JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

In Vivo and *in Silico* analysis of anti-inflammatory activity of *Acanthus ilicifolius* L. stem

Chinchu E. R.[a], Shanitha A[a*], Ancy Simon[b], Helen Antony[b], Achuthsankar S. Nair[a]

[a]*Department of Computational Biology and Bioinformatics, Karyavattom Campus, University of Kerala, India*

[b]*Department of Biochemistry, Karyavattom Campus, University of Kerala, India*

**Corresponding author. Department of Computational Biology and Bioinformatics, Karyavattom campus, University of Kerala, Inida,*

Abstract

Objective: To investigate the antioxidant and anti-inflammatory activity of the stem of *Acanthus ilicifolius* L., a mangrove commonly found in Vypin Island, India. **Methods:** Qualitative analysis and quantification of Total Phenol Content, Total Flavonoid Content, and Total Antioxidant Capacity was carried out by chemical methods. The compounds from the stem extracts were analyzed by Gas-Chromatography and Mass Spectroscopy. The in-vitro antioxidant property was determined by 2,2-diphenyl-1-picrylhydrazl assay and the *in vivo* anti-inflammatory activity was determined by carrageenan-induced rat paw edema in *Wistar albino* rats. The in silico ADME prediction and anti-inflammatory activity in different inflammatory was done using Discovery Studio2018. **Result:** *Acanthus ilicifolius* L. stem has steroids, flavonoids, alkaloids, and phenolic compounds. The crude methanol extract showed the highest yield of Total Phenolic Content (27.87 mg), Total Flavonoid Content (12.12 mg), Total Antioxidant Capacity (45.45 mg), and significant antioxidant efficiency against 2,2-diphenyl-1-picrylhydrazl radicals. The GC-MS analysis of the methanol extract revealed 28 compounds. The *in vivo* experiments showed potent anti-inflammatory activity at a concentration of 100 mg/kg. ADME estimation showed that five compounds have drug-like properties. The *in silico* study confirmed the strong affinity of five compounds selected based on ADME properties with inflammatory targets such as COX-2, LOX-5, TNF-α, and IL-2. Conclusions: The active phytochemicals of methanol and aqueous extracts of the stem of *Acanthus ilicifolius* L. have both free radical scavenging and anti-inflammatory activity. Further, scientific studies of the *Acanthus ilicifolius* L. stem extract will be essential to identify the pharmacological potential.

Key words: *Acanthus ilicifolius*, DPPH, Paw oedema, ADME, Molecular Docking

Abbreviations: ADME- Absorption, Distribution, Metabolism and Excretion; BW-Body Weight; CNS - Central Nervous System; COX-2 - Cyclooxygenase-2; DPPH - 2,2-diphenyl-1-picrylhydrazl; GAE-Gallic acid equivalents; GC-MS-Gas Chromatography-Mass Spectrometry; IC_{50} -half maximal inhibitory concentration; IL-2-Interleukin-2; OECD-Organisation for Economic Co-operation and Development; PDB-Protein Data Bank; QE- Quercetin equivalent; RT- Retention Time; SEM-Standard Error Mean; TAC-Total Antioxidant Capacity; TFC-Total Flavonoid Content; TNF-α-Tumor Necrosis Factor α ; TPC-Total Phenolic Content .

1. INTRODUCTION

Cytokines are the biomarkers of inflammation have a specific effect on the interactions and communications between cells. Pro-inflammatory cytokines and anti-inflammatory cytokines are there. The pro-inflammatory cytokines are involved in the up-regulation of inflammatory reactions. E.g.:-IL-β, IL-6 and TNF-α. Pro-inflammatory cytokine responses controlled by a series of immune-regulatory molecules are anti-inflammatory cytokines. The cytokines include interleukin (IL)-1 antagonist, IL-4, IL-10, IL-11 and IL-13 are includes major anti-inflammatory cytokines. Among all these, IL-10 has the potent anti-inflammatory properties. IL-10

activates the macrophages and repressing the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1. Also, up-regulation of endogenous anti-cytokine and down regulation of pro-inflammatory cytokine receptors is able to do by IL-10.[1]

Inflammation is a defensive mechanism developed in higher organisms in response to harmful stimuli such as microbial infection, tissue injury, and noxious conditions. It is considered as an "adaptive response" to reinstate cellular homeostasis from any damaging conditions. The four classical symptoms of inflammations are redness, pain, swelling, and heat.[1] To suppress inflammation, Non-steroidal anti-inflammatory agents and corticosteroids are the two major classes of drugs. However, their use is limited as they have toxic adverse effects.[2] Thus, it is necessary to investigate new anti-inflammatory agents with the least side effects. Currently, medicinal plants are used as alternative therapeutic agents to treat and prevent various diseases including inflammation and its related disorders.[3] *Acanthus ilicifolius*, a glossy green small shrub[4] inhabits modest saline areas around the palms of mangroves.[5] They are found in India to southern China, tropical Australia, and the western Pacific Islands, including New Caledonia and the Solomon Island.[6] It has a prominent role in traditional medicine[7]and is endowed with potential pharmacological activities such as anti-inflammatory,[8] antimicrobial,[9] antioxidant,[10] etc. In Ayurvedic medicine, it is one of the 9 plants equated to the drug "Sahachara" which is used for rheumatic complaints.[11]The present study aimed to identify the antioxidant and anti-inflammatory potential of methanol and aqueous crude extracts of *Acanthus ilicifolius* L. stem. Furthermore, to characterize the bioactive compounds in the methanol extract of *Acanthus ilicifolius* L. stem.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, Missouri, USA) of highest analytical grade. Methanol was obtained from Merck, Life sciences.

2.2. Collection and preparation of plant extract

The stem of *Acanthus ilicifolius* was collected from the Puthuvype village (9.9950° N, 76.2247° E), Vypin Island, Ernakulam, in the month December 2017. Authenticity and taxonomical identification of the plant was made by the Department of Botany, University of Kerala, India, and Department of Botany, Maharaja's College, Ernakulam, India.

The stem was collected and washed thrice with double distilled water, and dried under shade. The dried materials were crushed using a mechanical grinder and preserved carefully. The powdered sample (20g) was extracted with 250 ml methanol or with water by soxhlet extraction. The methanolic extract was evaporated using a rotary vacuum evaporator at 55^0C. The aqueous extract was lyophilized at -52^0 to -54^0 C.

2.3. Qualitative analysis of phytochemicals

Qualitative analysis of the phytochemicals of the stem extract was conducted using standard phytochemical methods of Harbone, to identify the following compounds: terpenoids, steroids, flavonoids, alkaloids, carbohydrates, phenolic compounds, saponins, and fixed oils and fats.[12]

2.4. Quantitative analysis of phytochemicals

2.4.1. Total Phenolic Content (TPC)

The total natural phenolics present in the extracts were measured by Folin-Ciocalteu reagent.[13] Gallic acid was used as the reference standard. TPC was expressed as milligram Gallic acid equivalents (mg GAE) per gram dry weight.

2.4.2. Total Flavonoid Content (TFC)

Aluminium chloride colorimetric method proposed by Woisky and Salatino[14] was used to evaluate the total flavonoids in the extract. Quercetin was used as the reference and TFC was expressed as milligram Quercetin equivalent (mg QE) per gram dry weight.

2.4.3. Total Antioxidant Capacity (TAC)

Phosphomolybdic acid method was used to evaluate the total antioxidant capacity of stem extracts.[15] Gallic acid was used as the reference standard. TAC was expressed as milligram Gallic acid equivalents (mg GAE) per gram.

2.5. GC-MS analysis for bioactive compounds of Stem

GC-MS analysis of the methanol extract was done using Shimadzu GC-MS (model no: QP2010S) equipped with Rxi-5Sil MS column having 30 m length, 0.25 mm ID, and 0.25um thickness. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2µl was employed (a split ratio of 50.0). Injection temperature was maintained at 260^0 C. The

oven temperature was programmed from 80^0 C for 4 min isothermal with an increase to 280^0C, ending with a 6 min isothermal at 280^0C. The ion source temperature was 200^0C. GC-MS solution software was used for the identification of compounds based on the comparison of the library within the mass spectra, NIST 11 and WILEY 8.The analysis was done with the help of the instrumentation unit of Kerala Forest Research Institute (KFRI), Thrissur, India.

2.6. Antioxidant activity

DPPH radical scavenging assay was used to determine the antioxidant activity.

Quantitative measurement of the radical scavenging property of stem extracts was carried out by the method of Mensor et al.[16] Ascorbic acid was taken as the reference standard. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

2.10. Animals used

Adult female *Wistar albino* rats (150-180 g**)** were used for the study. They were bred and kept in the animal house of the Department of Biochemistry, Karyavattom Campus, University of Kerala. The animals were fed with standard pellets (Hindustan lever limited, Maharashtra, India) and tap water ad libitum. The guidelines prescribed by the Institutional Ethical Committee (registration no. IAEC 3-KU-09/2018-BIN-ACH (1)) were in compliance to the care and use of laboratory animals.

2.11. Limit test: determining acute toxicity in animal models

According to OECD test guidelines 425,[17] nulliparous, non-pregnant female *wistar albino* rats weighing 150-180 g having age 1-2 months were selected randomly. Animals were kept under standard conditions for acclimatization. The animals were kept without food for 3-4 h before dosing but had access to water ad libitum. The limit test was performed at 2000 mg/kg as a single dose, administered according to their body weight to 3 female rats. They were observed for the first 30 minutes, then for 4h. They were allowed food after 1-2 h of dosing. The same dose under the same condition was administered again for the next 3 animals after the survival of the prior treated group. The animals were observed closely within the first 6h and then at regular intervals for a total of 14 days to check for any toxic effect. Then the weight of animals was monitored and documented.

2.12. Carrageenan induced rat paw oedema

The method of Winter *et al.* was adopted to experiment.[18] The animals were divided into 5 groups. Each group contained 3 animals each (n=3). Acute inflammation was induced on the right hind paw of the rat by aponeurosis injection of 0.1 ml of 1% carrageenan in 0.9% saline.

- Group 1: Normal
- Group 2: Carrageenan
- Group 3: Carrageenan + Methanol extract
- Group 4: Carrageenan + Aqueous extract
- Group 5: Carrageenan + Indomethacin

The crude extracts were administered orally at doses - 100mg/kg, 250mg/kg and 500mg/kg and standard drug (Indomethacin: 3mg/kg) 1 hour before the carrageenan injection. The paw volume was measured before injection and 1st, 3rd, 5th, and 24th hour after inflammation induction. The paw volume was increased due to the carrageenan injection. The difference in the paw volume was measured using a plethysmometer before and after injection. The percentage inhibition of rat paw volume was calculated.

2.13. ADME Prediction

Lipinski's rule of five was used to describe the drug-like properties of the GC-MS analyzed compounds of *Acanthus ilicifolius* L. stem. ADME properties of the phytochemicals were evaluated using Discovery studio 2018.[19]

2.14. Molecular Docking

The crystal structure of inflammatory targets such as COX-2 (PDB ID: 5IKQ), TNF-α (PDB ID: 2AZ5), IL-2 (PDB ID: 2ILK) was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).[20] The crystallographic water molecules and hetero atoms were removed from proteins. Hydrogen atoms were added using the CHARMm force field. The prepared targets were further used for docking analysis. The active sites of target proteins were predicted from PDB ligand interactions. The structure of bioactive compounds of crude methanol extract of *Acanthus ilicifolius* L. stem satisfied

Lipinski's rule of five was retrieved from PubChem database ((http://pubchem.ncbi.nlm.nih.gov/)). The ligand preparations were carried out by energy optimization and adding hydrogen atoms. Various ligand conformations were generated based on different energy values. Discovery studio 2018 was used for protein preparation, ligand preparation, and molecular docking.

2.18. Statistical analysis

One-way and two-way analysis of variance (ANOVA) was used for performing the statistical analysis and followed by Pearson's coefficient of correlations and post hoc Dunnett's test. Experimental results were compiled as mean ± SEM. Quantitative and graphical data were analyzed using GraphPad Prism 8.0.2 and IBM SPSS 22. The *P*-value <0.001 was considered significant.

3. RESULTS

3.1. Qualitative analysis

Qualitative analysis of the methanol and aqueous extract of *the Acanthus ilicifolius L.* stem indicated a positive reaction for different classes of phytochemicals (Table1).

Table SEQ Table * ARABIC 1: Qualitative analysis of the methanol and aqueous extract of *Acanthus ilicifolius* L. stem

Qualitative Test	Phytochemical	Methanol	Aqueous
Leibermann's Burchard's test	Steroid	+	+
	Terpenoid	-	-
Shinoda test	Flavonoid	±	±
Mayer's test	Alkaloids	±	±
Molisch's test	Carbohydrates	-	±
	Phenolic compounds	+	±
Foam test	Saponins	-	±
Spot test	Oils & fats	-	+

+: Present, - : Absent, ±: Weakly present

Table SEQ Table * ARABIC 2: Quantitative estimation of

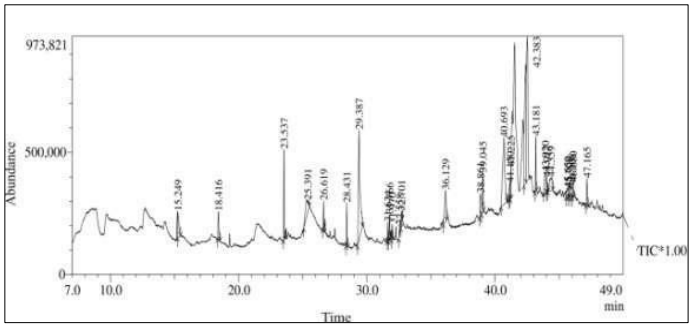
*Values expressed as Mean ± SEM. *** Significant F value at *P*<0.001. TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TAC: Total Antioxidant Capacity, GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents.

Fragments	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg GAE/g)
Methanol	27.87±0.20	12.29±0.11	45.45±0.16
Aqueous	13.31±0.09	10.62±0.06	20.32±0.20

3.2. Quantitative analysis

Table 2 shows the total phenolic, flavonoid, and antioxidant capacity of the crude methanol and aqueous extracts of *Acanthus ilicifolius* L stem. The highest content of phenolic, flavonoid, and antioxidant capacity was observed in crude methanol compared to crude aqueous extract.

3.3. GC-MS analysis



*R. Time: Retention Time. The compounds from GC-MS spectra were retrieved from Classyfire; a web-based application.[21]

Figure SEQ Figure * ARABIC 1: GC-MS analysis of the methanol extract of *Acanthus ilicifolius* L. stem done using Shimadzu GC-MS (model no: QP2010S) equipped with Rxi-5Sil MS column having 30 m length, 0.25 mm ID, and 0.25um thickness. Maintaining the injection temperature at 2600 and then temperature 800C (4 min) - 2800C (6 min). The flow rate of column was 1ml/min with 99.99% Helium gas.

The GC-MS chromatogram of the crude methanol stem extract of *Acanthus ilicifolius* L. presented in Figure 1 shows the retention time in the column and peaks detected, which corresponds to the bioactive components present in the crude methanol extract. A total of 28 peaks were observed in the chromatogram for the methanol extract of *Acanthus ilicifolius* L. stem.

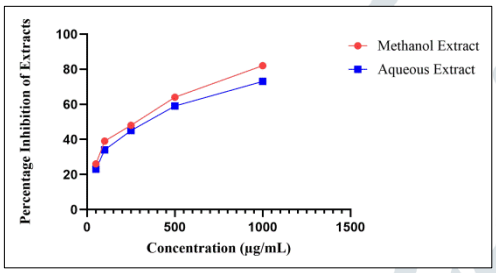
Table 3 shows the different classes of compounds analyzed by the GC-MS analysis of the methanol extract of *Acanthus ilicifolius* L. stem.

Sl. No.	Class	Name	R.Time
1	Phenols	Dimethoxyphenol	15.249
2	Fatty acyls	1-Dodecanol	18.416
		Tridecyl Acrylate	23.537
		Methylpalmitate	28.431
		Hexadecanoic Acid	29.387
		Methyl Linolelaidate	31.634
		9-Octadecenoic Acid (Z)-, Methyl Ester	31.766
		Palmitoleic Acid	32.701
3	Prenol lipids	Phytol, Acetate	26.619
		Phytol	31.97
		Squalene	43.181
4	Phenol ethers	1,3-Di(2'-Methoxyphenoxy)-2-Propanol	25.391
5	Unsaturated hydrocarbons	1,E-11,Z-13-Octadecatriene	32.558
6	Steroids and steroid derivatives	Stigmasta-5,22-Dien-3-Ol,(3.Beta.,22e)-	36.129
		.Gamma.-Sitosterol	40.693
		14-.Beta.-H-Pregna	41.15
		9, 19-Cyclolanost-24-En-3-Ol, Acetate, (3.Beta.)-	43.92
		7-Oxocholesteryl Isocaproate	44.359
		Cholesta-4, 6-Dien-3-Ol, (3.Beta.)-	46.08
		Cholesta-4,6-Dien-3-ol, Benzoate, (3.Beta.)-	47.165
7	Glycerolipids	Glycerol .Beta.-Palmitate	38.861

8	Benzene and substituted derivatives	1,2-Benzenedicarboxylic Acid	39.045
9	Organic metal salts	tert-Hexadecanethiol	41.225
10	Diarylheptanoids	2-Tert-Butyl-4,6-Bis(3,5-Di-Tert-Butyl-4 Hydroxybenzyl)Phenol	42.383
11	Alkyl halides	OctatriacontylPentafluoropropionate	46.008
12	Nitriles	3-Methyldecanenitrile	45.708
13	Unclassified	Methyl 4-Acetylhydroxypalmitate	44.017
		3.Alpha.-Methoxy-21-Keto-.Delta.13-Serratene	45.65

3.4. Antioxidant assay

DPPH radical scavenging assay was used to examine the antioxidant activity of the test sample. It has the ability to abstract hydrogen atoms from polyphenols.[22] The IC_{50} value of the standard ascorbic acid was 12.59µg/ml. The observed IC_{50} value of methanol extract



was 336.55µg/ml, the aqueous extract was 436.11µg/ml, which depicts a significant correlation (Figure 2).

3.5. Animal Study

3.5.1. Acute toxicity

The acute toxicity study with the methanol and aqueous extract of *Acanthus ilicifolius* L. stem extract did not show any symptom of toxicity up to 2000 mg/kg body weight of a female *Wistar albino* rats (Table 4). Hence, it was considered to be safe.

Table SEQ Table * ARABIC 4: Behavioral pattern of rat in extract treated (2000 mg/kg p.o.) and vehicle treated group

DPPH reduction (Standard: Ascorbic acid) of both methanol and aqueous crude extracts of *Acanthus ilicifolius L. stem* tested with different concentrations: 50, 100, 250, 500, and 1000 µm. Values expressed as Mean ± SEM. The absorbance read at 517 nm with *P-value* < 0.05.

Parameters	30 Minutes		4 h		24 h		48 h		7 days		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG	CG	TG
Fur & Skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Respiration	N	|	N	|	N	|	N	N	N	N	N	N
Urination (color)	N	P	N	P	N	P	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N	N	N	N	N	N	N
Convutions & Tremours	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.
Itching	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.
Coma	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.
Mortality	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.

*CG: Vehicle control group, TG: Acanthus ilicifolius extract treated group, N= normal, P= present, |=increased, N.F.: not found.

3.5.2. Carrageenan induced rat paw oedema

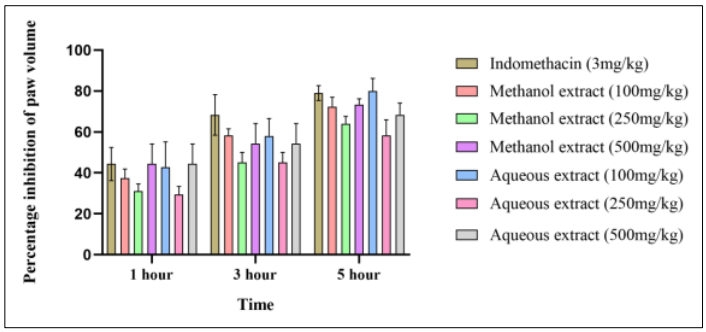


Figure 4: Anti-inflammatory activity test by carrageenan-induced rat paw edema. Values expressed as Mean ± SEM (n=3). Significant at *P*-value < 0.001.

Both methanol and aqueous extracts of *Acanthus ilicifolius* L. stem significantly decreased the carrageenan-induced rat paw edema (Figure 4). Compared to the reference standard indomethacin, the crude methanol and aqueous extracts of *Acanthus ilicifolius* L. stem showed significant activity at a concentration of 100 mg/kg BW.

3.6. ADME prediction using Discovery Studio 2018

In silico ADME estimation, 5 phytochemicals showed good aqueous solubility, undefined blood-brain-barrier penetration, low intestinal absorption, <90% plasma protein binding, no inhibition effect on cytochrome P450 and extreme low hepatotoxicity.

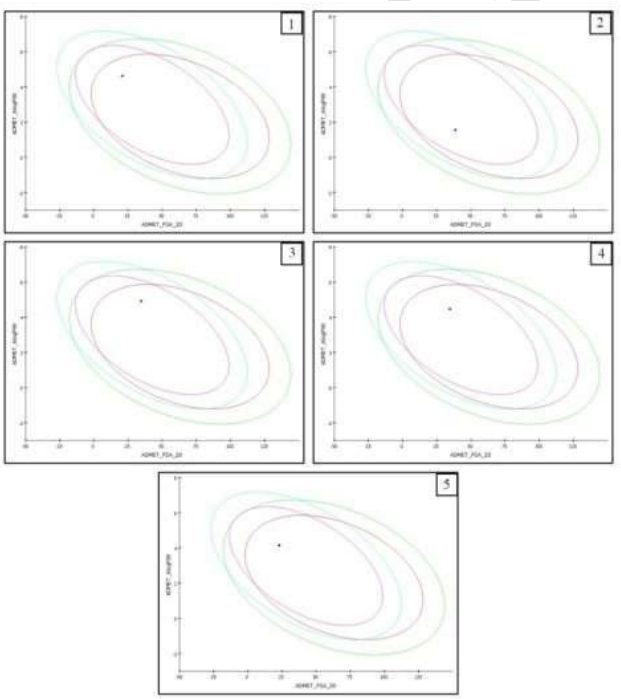


Figure 3: ADME plots of polar surface area (PSA) vs. A log P: 1) 3-Methyldecanentrile, 2) Hexadecanoic acid, 3) 1-Dodecanol, 4) Palmitoleic acid, and 4) Dimethoxyphenol. Prediction of blood-brain-barrier penetration and 95% and 99% confidence ellipses in the ADMET_PSA_2D, ADMET_AlogP98 plane.

3.7.

Molecular docking

The hydrogen bond interaction of compounds that show promising ADME profiles with inflammatory targets and their dock scores are summarized in Table 5.

Table SEQ Table * ARABIC 5: Docking results of inflammatory markers with the phytocompounds of *Acanthus ilicifolius* L. stem

		Docking Score (Kcal/mol)			
		5IKQ	3O8Y	2AZ5	2ILK
1	1-Dodecanol	98.87	-	87.86	76.89
2	Dimethoxyphenol	53.8	58.55	68.74	-
3	Hexadecanoic acid	109.8	-	113.3	98.29
4	Palmitoleic acid	103	-	111.5	101.1
5	3-Methyldecanenitrile	-	59.31	90.45	72.05

*The docking score was expressed in Kcal/mol. '-': No H- bond interaction; 5IKQ: COX-2, 3O8Y: LOX-5, 2AZ5-TNF-α, 2ILK-IL-2

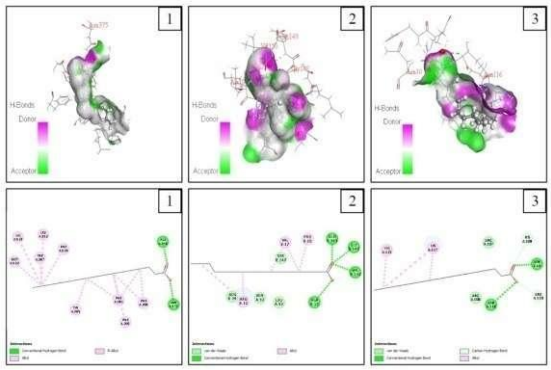


Figure 5: Stereoview (on top) and 2D images (on bottom) of best docking scores of Hexadecanoic acid. Diagrams were drawn in Discovery Studio Visualizer, green-dashed line along with distance in A^0 shows hydrogen bond. 3-letter amino acid code used to annotate the interactive residues. Ball and stick style represents Hexadecanoic acid. 1)Hexadecanoic acid-5IKQ, 2) Hexadecanoic acid-2AZ5 , 3)

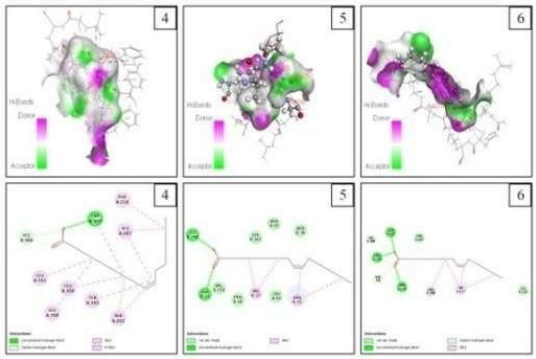


Figure 6: Stereoview (on top) and 2D images (on bottom) of best docking scores of Palmitoleic acid. Diagrams were drawn in Discovery Studio Visualizer, green-dashed line along with distance in A^0 shows hydrogen bond. 3-letter amino acid code is used to annotate the interactive residues. Ball and stick style represents Palmitoleic acid. 4) Palmitoleic acid-5IKQ, 5) Palmitoleic acid-2AZ5, 6) Palmitoleic acid- 2ILK.

4. DISCUSSION

The study demonstrated that both the methanol and aqueous stem extracts of *Acanthus ilicifolius* L. contain steroids, flavonoids, alkaloids, and phenolic compounds. Steroids promote nitrogen retention in osteoporosis and animals with washing illnesses.[23] Flavonoids are antioxidants or free radical scavengers.[24] Alkaloids acts as anesthetics and CNS stimulants.[25] Phenolics are natural antioxidants and anti-inflammatory agents.[24] The Carbohydrate and the saponin were present only in a negligible amount in aqueous extract. Saponins have hypolipidemic and anticancer activity. Saponins are necessary for cardiac glycoside activity.[25] Indeed, both methanol and aqueous extracts showed a significant level of total phenolic content, total flavonoid content, and total antioxidant capacity.

Previous GC-MS analysis revealed that the hexane extract of the stem of *Acanthus ilicifolius* L. contained homologous series of fifteen saturated odd and even fatty acids.[26, 27, 28, 29] Peng et al. reported eight compounds from the stem of *Acanthus ilicifolius* L. as hexacosanoic acid, stigmasterol, tetratriacontanol, 2-benzoxazolinone, stigmasterol-3-o-beta-D-glucopyranoiside, vanillic acid, 4-hydroxy-2-benzoxazolone and quercetin.[30] The current study revealed that the stem of methanol extract of *Acanthus ilicifolius* L. has twenty-eight phytoconstituents including different classes such as fatty acyls, prenolic lipids, steroids and steroid derivatives, carboxylic acids and derivatives, phenol ethers, unsaturated hydrocarbons, alkyl halides, glycerolipids, benzene, and substituted

derivatives and diarylheptanoids. Two out of twenty- eight were unclassified and further spectral data studies are needed for their classification. However, some of the constituents disclosed by GC-MS analysis are scientifically proven as biologically active compounds which might contribute to the pharmacological activities of the stem of *Acanthus ilicifolius* L. stem. Aparna et al. suggested that n-hexadecanoic acid might function as an anti-inflammatory agent hence it is an inhibitor of phospholipase A2. In Ayurveda, they have indirectly confirmed the use of medicated oils rich in n-hexadecanoic acid for treating the symptoms of rheumatism.[31] In addition to that n-hexadecanoic acid acts as an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, antimicrobial and antiallergic.[31, 32, 33, 34, 35, 36] Phytol is a diterpene function as an anticancer (R26), antioxidant, anti-inflammatory, and diuretic agent.[31, 37, 38, 39]

Phenolics and flavonoids having antioxidant activity play an important role in stabilizing lipid peroxidation.[32] In a previous study, Sofia et al. reported that methanol, ethanol, ethyl acetate, and chloroform extracts of leaf, stem, and root of *Acanthus ilicifolius* showed significant DPPH scavenging activity.[36] *Acanthus ilicifolius* L. stem showed significant antiradical efficiency (P<0.001) both in methanol and aqueous extracts, though, it can be classified as a good and promising antioxidant agent.

Acute toxicity study of *Acanthus ilicifolius* L. stem extracts evaluated the toxicity potential in *Wistar albino* rats by following OECD guidelines 425.[37] The treated animals showed a few changes like increased respiration and color change in urine in the initial 24 hr. Hence, the intake of food and water was normal during 14 days of the evaluation period. Then the animals were retained for further studies.

The process of inflammation is characterized by the release of chemicals from tissue, migrating cells, production of prostaglandins, leukotrienes, histamine, bradykinin, and platelet-activating factor.[38] The present study establishes the anti-inflammatory activity of the methanol and aqueous extracts of *Acanthus ilicifolius* L. stem. The fractions produced significant inhibition (P-value <0.001) of carrageenan-induced rat paw edema in *Wistar albino* female rats.

To examine the anti-inflammatory activity, a broad computational approach was followed for the identified compounds in the present study. Commonly, *in silico* approaches are used for the prediction and confirmation of drug design as it is inexpensive, needs less time, and minimizes the isolation of inactive compounds using ligand-based target fishing.[39] Molecular docking results revealed that compounds such as 1-Dodecanol, Dimethoxyphenol, Palmitoleic acid, Hexadecanoic acid, and 3-Methyldecanentrile satisfies both ADME properties and possess potent anti-inflammatory activity. Hexadecanoic acid and Palmitoleic acid exhibited highest docking scores for inflammatory targets except LOX-5.

In conclusion, both methanol and aqueous extracts of *Acanthus ilicifolius* L. stem are potential source of phytochemicals that have significant antioxidant and anti-inflammatory activities. Supplementary studies are needed to classify and understand the pharmacological properties of unclassified components. In addition to that further studies have to focus on the precise compounds and anti-inflammatory activity as well as to identify the mechanism of action of the methanol and aqueous extracts of *Acanthus ilicifolius* L. stem to come up to an explicit conclusion.

ACKNOWLEDGEMENT

The authors are grateful to the Emeritus scientist Dr. P. R. Sudhakaran for giving constant support and guidance for this work. Also wish to admire our sincere thanks to Dr. Linoy Libini C, Assistant Professor & Head, Fisheries Station (KUFOS), Puthuvype, India for his suggestions and encouragement to do this work. The funding support from the State Inter-University Centre of Excellence in Bioinformatics is also deeply acknowledged.

REFERENCES

1. Zhang JM, An J. Cytokines, inflammation and pain. International anesthesiology clinics. 2007;45(2):27.
2. Ahmed AU. An overview of inflammation: mechanism and consequences. Front. Biol. 2011; 6:274-281
3. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA. Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of Albizzia lebbeck in rats. J tradit and complement med. 2016; 6:172-175.

4. Zhang H, Shang C, Tian Z, Amin HK, Kassab RB, Abdel Moneim AE, Zhang Y. Diallyl disulfide suppresses inflammatory and oxidative machineries following carrageenan injection-induced paw edema in mice. Mediat Inflamm. 2020; 2020:8508906.
5. Wöstmann R, Liebezeit G. Chemical composition of the mangrove holly *Acanthus ilicifolius* (Acanthaceae)—review and additional data. Senckenb. Marit. 2008; 38:031-037.
6. Saranya A, Ramanathan T, Kesavanarayanan KS, Adam A. Traditional medicinal uses, chemical constituents and biological activities of a mangrove plant, *Acanthus ilicifolius* Linn.: a brief review. Am Eurasian J Agric Environ Sci. 2015; 15:243-250.
7. Ragavan P, Saxena A, Mohan PM, Jayaraj RS, Ravichandran K. Taxonomy and distribution of species of the genus *Acanthus* (Acanthaceae) in mangroves of the Andaman and Nicobar Islands, India. Biodiversitas. 2015; 16: 225-237.
8. Singh A, Duggal S, Suttee A. *Acanthus ilicifolius* linn.-lesser known medicinal plants with significant pharmacological activities. Int. J. Phytomedicine. 2009; 1:1-3.
9. Mani Senthil Kumar KT, Gorain B, Roy DK, Samanta SK, Pal M, Biswas P et al. Anti-inflammatory activity of *Acanthus ilicifolius*. J.Ethnopharmacol. 2008; 120:007-012.
10. Khajure PV, Rathod JL. Antimicrobial activity of extracts of *Acanthus ilicifolius* extracted from the mangroves of Karwar coast Karnataka. Recent res. sci. 2010; 2:098-099.
11. Paul T, Ramasubbu S. The antioxidant, anticancer and anticoagulant activities of *Acanthus ilicifolius* L. roots and *Lumnitzera racemosa* Willd. Leaves, from southeast coast of India. J. Appl. Pharm. Sci.2017; 7:081-087.
12. Dharya Singh, Vidhu Averi. Phytochemical and pharmacological potential of *Acanthus ilicifolius*. J Pharm Bioallied Sci. 2013; 5: 017-020
13. Harborne JB, A guide to modern techniques of plant analysis. 1st ed. USA: Chapman and Hall;
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-158.
15. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res. 1998; 37:099-105.
16. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. Anal Biochem. 1999; 269: 337-341.
17. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001; 15:127-130.
18. OECD, Acute oral toxicity: Up and down procedure, Guideline for the Testing of Chemicals 425 OECD 2008, pp. 1-2.
19. Winter CA, Risley EA, Nuss CW. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 544–547.
20. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev.1997;23:3-25.
21. Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Feng Z, Gilliland GL, Iype L, Jain S, Fagan P. The protein data bank. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2002 Jun 1;58:899-907.
22. Feunang YD, Eisner R, Knox C, Chepelev L, Hastings J, Owen G et al. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. J Cheminform. 2016; 8:061.
23. Shahidi F, Ho CT. Antioxidant measurement and applications: an overview, ACS Symposium series 956, American Chemical Scoiety, Washington, DC (2007), pp. 002-007.
24. Maurya R, Singh G, Yadav PP. Antiosteoporotic agents from natural sources. In: AU Rahman, ed. Studies in Natural Product Chemistry, Bioactive Natural Products. New York: Elsevier, 2008: 517– 548.
25. Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Rao DV (ed) Phytochemicals—a global perspective of their role in nutrition and health. Intech Open Access Publisher, Rijeka, Croatia (2012), p. 283–359.

26. Madziga HA, Sanni S, Sandabe UK. Phytochemical and elemental analysis of *Acalypha wilkesiana* leaf. Am J Sci 2010; 6:510-514.
27. Oleszek W, Marston A, editors. Saponins in food, feedstuffs and medicinal plants. In Proceedings of the Phytochemical Society of Europe. Kluwer Academic Publishers, Dordrecht (2000), p. 001–095.
28. Phillipson JD. Phytochemistry and pharmacognosy. *Phytochemistry* 2007; 68:2960-72.
29. Daffre S, Bulet P, Spisni A, Ehret-sabatier L, Rodrigues EG, Travassos LR. Bioactive natural peptides. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry, Elsevier (2008). p. 597- 691.
30. Goswami S, Chatterjee B, Mallik M. Poof-of-presence of unusually naturally occurring homologous series of fifteen saturated odd and even fatty acids in *Acanthus ilicifolius* L. (Acanthaceae). J Indian Chem Soc. 2004, 81: 696-706.
31. Peng X, Long SJ. Chemical constituents in stem of *Acanthus ilicifolius*. *Chinese Traditional and Herbal Drugs* 2006; 37: 971-973.
32. Revathi P, Jeyaseelansenthinath T, Thirumalaikolundhusubramaian P. Preliminary phytochemical screening and GCMS analysis of ethanolic extract of mangrove plant-*Bruguiera cylindrica* (Rhizho) L. Int J Pharmacogn. 2014;6:729-740.
33. Rajeswari G, Murugan M, Mohan VR. GC-MS analysis of bioactive components of *Hugonia mystax* L.(Linaceae). Res J Pharm Biol Chem Sci. 2012; 3:301-308.
34. Jegadeeswari P, Nishanthini A, Muthukumarasamy S, Mohan VR. GC-MS analysis of bioactive components of *Aristolochia* krysagathra (Aristolochiaceae). J Curr Chem Pharm Sci. 2012; 2:226-32.
35. Elezabeth VD, Arumugam S. GC-MS analysis of ethanol extract of *Cyperus rotundus* leaves. IJCB. 2014; 2:019-023.
36. Omotoso AE, Kenneth E, Mkparu KI. Chemometric profiling of methanolic leaf extract of *Cnidoscolus aconitifolius* (Euphorbiaceae) using UV-VIS, FTIR and GC-MS techniques. J Med Plants Res. 2014; 2:006-012.
37. Sofia S. Merlee Teresa M V. Isolation of bioactive compounds by GC-MS and biological potentials of *Acanthus ilicifolius*. L. Int Res J Biol Sci. 2017;6:007-019.
38. Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, Mary S. Acute oral toxicity evaluation of aqueous ethanolic extract of Saccharum munja Roxb. roots in albino mice as per OECD 425 TG. Toxicol. Reports. 2017; 4:580-5.
39. Ratheesh M, Sindhu G, Helen A. Anti-inflammatory effect of quinoline alkaloid skimmianine isolated from Ruta graveolens L. Inflamm. Res. 2013;62:367-76.
40. Utami W, Aziz HA, Fitriani IN, Zikri AT, Mayasri A, Nasrudin D. In silico anti-inflammatory activity evaluation of some bioactive compound from ficus religiosa through molecular docking approach. *InJournal of Physics*: Conference Series 2020 Jun 1 (Vol. 1563, No. 1, p. 012024). IOP Publishing.