zone of inhibition of 16 mm and 18 mm, respectively, at a concentration of 2 mg/ml. The chloroform and hexane extracts of the leaves did not demonstrate any significant activity. Based on GC-MS analysis and literature review, 12 phytochemicals were identified to be present in the ethanol extract of the *T. catappa* leaves. These molecules were subjected to *in silico* protein–ligand docking study against common drug target proteins of SA and MRSA. Among the studied ligands, granatin A demonstrated the highest significance to inhibit topoisomerase IV with a binding energy of −11.3 kcal/mol and produced 7 hydrogen bonds, followed by punicalin with −10.7 kcal/mol binding energy toward penicillin-binding protein 2a with 6 hydrogen bonds.

Conclusion: Phytochemicals of *T. catappa* demonstrates significant drug ability potential against drug-resistant MRSA pathogen and demands further investigation on their individual activity and mechanism.

Keywords: *Terminalia catappa*, *Staphylococcus aureus*, Antibacterial activity, Protein–ligand docking, Gas chromatography–mass spectrometry.

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INTRODUCTION

The folk and traditional medicine practices are based on the properties and uses of plants and its extracts and are recognized as a great potential for the development of new medicines. Plants have inherent potential to synthesize chemical compounds that are useful and aid to uphold themselves against attack from extensive predators such as fungi, insects, and herbivorous mammals. Since decades, humans have been using spices and herbs in their day-t- day lives, which possess many medicinal compounds [1]. The most cost-effective and rapid method for researchers toward exploring the plants' bioactivity potential is reported to be *in vitro* antibacterial activity assay [2]. Since decades, many researches are being reported on antiviral, antifungal, antimolluscal, antibacterial, anthelmintic, and anti-inflammatory activities of several medicinal, food, and commercial plants [3]. Considering the enormous number of medicinal and medicinally valuable plants present in India, it is surprising that there are no market available antibiotics that are derived from plants. The present study was carried with an objective to assess the antibacterial potential of *Terminalia catappa*.

T. catappa, commonly known as tropical almond, is one of the large, spearing tropical trees, distributed throughout the warmer parts of Indian and other tropical regions. Various parts of *T. catappa* such as leaves, fruits, and seeds are studied for multiple bioactivities, since they contain diverse variety of compounds that process many medical benefits. Research on medicinal uses of *T. catappa* has been reported by several researchers over decades. Leaves and fruits of *T. catappa* are reported to contain bioactive tannins that astringent in nature [4]. *T. catappa* leaves and bark extracts are reported to possess anticancer [5], antioxidant [5], antigenotoxic [6], anti-HIV reverse transcriptase [7], anti-inflammatory [8], aphrodisiac [9], hepatoprotective[10],anticlastogenic[11],andantihepatitisactivities[6]. The ethanol extract of *T. catappa* leaves is reported to inhibit osmotically induced human erythrocyte hemolysis in a dose-dependent manner. Phytochemical molecules "punicalagin" and "punicalin" obtained from extracts of the leaves are reported beneficial for treating dermatitis and hepatitis, since they possess antioxidative activity. *T. catappa* leaves also function as a vermifuge, and also, the leaves along with mixture of kernel oil are reported to be useful in leprosy treatment [12]. The raw leaf juice is used as ingestion for cough [13]. The leaves are also reported to be beneficial in treating, indigestion, jaundice [13], and bronchial asthma [14] and to cure headaches and colic in babies [12]. Leaves, fruit, and bark are also used to treat yaws [13].

The bark of the plant is useful for bilious fever, thrush, diarrhea, sores, and abscesses [12,13]. The bark is also used for treating diabetes and stomach ache [13]. On the other hand, the fruit of this plant researched to possess cyanidin-3-glucoside and corilagin that are said to be topoisomerase I and II inhibitors and also xanthine oxidase inhibitor [15,16], gallic acid [17], ellagic acid [7], anti-asthmatic compound [17], and pentosans. The seed kernel consumption is reported to be useful for the treatment of sexual dysfunctions in men.

Despite the multiple reports of valuable medicinal properties of this commonly available plant, there are no significant publications in the recent decades and are being neglected as a potential source of antibiotic. In this current study, the antibacterial potential of *T. catappa* leaves is analyzed by *in vitro* examination against a common pathogen *Staphylococcus aureus* (SA) and methicillin-resistant SA (MRSA) a

drug-resistant bacterial pathogen that is a common threat to Indian population. The list of identified compounds that are present in the extract is then analyzed using protein–ligand *in silico* docking studies, to predict the possible mechanism of action.

METHODS

Plant extraction

Shade-dried leaves of *T. catappa* were mixed with 1:10 ratio of different solvents (EtOH, chloroform, and hexane) and were extracted by maceration for 24 h at room temperature. The mixture was then filtered and the filtrate was concentrated in rotary vacuum evaporator. The concentrated crude extract was dissolved in dimethyl sulfoxide at a concentration of 10 mg/ml for biological studies [18].

Agar well diffusion

The bacterial cultures were freshly prepared before antibiotic susceptibility test using agar well diffusion method assay. The bacterial cultures used were SA (MTCC 6) and clinical MRSA isolate. The isolates were inoculated as a lawn culture on the freshly prepared Mueller-Hinton agar plates. Wells were punched onto these Mueller-Hinton agar plates using well borers. A total of 200 µl of test sample was loaded into each well, with a concentration of 10 mg/well. The plates were incubated at 37°C overnight, and diameter of the zone of inhibition was measured [19-21].

Gas chromatography–mass spectrometry (GC-MS)

The ethanol extract of *T. catappa* leaves was subjected to GC-MS analysis, at Vellore Institute of Technology University. The sample was also evaluated for area percentage analysis [22-24].

Protein–ligand docking

The three-dimensional (3D) structure of the protein molecules penicillin-binding protein 2a (PBP2a), topoisomerase IV, dihydropteroate synthase (DHPS), and dihydroorotate dehydrogenase was retrieved from RCSB website www.rcsb.org. The protein molecules were cleaned, by removing all non-amino acid derivatives from the PDB file. The PDB ID of the protein is topoisomerase IV – 2INR, PBP2a – 3ZG5, dihydrofolate reductase (DHFR) – 4FGG, and DHPS – 1AD1. The ligand molecules were downloaded from the PubChem website with the respective ID. Protein–ligand docking was performed using AutoDock Vina. The results of the docking study were visualized using LigPlot-Plus and Pummel tools to study the two-dimensional and 3D interactions, respectively [25].

RESULTS

Antibacterial potential of *T. catappa*

Among the 3 studied extracts of *T. catappa* leaves (ethanol, chloroform, and hexane), the ethanol extract demonstrated significant antibacterial activity against both SA and MRSA, with a zone of inhibition of 16 mm and 18 mm at 2 mg/well concentration, respectively. The agar well diffusion method antibacterial activity results are shown in Fig. 1. The results show that the ethanol extract possesses significant antibacterial activity against both the drug-sensitive and drug-resistant SA. The extract demonstrated a slight increase in the activity toward MRSA, suggesting its specificity toward to the drug-resistant pathogen.

GC-MS analysis

The ethanol extract of *T. catappa* leaves was subjected to GC-MS analysis to identify its phytochemical constituents. Area% analysis was done to identify the percentage contribution of individual compounds in the crude extract. The chromatogram image of GC-MS along with the table for area% analysis is shown in Fig. 2.

Phytochemical constituents

Based on the mass spectrum data search in the NIST library from GC-MS and based on the literature reports, the following 12 phytochemical molecules were identified to be present in the ethanol extract *T. catappa* leaves. The compounds are tabulated in Table 1.

Protein–ligand docking study

The identified phytochemicals present in the ethanol extract of *T. catappa* leaves are subjected to protein–ligand docking study, to predict the mechanism of action of the observed activity. The results of the docking analysis are tabulated in Table 2. A total of 28 dockings were performed, i.e. 12 ligands with 4 proteins. Among the studied drug targets, significant interaction between protein and ligand were observed with topoisomerase IV and PBP2a proteins.

Topoisomerase IV protein is a key enzyme in the DNA replication, playing a key role in the unwinding of DNA. Inhibition of topoisomerase would produce a bacteriostatic effect, by preventing bacterial cell multiplication. The results of this analysis show that granatin A and terflavin A ligands present in the leaf extract shows the highest significance in inhibiting topoisomerase IV, with a free binding energy of −11.3 kcal/mol and −11.2 kcal/mol, respectively. Granatin A demonstrated a strong affinity toward the protein, with formation of 7 hydrogen bonds (Ser-108, Lys-266, Ile-109, Thr-216, Pro-215, and Arg-294) along with 9 hydrophobic interactions. The molecular interactions between granatin A and topoisomerase IV are graphically represented in Fig. 3.

PBP2a is a cell wall biosynthesis protein that plays a key role in the drug resistance of SA and characteristic feature of identification of MRSA. PBP2a protein is a low-affinity protein that has poor binding potential with beta-lactam antibiotics. Inhibition of PBP2a would result in bactericidal effect by disruption of the bacterial cell wall. The results of this analysis show that punicalin and terflavin A molecules present in the *T. catappa* leaves shows significant binding affinity toward inhibition of PBP2a, with a binding energy of −10.7 kcal/mol and −10.4 kcal/mol, respectively. Punicalin produced a strong protein–ligand binding, with 6 hydrogen bonds (Tyr-196, Lys-148, Thr-238, Ser-149, His-293, and Met-372) along with 22 hydrophobic interactions with the residues in the binding pocket. The interactions between punicalin and PBP2a are graphically represented in Fig. 4.

DHPS is a protein involved in the biosynthesis of folic acid. Inhibition of this enzyme would result in a bacteriostatic effect as it would reduce the metabolism of the bacterial cells and prevent further growth/multiplication of the bacterial cells. The results of this docking study showed that the ligand mupirocin showed significance to inhibit DHPS enzyme with a free binding energy of −10.2 kcal/mol.

The protein–ligand docking study suggests that the phytochemical molecules present in the leaves of *T. catappa* have significant potential to inhibit key metabolic enzymes of the Gram-positive bacterium, correlating to the observed *in-vitro* antibacterial activity.

DISCUSSION AND CONCLUSION

In silico prediction of the bioactivity of target molecules and prediction of mechanism of action of observed activity is a popular approach that is attracting spotlight in the recent years toward acceleration

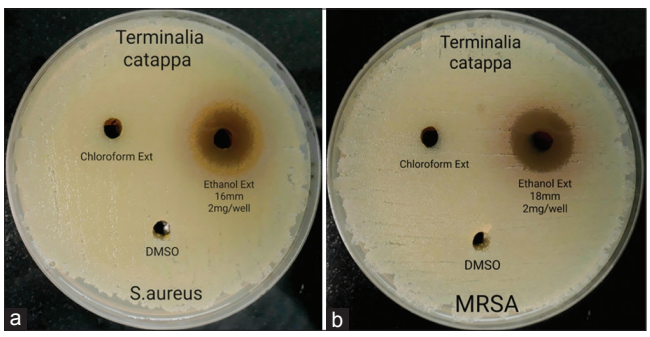
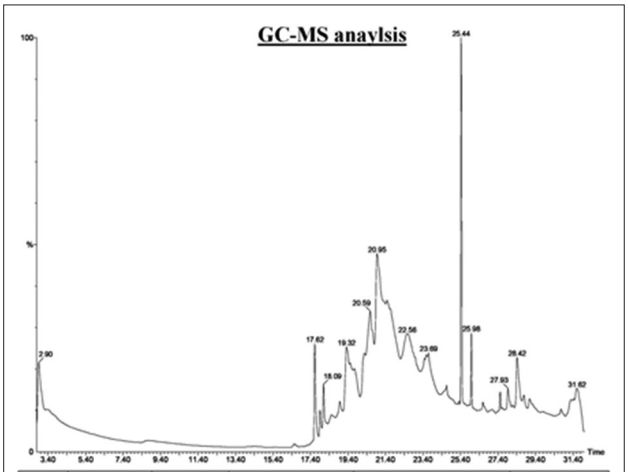


Fig. 1: (a and b) Antibacterial potential of *Terminalia catappa* against *Staphylococcus aureus* (SA) and methicillin-resistant SA



#	RT	Scan	Height	Area	Area %
1	17.610	2961	186,814,976	9,425,033.0	3.726
2	19.295	3298	84,491,504	15,368,836.0	6.076
3	19.515	3342	57,352,940	11,651,242.0	4.606
4	19.740	3387	46,543,048	8,516,503.0	3.367
5	20.261	3491	57,162,408	11,185,218.0	4.422
6	20.586	3556	105,465,656	32,323,818.0	12.778
7	20.941	3627	173,528,064	113,307,608.0	44.793
8	22.487	3936	41,374,644	18,029,348.0	7.127
9	25.433	4525	975,548,800	23,948,596.0	9.467
10	28.419	5122	66,539,392	9,200,430.0	3.637

Fig. 2: Gas chromatography–mass spectrometry analysis of the ethanol extract of *Terminalia catappa* leaves, with area% analysis

of pharmaceutical research [26-29]. India is a tropical country filled with a large amount of pharmaceutically valuable plants that demonstrate preferred bioactivity better than the available standard pure compound molecules. The fact that these plants consist of multiple bioactive molecules of similar and/or distantly related chemical molecules provides an aggregated and synergistic effect that supersedes the activity of single mode of action of pure chemically synthesized molecules [30-32]. The difficulty in purification and mass production of the phytochemical molecules is the greatest hurdle in developing marketable drugs from the plant sources. However, these phytochemicals have great potential to be developed into a marketable drug. The results of this current study strongly suggest that the phytochemicals of *T. catappa* leaves have significant antibacterial activity against Gram-positive SA and drug-resistant MRSA bacterial pathogens. Further analytical study and literature review identified the phytochemical molecules present in the *T. catappa* leaves. Such identified molecules were further subjected to protein–ligand docking study, to identify the possible mechanism of action for the observed antibacterial activity. The *in silico* studies suggest that the crude ethanol extract of *T. catappa* exhibits a synergistic effect by targeting multiple proteins such as topoisomerase IV, DHPS, and PBP2a protein, conferring both bactericidal and bacteriostatic effects on the bacterium. Further purification and identification of the individual phytochemicals would greatly benefit understanding the mechanism of action individually. Studies are currently being pursued in purification and analysis of pure phytochemicals from *T. catappa* leaves. The results of this study prove that *T. catappa* leaf extract demonstrates significant anti-MRSA activity, with potential for medicinal values.

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Table 1: List of identified phytochemicals present in leaves of *T. catappa*

Phytochemical	Molecular weight	References
Geraniin	952.648 g/mol	Griffiths LA. On the distribution of gentisic acid in green plants. J Exp Biol 1959;10:437.
Gentisic acid	194.118 g/mol	
Kaempferol	286.239 g/mol	List PH, Horhammer L. Hager's Handbuch der Pharmazeutischen Praxis. Vol. 2-6. Berlin: Springer-Verlag; 1969-1979.
Quercetin	302.238 g/mol	
Chebulagic acid	954.664 g/mol	Tanaka T, Nonaka GI, Nishioka I. Tannins and related compounds. XLII. Isolation and characterization of four new hydrolyzable tannins, terflavins A and B, tergallagin and tercatain from the leaves of *T. catappa* L. Chem Pharm Bull 1986;34:1039-49.
Corilagin	634.455 g/mol	
Granatin A	784.544 g/mol	
Punicalagin	1084.722 g/mol	
Punicalin	782.528 g/mol	
Tercatain	786.56 g/mol	
Terflavin A	1086.738 g/mol	
Terflavin B	784.544 g/mol	

T. catappa: *Terminalia catappa*

Table 2: Binding energies of ligands present in *Terminalia catappa* against bacterial drug targets

Phytochemical	PubChem ID	Topoisomerase IV	DHFR	DHPS	PBP2a
		Binding energy (kcal/mol)			
Corilagin	5578	−5.8	−7.2	−5.8	−5.3
Chebulagic acid	73568	−9.1	−7.4	−8.5	−9.6
Mupirocin	250397	−9.3	−7.4	−10.2	−8.9
Quercetin	3001497	−8.6	−7.3	−9.2	−7.2
Kaempferol	5280343	−7.7	−8.7	−7.7	−7.8
Tercatain	5280863	−7.1	−8.4	−7.3	−7.7
Terflavin B	14411426	−9.1	−8.6	−9.3	−9.1
Gentisic acid	14886031	−8.9	−8.9	−9.5	−8.3
Punicalagin	44584733	−10.1	−8.4	−9.3	−9.1
Punicalin	92131301	−10	−8.3	−9.6	−10.7
Terflavin A	101589226	−11.2	−8.7	−9.8	−10.4
Granatin A	131752596	−11.3	−7.8	−9.5	−9.4

DHFR: Dihydrofolate reductase, DHPS: Dihydopteroate synthase, PBP2a: Penicillin-binding protein 2a

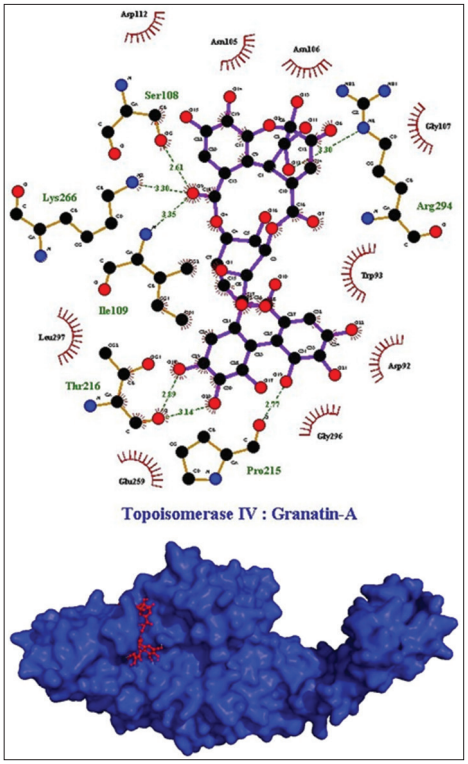

Fig. 3: Interaction between topoisomerase IV and granatin A

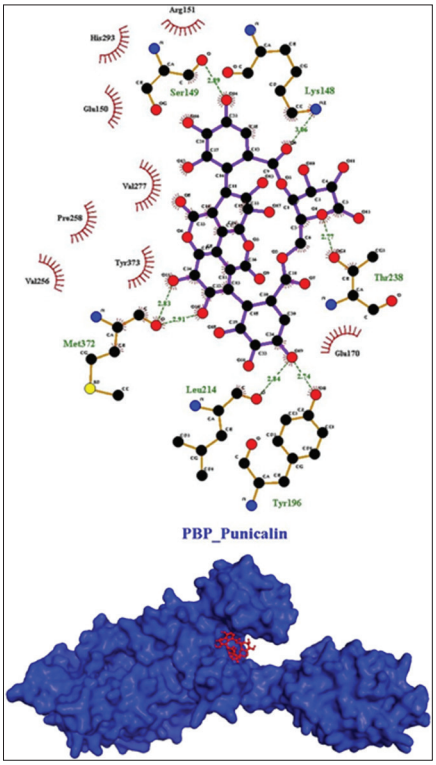

Fig. 4: Interactions between penicillin-binding protein 2a and punicalin

AUTHOR CONTRIBUTIONS

All authors provided equal contribution in research and manuscript preparation.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest for this research work.

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