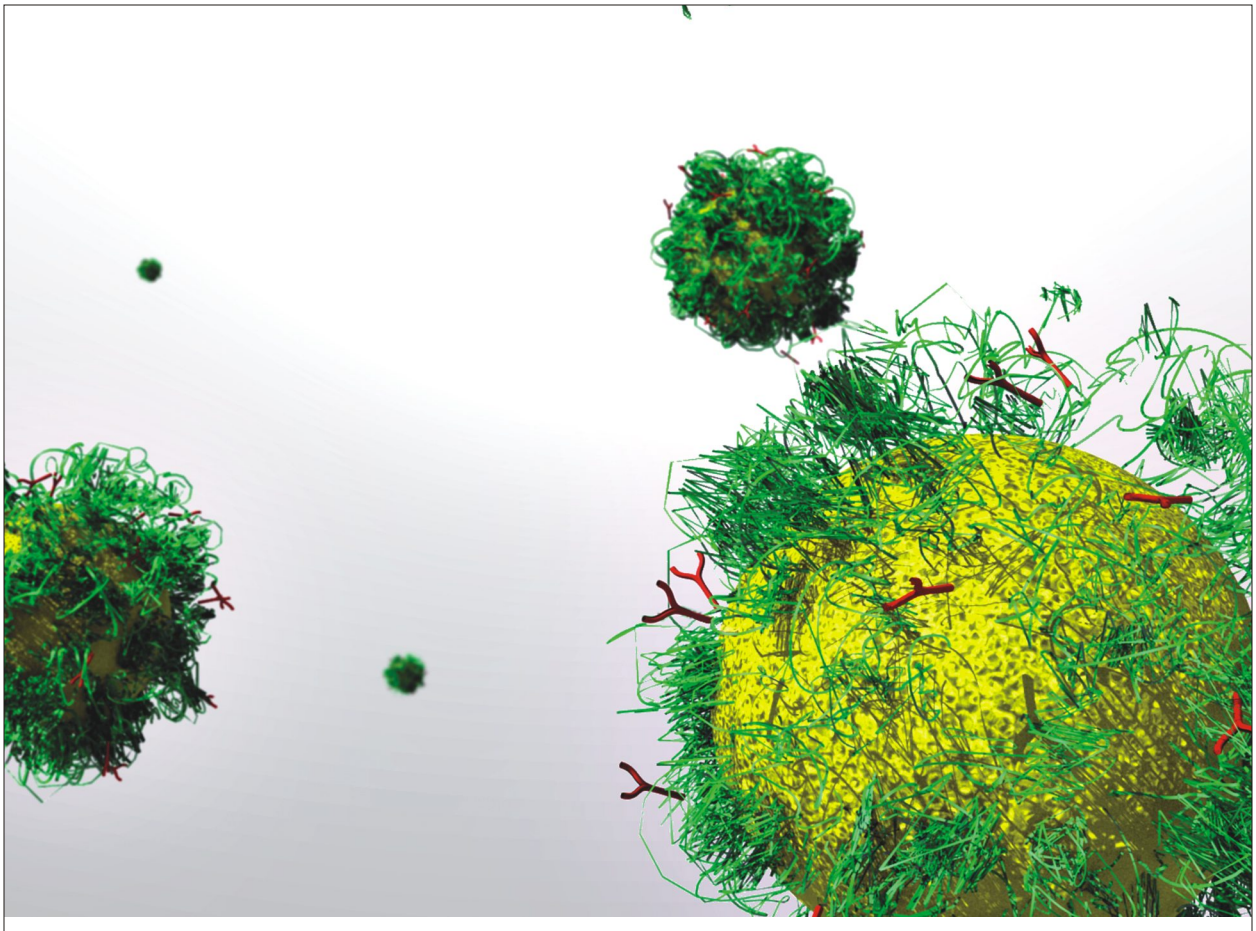

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Antimicrobial Activity of Phytochemicals Extracted from *Acanthus ilicifolius* Leaves Against *Staphylococcus aureus*: An *In silico* Approach

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ABSTRACT

Acanthus ilicifolius is lesser-known medicinal plant is used as medicine in traditional systems. *Staphylococcus aureus* is inducing many infections and it can infect tissues when the skin and mucosal barriers. The plant *Acanthus ilicifolius* GC-MS have reported the following phytochemicals, 26.27-Di (nor)-cholest-5,7,23-trien-22-ol, 3-methoxymethoxy, 9H-purin-6-amine, N, 9-bis (trimethylsilyl)-8-((trimethylsilyl) Oxy), Cyano colchicines and 3beta-methoxy-5-choleston-19-oic acid against a 12 essential protein and synthetic drug of *S. aureus* was collected through literature survey. *S. aureus* was studied zone of inhibition in different concentrations against microorganisms and essential proteins were used as target which were docked using phytochemicals (ligands) found in *A. ilicifolius* leaves. In the present study, to analyze the effect of *A. ilicifolius* against *S. aureus* through docking and inhibition studies, that *A. ilicifolius* has significant activity against *S. aureus*. This research was helpful attempt to the drug discovery in *S. aureus*.

Key words: *Staphylococcus aureus*, *Acanthus ilicifolius*, steroid components, target proteins

INTRODUCTION

Acanthus ilicifolius Linn. (Acanthaceae), locally known as 'sea holly' is a spiny shrub found in the low lying coastal areas of southern districts in Bangladesh and the vast area of mangrove forest, the Sunderbans and it is also widely distributed. It is a viny shrub or tall herb and bushy with very dense growth (Lakshmi *et al*., 1997). Many phytochemicals are present in *A. ilicifolius* which is responsible for antimicrobial, chemo preventive, hepatoprotective, anti-osteoporotic activity and anti-inflammatory activities (Babu *et al*., 2001; Agshikar *et al*., 1979). Long back, the leaf juice of *A. ilicifolius* was commonly used both internally and externally for treating rheumatism, wounds, neuropathy and snake bites. The seeds were ground and their paste was administered for boils. In Ayurvedic medical systems, *A. ilicifolius* is as an astringent, nervine tonic, stimulant and expectorant.

Earlier GC-MS studies was done in our laboratory shown that the following major components present were in the *A. ilicifolius* leaves-26,27-di (nor)-cholest-5,7,23-trien-22-ol, 3-methoxymethoxy, 9H-purin-6-amine, N, 9-bis (trimethylsilyl)-8-((trimethylsilyl) Oxy), Cyano colchicines and 3beta-methoxy-5-cholesten-19-oic acid (Bala *et al*., 2011).

S. aureus has increased the risk of the infection development, both nosocomial and community-acquired (DeLeo *et al.*, 2010; Rafee *et al.*, 2012; Wang *et al.*, 2010). In 1960 methicillin-resistant *S. aureus*. MRSA, has become one of the disease-producing agent for healthcare associated infections and it's become very difficult to treat (Bigos and Denys, 2008).

The present study aims to analyze the phytochemicals by GC-MS and evaluate the antimicrobial activity of most relevant compounds identified and quantified in *A. ilicifolius*. Furthermore, a Structure-activity Relationship (SAR) analysis and molecular docking studies against *S. aureus* were performed, in order to provide the mechanism of potential antimicrobial drugs for resistant microorganisms. Molecular docking is an *in silico* tool that predicts how a ligand interacts with a receptor and has been successfully applied in several therapeutic programme at the lead drug discovery (Ghosh *et al.*, 2006).

MATERIALS AND METHODS

Antibacterial activity

Zone of inhibition: *Acanthus ilicifolius* were botanically identified by the Botanical survey of India, Tamil Nadu Agriculture University, Coimbatore. A voucher specimen of the plant has been deposited at the botanical survey of India herbarium (Voucher number-53582). Fresh leaves of *A. ilicifolius* were cleaned with deionized water and dried in the shade. The dried leaves were ground and sieved. Soxhlet extrtaction was carried out for the *Acanthus ilicifolius* leaf samples with solvent methanol (Okunade *et al.*, 2007).

Microorganism was grown overnight at 37°C in nutrient broth (Perez *et al.*, 1990). The antimicrobial activity of the crude leaf extract of *A. ilicifolius* was studied in different concentrations against bacterial strain, the antimicrobial potential of leaf extract was assessed in terms of zone of inhibition of bacterial growth.

Antibacterial activity

***In silico* analysis:** The list of 12 genes involved in *S. aureus* infection pathway in human was collected from literature (Bartlett and Hulten, 2010). These were found to be essential for the survival of the organism as reported in the DEG database (www. deg. Org). They are Alpha hemolysin, Panton Valentine leukocidin, Enterotoxin, Toxic shock syndrome toxin-1, Staphylokinase, Aureolysin, Clumping factor A, Clumping factor B, Sec A protein translocase, Serine-aspartate repeat-containing protein C, 30S ribosomal protein S4, Serine-aspartate

Table 1: Genes and proteins of *S. aureus* responsible for infection

Genes	Proteins	Uniprot ID
Hla	Alpha hemolysin	Q7A632
Luk	Panton valentine leukocidin	
EntG	Enterotoxin	P0A0L7
Tst	Toxic shock syndrome toxin-1	Q7A4K7
Sak	Staphylokinase	Q99SU7
Aur	Aureolysin	Q7A378
ClfA	Clumping factor A	Q99VJ4
ClfB	Clumping factor B	Q7A382
Sec A	Sec A protein translocase	Q7A6R5
Sdrc	Serine-aspartate repeat-containing protein C	Q7A781.
Sas G	30S ribosomal protein S4	P66563
Can	Serine-aspartate repeat-containing protein D	P66563

repeat-containing protein D (Table 1). These proteins were taken as targets for docking with phytochemicals identified in *A. ilicifolius*. The structure of all target proteins for *Staphylococcus aureus* was retrieved from PDB (Protein Data Bank). The binding site of all target proteins was found using the Q site finder for grid generation in Schrodinger. Identifying the ligand binding sites on a protein is the functional importance of many applications including molecular docking, *de novo* drug design and structural identification and comparison of functional sites.

ISIS Draw 2.3 software was used to design the phytochemicals. Analogues were changed in MOL files and 3D optimization was done by ChemSketch 3D viewer of ACDLABS 8.0. The MOL files were converted into PDB format using VEG ZZ software. The docking studies have been used with Schrodinger Glide program version 4.0. The best 10 poses and corresponding scores have been evaluated using Glide in single precision mode (GlideSP) for each ligand. For each screened ligand, the pose with the lowest Glide SP score has been taken as the input for the Glide calculation in extra precision mode (Glide XP). The docking was carried out with Glide SP and Glide XP.

RESULTS AND DISCUSSION

A. ilicifolius methanolic extracts has shown significantly higher when compared to methanolic control ($p>0.05$). In order to verify the results, the experiment was triplicated and Mean±SD was reported (Table 2). The phytochemical reported in *A. ilicifolius* were considered as ligands for docking. These essential proteins in *S. aureus* were used as target proteins which were docked using phytochemicals (ligands) found in *Acanthus ilicifolius* leaves using Schrodinger software. (Table 3).

Although the phytochemicals exhibited bonding with proteins present in *S. aureus*, their interaction was found to be less when compared to common drug Penicillin G. So it is suggested that although the phytochemicals present in *Acanthus ilicifolius* leaves has individual insignificant activity against *S. aureus* ($p>0.05$), there may be a synergy effect of phytochemicals together in inhibiting the growth of *S. aureus* as seen in the zone of inhibition. This research was an attempt to take a one step towards the drug discovery for *Staphylococcus aureus*.

Table 2: Antibacterial activity of *A. ilicifolius*

Concentration (mg mL^{-1})	Zone of inhibition (mm)
60	13.333±2.08
80	13.333±2.08
100	16.333±1.52
Control (methanol)	7±0.5700000

Table 3: Docking of phytochemicals present in the leaves of *A. ilicifoilus* with *S. aureus* target proteins

S. No.	Ligand	Proteins	No. of bonds	Docking score	Glide score
1	3β-methoxy-5-cholesten-19-oic acid	Aur	2	-3.629007	-31.612565
		Can	1	-2.958666	-23.785371
		ClfA	1	-1.950176	-35.926784
		EntG	2	-4.087633	-31.674371
		Sas	1	-1.052580	-39.209316
		clfB	2	-1.406307	-30.728404
2	9H-purin-6-amine, N, 9-bis	Aur	2	-3.409015	-43.811679
	(trimethylsilyl)-8-((trimethylsilyl) oxy)	ClfA	1	-3.541631	-43.589035
		EntG	3	-3.786835	-40.362101
		Sak	1	-2.792525	-38.602261

Table 3: Countinue

S. No.	Ligand	Proteins	No. of bonds	Docking score	Glide score
		SasG	1	-2.181615	-43.424216
		clfB	2	-3.513492	-33.670037
3	26.27-Di (nor)-cholest-5, 7,	Aur	2	-1.737733	-40.038974
	23-trien-22-ol, 3-methoxymethoxy	Can	1	-2.427747	-19.748545
		ClfA	2	-4.080783	-36.320812
		EntG	2	-3.746605	-26.153047
		Sak	1	-3.046489	-32.417226
		SasG	1	-2.512295	-29.181025
		ClfB	3	-5.331937	-30.680526
4	Cyano colchicines	clfB	1	-2.137746	-38.090149
		ClfA	3	-2.302054	-26.866772
		EntG	1	-2.198476	-28.403514
		Sak	1	-1.851170	-27.809389
		SasG	2	-1.886397	-39.627417
5	Penicillin G	clfA	4	-4.279696	-37.523643
		SasG	3	-2.859226	-29.575121
		Aur	3	-5.791304	-72.369454
		Sak	4	-2.710948	-24.753152
		clfB	3	-5.111300	-43.262107
		Sdr C	2	-2.286182	-29.668813
		SecA	3	-5.498118	-34.958509
		Luk	2	-5.140295	-34.958509

REFERENCES

Agshikar, N.V., V.R. Naik, G.J. Abraham, C.V. Reddy, S.W. Naqvo and P.K. Mittal, 1979. Analgesic anti-inflammatory activity of *Acanthus illicifolius* Linn. Indian J. Exp. Biol., 17: 1257-1258.

Babu, B.H., B.S. Shylesh and J. Padikkala, 2001. Antioxidant and hepatoprotective effect of *Acanthus iliciofilus*. Fitoterapia, 72: 272-277.

Bala, J.M., J.J. Vennila, S. Ganesh and S. Singh, 2011. Analysis of active components in *Acanthus ilicifolius* leaves for their potential chemoprotective activity against Hepatocellular carcinoma: An *In silico* Approach. J. Pharm. Res., 4: 1809-1812.

Bartlett, A.H. and K.G. Hulten, 2010. *Staphylococcus aureus* pathogenesis: Secretion systems, adhesins and invasins. Pediatr. Infect. Dis. J., 29: 860-861.

Bigos, M. and A. Denys, 2008. The MRSA hospital infections. Int. Rev. Allergol. Clin. Immunol., 14: 101-109.

DeLeo, F.R., M. Otto, B.N. Kreiswirth and H.N. Chambers, 2010. Community-associated methicillin resistant *Staphylococcus aureus*. Lancet, 375: 1557-1568.

Ghosh, S., A. Nie, J. An and Z. Huang, 2006. Structure-based virtual screening of chemical libraries for drug discovery. Curr. Opin. Chem. Biol., 10: 194-202.

Lakshmi, M., S. Rajalakshmi, M. Parani, C.S. Anuratha and A. Parida, 1997. Molecular phylogeny of mangroves I. Use of molecular markers in assessing the intraspecific genetic variability in the mangrove species *Acanthus ilicifolius* Linn. (Acanthaceae). Theor. Applied Genet., 94: 1121-1127.

Okunade, M.B., J.A. Adejumobi, M.O. Ogundiya and A.L. Kolapo, 2007. Chemical, phytochemical compositions and antimicrobial activities of some local chewing sticks used in South Western Nigeria. J. Phytopharmacother. Nat. Prod., 1: 49-52.

Perez, C., A. Pauli and P. Bazerque, 1990. An antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp., 15: 113-115.

Rafee, Y., N. Abdel-Haq, B. Asmar, T. Salimnia, C.V. Pharm, M.J.R. Pharm and M. Amjad, 2012. Increased prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization in household contacts of children with community acquired disease. BMC Infect. Dis., Vol. 12. 10.1186/1471-2334-12-45

Wang, J.T., C.H. Liao, C.T. Fang, W.C. Chie, M.S. Lai, T.L. Lauderdale and S.C. Chang, 2010. Incidence of and risk factors for community-associated methicillin-resistant *Staphylococcus aureus* acquired infection or colonization in intensive-care-unit patients. J. Clin. Microbiol., 48: 4439-4444.