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Isolation of bioactive compounds by GC-MS and biological potentials of *acanthus ilicifolius*, L.

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Abstract

Mangroves are ecological group of halophytic plant species, which provide a wide range of pharmaceutical and economic products and services. The present work focussed on a study of Acanthus ilicifolius, L.a mangrove plant from Kerala. Phytochemical constituents were determined by UV-Visible spectrophotometer. Free radical scavenging activity was estimated using in vitro methods like DPPH, Nitric Oxide Radical, ABTS and Super Oxide Dismutase while minimum inhibitory concentration of leaf extracts were determined by resazurin based microtiter dilution assay and the characterisation of bioactive components by GC-MS analysis. The methanol extract of A. ilicifolius, L. leaf showed better scavenging activity in SOD method (419 µg/ml) followed by ABTS (450.7 µg/ml), NO (556 µg/ml) and DPPH (562.5 µg/ml). The crude methanolic extract showed remarkable MIC of 0.469 mg/ml against Staphylococcus aureus, 0.521 mg/ml against Klebsiella pneumonia, 0.416 mg/ml against Candida albicans and Pencilliumnotatum. Phenols, flavonoids and tannins had higher correlation with antioxidant activities. The GC-MS of active column fraction revealed that the active principles were a mixture of Lupeol, decanoic acids, cyclolignan glycosides, glycine, cyano colchicine and other therapeutically active compounds. It could be concluded that A .ilicifolius, L. contains compounds with various biological activities and so recommended as a plant of phyto pharmaceutical importance.

Keywords: GC-MS, spectrophotometer, lupeol, resazurin, antioxidant.

Introduction

Acanthusilicifolius, L. is the only genus of family Acanthaceae which occupies mangroves habitat[1]. Moreover, the plant possesses anti-inflammatory, antioxidant, antileishmanial, osteoblastic, hepatoprotective, anticancer, antiulcer and antimicrobial activities[2]. The plant is employed in traditional systems of medicine, including traditional Indian medicine or Ayurveda and traditional Chinese medicine for treating various ailments[3,4]. This plant speciescontains many bioactive compounds like triterpenoids, alkaloids, phenolic compounds, lignans, flavonoids, steroids, and tannins[3].The phytochemical analysis of the plants is important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing various diseases[5].

Numerous mangrove plants are being used in folklore medicine, and recently, extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens, but only limited investigations have been carried out to identify the metabolites responsible for their bioactivities as well as cytotoxic and scavenging activities[6-9]. The phytochemical literature of *A.ilicifolius,* L. reveals the presence oflignanglucosides, benzoxazinoideglucosides, flavone glycosides and phenylethanoid glycosides[10,11]. GC-MS is a method to identify different substances within a test sample and can provide meaningful information for components that are volatile, non-ionic, and thermally stable and have relatively low molecular weight. The aim of the present study is to screen the phytochemicals present in the leaf extract of *A. ilicifolius,* L. and analyse the bioactive components present in it by GC-MS.

Figure-1: *Acanthus ilicifolius*, L.

Acanthus ilicifolius, L. is an erect herb. Leaves pinnatifid, toothed, spinous, the petioles with spines at their bases. Flowers in terminal, strobilate, bracts ovate, large, decussate bracteoles large lanceolate. Calyx 4-partite, the outer 2 lobes are larger. Corolla-tube short, horny, upper lip obsolete, stammens 4, didynamous, shorter than the lower lip; filaments stout; anthers 1-celled. Ovary 2-celled, ovules 2 in each cell, style slender, stigma bifid[12].

Materials and methods

The plant *Accanthusilicifolius*, L. were collected from Chirackal of Ernakulam District, Kerala. The collected plants were washed with tap water and shade dried at room temperature. The dried samples were powdered using electrical blender. Ten grams of material was stirred overnight for 72 hours in different solvents (100 mL) like methanol, ethanol, ethyl acetate, chloroformand then centrifuged at 10,000 rpm for 10 min at 40C. The resultant supernatant was collected and the solvents were removed by evaporation. This extract was used for further phytochemical analysis.

Qualitative profiling: Extracts of *A,ilicifolius*, L. leaf, stem and root were used for qualitative assessment for the major classes of phytochemicals namely phenols, flavonoids, steroids, amino acids, tannins, terpenoids, glycosides, saponins, alkaloids in addition to the major biomolecules namely lipids, carbohydrates and proteins. The tests were performed according to various standard methods[13-15].

Quantitative profiling: Quantitative estimation of various phytohemicals viz. Total Phenols[16], Total Tannins and Lignin[17], Total Flavonoids[18], DPPH Antioxidant Assay[19], Superoxide Dismutase Radical Scavenging Assay[20], Nitric Oxide Radical Scavenging Assay[21], ABTS Radical Scavenging assay[22], Microtitre Dilution Assay[23]and GC-MS Analysis present in the plant of *A.ilicifolius,* L. was analyzed according to the standard protocols.

Results and discussion

Mangroves are considered as a valuable source for chemical constituents with potential medicinal and agricultural values[24]. Qualitative and quantitative screening revealed the strong phytochemical relation within the plant under investigation. Mangrove plants can produce metabolites and toxins that are unique to these plants, which suggest that they may be a source of novel compounds[25].

They are biochemically unique, producing a wide array of natural products. They possess new agrochemical products, compounds of medicinal value and biologically active compounds. Mangroves are a promising source of natural products. They have been a source of several bioactive compounds[26]. Phenolic compounds are a large group of phytochemical components widespread in the plant kingdom and characterized by having at least one aromatic ring with one or more hydroxyl groups attached which directly contribute to the antioxidant properties[27].

Total phenol content: Phenolic compounds from plants are known to be good natural anti-oxidants. Itcontributes to quality and nutritional value of modifying colour, taste, aroma and flavour and in providing health beneficial effects. They counteract reactive oxygen species in plant defense mechanisms[28,29]. In plants, they also serve as defence mechanism to counteract reactive oxygen species (ROS) in order to prevent the molecular damage in cells[30]. Phenolics are aromatic secondary plant metabolites associated with colour, sensory qualities, nutritional, and antioxidant properties[31]. The total phenols in different solvent extracts of three parts of *A.ilicifolius,* L. showed wide variation in terms of extracts and plant parts which ranged from 39.1 to 87.4 mg/g. Among all the solvent extracts, the methanol extract of *A.ilicifolius,* L. leaf possesses significantly higher amount of total phenolsand have the following sequence, 53.8, 69.4, 72.6 and 87.4 mg/g in ethanol, ethyl acetate, chloroform and methanol extracts respectively. Stem samples showed the sequence as 39.1, 52.4, 58.3 and 64.9 mg/g in different solvents and root also showed good results like 41.6, 44.5, 46.9 and 64.4 mg/g that are responsible for high antioxidant activity. It was seen from the result that more amount of phenol is extracted by methanol solvent. In general, methanol and ethyl acetate extracts showed higher results than ethanol and chloroform extracts.

Total flavanoid content: Phenolics and flavonoids play an important role in stabilizing lipid peroxidation and by their antioxidant activity[32]. Ascorbic acid is naturally available form of vitamin C and is consequently the most important water soluble antioxidant vitamin in cells, effectively scavenging reactive oxygen species (ROS)[33]. Vitamin C can act as an oxidant and pro-oxidant so as to protect DNA from free radical damage and mutagens[34,35]. Total flavonoid content varied from solvent to solvent owing to the differences in their polarities. Methanol was found to be most effective for the extraction of total flavonoids as was found to contain maximum of 39.3, 31.4 and 29.8 mg of catechin equivalent/g in leaf, stem and root samples of solvents like methanol, ethyl acetate and methanol respectively and the minimum level was found in that of ethanol and chloroform with 20.4, 12.7 and 17.9 mg/g.

Total tannin content: Total tannin content in different solvent extracts of the plant varied with plant parts. Values ranged from 39.4 to 54.1 mg/g in leaf, 41.5 to 48.7 mg/g in stem and 34.9 to 44.8 mg/g in root showed a sequence of methanol, ethyl acetate, ethanol and chloroform extracts. Maximum tannin was found in the methanol extract of leaf (54.1 mg/g) followed by stem (48.7 mg/g) and in chloroform extract of root (44.8 mg/g).Total phenols, Total flavonoids and Total tannins of leaf, stem and root of *A.ilicifolius,* L. in different extracts are given in Table-1 and Figures-2- 4.

Table-1: Phenol, Flavanoid and Tannin contents of *A.ilicifolius,* L. in different extracts.

		Leaf (mg/g)	Stem (mg/g)	Root (mg/g)
Phenols	Methanol	84.477±1.548	41.447±1.92	54.367±1.518
	Ethanol	51.933±1.23	30.06±0.847	42.877±0.698
	E.acetate	67.4±1.617	35.773±0.485	46.133±0.769
	Chloroform	70.833±1.071	22.12±0.863	39.077±1.412
Flavanoids	Methanol	64.567±1.625	31.41±2.779	48.073±0.343
	Ethanol	40.4±0.929	30.177±1.51	36.017±1.3
	E.acetate	59.727±0.783	32.04±0.999	39.77±0.896
	Chloroform	50.733±0.851	14.723±1.06	43.267±0.406
Tannins	Methanol	42.077±0.889	32.067±1.146	43.2±0.808
	Ethanol	45.2±0.4	18.88±0.61	45.55±0.558
	E.acetate	39.167±0.561	27.0133±1.296	62.867±0.775
	Chloroform	35.367±0.328	20.73±0.698	47.267±0.524

Each value is expressed as mean± standard error done in triplicates. Data were analysed by ANOVA SPSS version 20.0 for windows followed by Duncan Multiple Range Test (DMRT) for comparison at P= 0.05 level of significance.

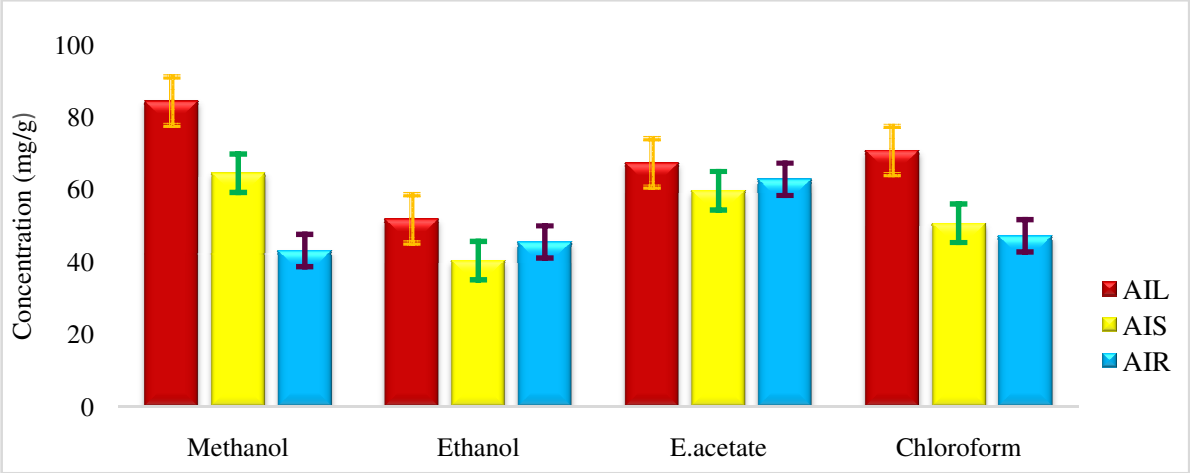

Figure-2: Total phenolic content of *A.ilicifolius,* L.in different extracts (mg/g).

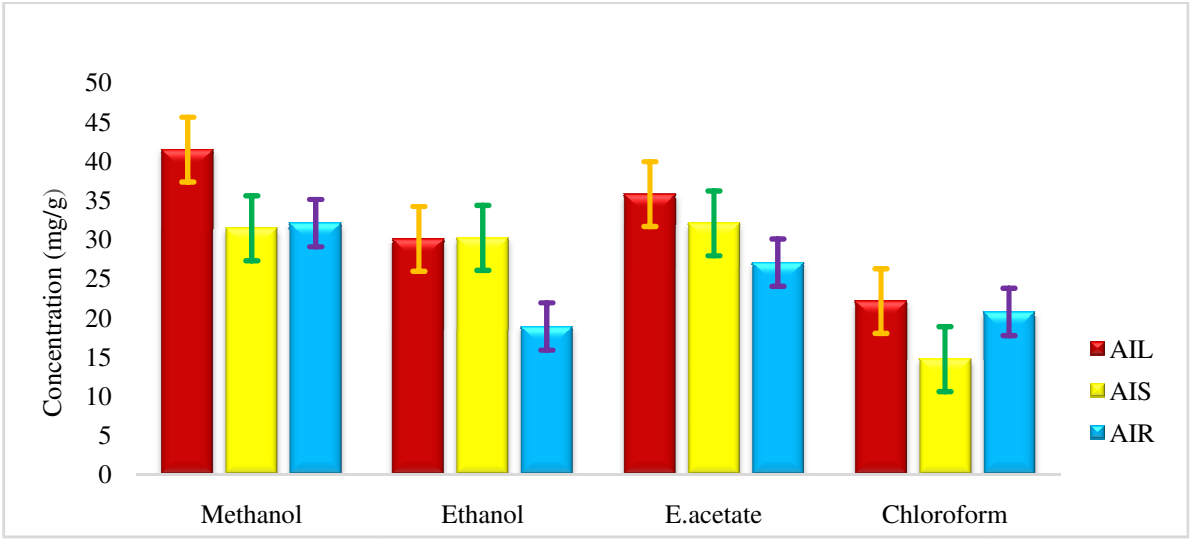

Figure-3: Total flavanoid content of *A.ilicifolius,* L.in different extracts (mg/g).

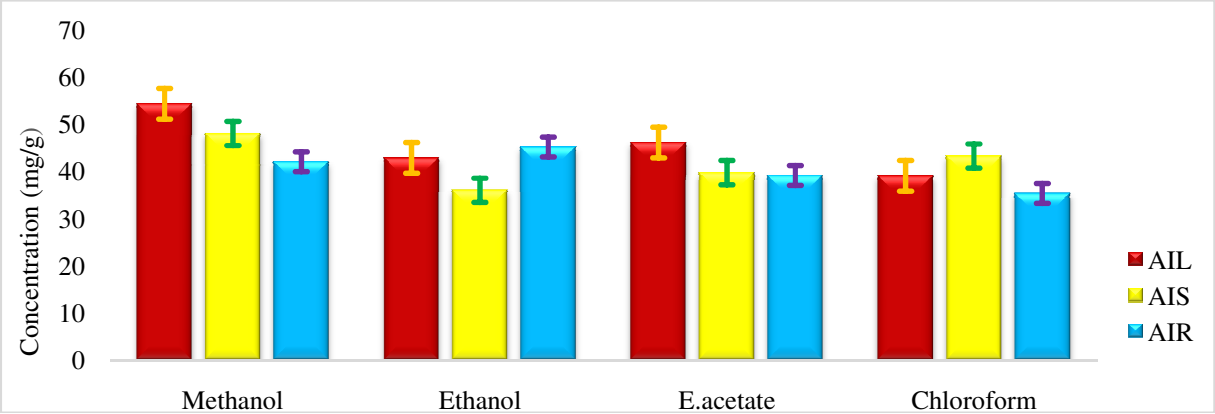

Figure-4: Total tannin content of *A.ilicifolius,* L.in different extracts (mg/g).

Antioxidant assay: The comparative evaluation of the antioxidant potential of different solvent extracts of *A.ilicifolius,* L. parts viz, leaf, stem and root was determined by various antioxidant assays such as total phenol contents, total flavonoid contents, total tannin contents, DPPH, NO, SOD and ABTS. Recently, it has been strongly recommended that Environmental stress factors affect plant growth. The plant *A. ilicifolius,* L. leaf, stem and root have been used to prevent tumour growth and cancer progression[41,42]. In this species, methanol extract showed the highest antioxidant and cytotoxic activity. Methanol is effective to dissolve active compounds in cells. Hence, easy to penetrate the cellular membrane to extract the intracellular ingredients from plant materials. Several bioactive compounds have been obtained when methanol used as solvent in the extraction technique of secondary metabolites. They are known to act as free radical scavenger, reactive species quencher, hydrogen donor, antioxidant enzymes activator, detoxification inducer, normal cell differentiation promoter, tumour production and proliferation cell inhibitor, and apoptosis[43].

The methanol extract of *A.ilicifolius,* L.exhibited the highest antioxidant activities than the other solvent extracts. The bioactive compounds in the leaf, stem and root of this plant have been revealed as a scavenging radical which able to inhibit carcinogenesis. Hence, the best method to extract the antioxidant is methanol extraction[44]. Extracts of *A.ilicifolius,* L. are rich source of natural antioxidant with good antimicrobial activities. Though the plant contains most of the phytochemicals in different proportion, the presence of phenolic compounds, flavonoids and tannins are particularly important for expressing various bioactivities. The key role of phenolic compounds and flavonoids in scavenging of free radicals has been well studied[45-47]. Apart from having antioxidant activity, phenols and flavonoids are also known to be associated with other biological activities[48,49].

DPPH radical scavenging activity: DPPH is a stable free-radical accept an electron or hydrogen radical becoming a stable diamagnetic molecule[50]. DPPH is purple in colour which turns yellow; the intensity of the yellow colour depends upon the amount and nature of radical scavenger present in the sample and standard compounds. Methanol leaf extract of *A.ilicifolius,* L. has shown good free-radical scavenging activity at all tested samples. The scavenging activity increases with an increase in concentration of the extract, as well as ascorbic acid, and the IC 50 value of ethanol and methanol extract of leaf is 557 µg/ml and 569 µg/ml. The residual concentration of DPPH depends exclusively on the structure of the phenolic compound. The accessibility of the radical centre of DPPH to each polyphenol could influence the order of the antioxidant power[51,52]. Stem and root also showed good IC 50 value in ethanol (564.7 µg/ml and 629.8 µg/ml) and methanol (596.9 µg/ml and 649 µg/ml) extracts. Ethyl acetate and Chloroform showed comparatively less results in DPPH.

Superoxide anion radical scavenging activity: Superoxide radical is a highly toxic species, which is generating by numerous biological and photochemical reactions[53]. Superoxide radical can further interact with other molecules to generate secondary ROS either directly or prevalently through enzyme or metal catalyzed processes[54]. The inhibition percentage of superoxide radical generation by *A.ilicifolius,* L. plant extracts at different plant parts and solvents were compared with L-ascorbic acid, BHT and Trolox as standards at the same concentrations and solvents. All sample extracts showed good scavenging activity while standards showed high scavenging activity at the same concentration. Superoxide anion radical inhibition percentage was increased gradually with increasing extract concentration.

A.ilicifolius, L. leaf showed the highest anion radical scavenging activity in all concentrations. Inhibition percentages of plant extract are arranged in the order: leaf >stem >root and solvents are Methanol > Ethanol > Ethyl Acetate >Chloroform. IC 50 values of the leaf sample in methanol extract was 419 µg/ml followed by 453 µg/ml, 507 µg/ml and 576 µg/ml in ethanol, ethyl acetate and chloroform respectively. The stem and root samples showed good activity in ethyl acetate (443 µg/ml and 631 µg/ml), ethanol (503 µg/ml and 489 µg/ml) and methanol (518 µg/ml and 423 µg/ml) extracts. Chloroform extracts (584 µg/ml and 507 µg/ml) showed comparatively less results in stem and root samples.

Nitric Oxide radical scavenging activity: Nitric oxide acts both as a pro-oxidant and oxidant, the pro-oxidant effect of nitric oxide is by the formation of peroxynitrite, which is formed by its reaction with a superoxide free radical. Nitric oxide is implicated in inflammation, cancer and other pathological conditions like reactive oxygen species[55]. Nitric oxide radical scavenging activity was found higher in the ethanol extract of *A. ilicifolius,* L. The IC 50 value of ethanol extract of leaf was 434.8 µg/ml, followed by chloroform (494.6 µg/ml), methanol (560.9 µg/ml) and the least of activity was observed in *ethyl acetate* (610 µg/ml) extracts. Stem exhibited the IC 50 value in different ranges in the order as 514.2 > 583.2 > 619.8 > 652 µg/ml in ethanol, methanol, chloroform and ethyl acetate respectively. Ethanol extract showed the high IC 50 value (561 µg/ml) in root also.

ABTS radical scavenging activity: ABTS is another widely used synthetic radical for both the polar and non-polar samples. The $ABTS^{\bullet+}$ scavenging abilities of the crude extract of *A. ilicifolius*, L. leaf exhibited a maximum scavenging activity. The IC 50 value of ABTS radical scavenging ability of leaf in different solvents are 451.6, 527.3, 579.1 and 617 µg/ml in methanol, ethanol, ethyl acetate and chloroform respectively. Stem and root showed radical scavenging activity in different extracts are ethanol (494 and 546.7 µg/ml), methanol (562.4 and 580 µg/ml), ethyl acetate (602.7 and 584.9 µg/ml) and chloroform (614.5 and 719.2 µg/ml).Methanol extract showed the highest antioxidant activity, while the lowest one was detected in the chloroform extract.

The reduction effectiveness of free radicals by polyphenols is affected a number of hydroxyl groups on the compound[56,57]. The effectiveness and strength of activity antioxidant is determined by the ability to move the hydrogen atom and transfer of a single electron[58].The antioxidant assays showed that, minimum concentration of the IC50 value was identified in the SOD (423±1.202 µg/ml) inhibitory assay followed by ABTS (438±0.577 µg/ml) inhibitory assay, nitric oxide (560.67±0.882 µg/ml) inhibitory assay and DPPH (562.47±3.841µg/ml) inhibitory assay, but the vitamin C, BHT and Trolox (positive control) showed minimum concentration of IC50 values in SOD, NO, ABTS and DPPH radical scavenging activity assay.

Table-2: IC 50 value of DPPH and SOD scavenging activity of *A.ilicifolius,* L. with standards (µg/ml).

	Leaf	Stem	Root	A.Acid	BHT	Trolox	Leaf	Stem	Root	A.Acid	BHT	Trolox
Methanol	562.5 ±3.84	562 ±1.155	433.3 ±1.2	422.3 ±1.45	452.3 ±0.88	472 ±1.15	419 ±1.15	515 ±1.73	417.7 ±0.88	406.3 ±0.66	441.3 ±0.88	472 ±0.57
Ethanol	576 ±6.51	592.3 ±1.86	626.7 ±1.45	446.3 ±0.88	461 ±1.55	467 ±3.05	451.3 ±0.88	501.3 ±0.88	487.7 ±0.88	395.7 ±0.88	429 ±1.15	494 ±1.85
E.acetate	678.4 ±2.52	593 ±0.58	593 ±1.15	435 ±1.53	442 ±0.58	484 ±1.53	503 ±2.08	442.7 ±0.88	626.3 ±0.88	412.3 ±1.45	417.7 ±1.2	467 ±2.31
Chloro-form	688.7 ±1.45	759.7 ±0.88	883.3 ±2.03	422 ±1.2	459.7 ±0.88	471 ±1.15	572.7 ±1.76	582 ±1.15	504.7 ±1.2	450 ±0.57	405.3 ±1.2	428 ±0.88

Each value is expressed as mean± standard error done in triplicates. Data were analysed by ANOVA SPSS version 20.0 for windows followed by Duncan Multiple Range Test (DMRT) for comparison at P= 0.05 level of significance.

Table-3: IC 50 value of NO and ABTS scavenging activity of *A.ilicifolius,* L.with standards (µg/ml).

			NO						ABTS			
	Leaf	Stem	Root	A.acid	BHT	Trolox	Leaf	Stem	Root	A.acid	BHT	Trolox
Methanol	556 ±1.73	512 ±1.15	608.7 ±1.2	394 ±1.15	427 ±0.58	505.7 ±1.7	450.7 ±0.3	490.7 1.7	561.3 ±0.8	328 ±1.15	411.7 ±1.2	292.3 ±1.2
Ethanol	434 ±0.58	581.3 ±0.8	560.7 ±0.3	415.3 ±1.4	445.7 ±1.2	489.3 ±1.2	525.3 ± 0.8	561 ±0.58	578.7 ±0.8	351 ±0.58	424.3 ±1.4	327.7± 1.7
E.acetate	602 ±3.8	652 ±0.58	601.3 ±0.8	462 ±1.15	472.3 ±1.2	490.7 ±1.4	577.3 ±1.2	600 ±1.15	584 ±1.15	391 ±1.53	394.7 ±1.2	362± 1.73
Chloro-form	494 ±0.58	617.7 ±1.2	681.7 ±1.2	520 ±0.58	518.7 ±1.52	504 ±1.52	615.7 0.8	612 ±1.15	715.7 ±2.0	402 ±1.53	417.3 ±1.2	387.7± 0.6

Each value is expressed as mean± standard error done in triplicates. Data were analysed by ANOVA SPSS version 20.0 for windows followed by Duncan Multiple Range Test (DMRT) for comparison at P= 0.05 level of significance.

In this study suggest that methanol extract of *A. ilicifolius,* L.contains significantly high free radical scavenging activity. This observation strongly supports the results that, as the methanol extract contain the highest amount of polyphenols out of the four organic extracts. There is a strong correlation between polyphenols and free radical scavenging potential of plant species due to the scavenging capability and hydrogen donating ability of hydroxyl groups of polyphenols. Flavonoids also act as scavengers of various "reactive oxygen species (ROS)" and quenchers of singlet oxygen[59]. The methanol extract had the highest content of phenolic compounds, flavonoids and tannins as well as the best antioxidant activity in DPPH, SOD, NO and ABTS assays, whereas ethyl acetate and ethanol extracts showed highest content of flavonoids and tannins followed by chloroform extract. These results showed that the antioxidant activity are in correlation with phenolic compounds concentration, a positive linear correlation between antioxidant activity and total phenol, total flavonoid and total tannin content was noticed. From the antioxidant assays, Pearson correlation coefficient confirmed that DPPH assay showed strong positive correlation with respect to the selected secondary metabolites that posses antioxidant property (Table-4).

Antimicrobial Activity: Resazurin Microtitre Dilution Assay: Resazurin is an oxidation–reduction indicator used for the evaluation of cell growth, particularly in various cytotoxicity assays. Ten reference strains of human pathogens including two gram-positive [*Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Bacillus subtilis* (*B.subtilis*) ATCC 6051], three gram-negative [*Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Escherichia coli* (*E. coli*) ATCC 25922, *Klebsiella pneumoniae* (*K.pneumoniae*) ATCC 700603] and five fungal strains [*Aspergillusniger* (*A.niger*) ATCC 16888, *Aspergillusflavus* (*A.flavus*) ATCC 9643, *Pencilliumnotatum* (*P.notatum*)ATCC 302300, *Rhizopusbiflorus* (*R.biflorus*) ATCC 9363 and *Candida albicans* (*C.albicans*) ATCC 3752] were used in this study. A resazurin reduction test has also been used for decades to demonstrate bacterial and yeast contamination of milk[36,37]. The colour changes in the tubes can be markedly visible and also obtained MIC for potential antibacterial extracts showing the values close to the antibiotic control wells.

Methanol extract of *A.ilicifolius,* L. leaf showed promising activity against the microbes. The MIC values ranged from 0.26 mg/ml to above 5 mg/ml for different solvent extracts of *A.ilicifolius,* L. leaves against test bacteria. The least MIC value of *A.ilicifolius, L.* was 0.416 mg/ml against fungus *P.notatum* and *C.albicans and* 0.469 mg/ml, 0.521 mg/ml and 0.625 mg/ml against *S.aureus*, *K.pneumoniae*, and *A.niger*was recorded for methanol extract respectively. Ethyl acetate and ethanol extracts also showed good results in *P. aeruginosa* (0.938 mg/ml and 0.521 mg/ml), *E. coli* (1.042 mg/ml and 1.042 mg/ml), *K. pneumoniae* (0.625 mg/ml and 1.042 mg/ml), *C. albicans* (0.833 mg/ml and 0.625 mg/ml), *A. niger* (1.67 mg/ml and 1.25 mg/ml) and *R. biflorus* (1.042 mg/ml and 2.08 mg/ml) respectively. For bacteria, Ampicillin and Gentamycin were used as positive standards; Clotrimazole and Fluconazole were used for fungus. Ampicillin showed good results (0.521 mg/ml and 0.938 mg/ml) against *B.subtilis, K.pneumoniae* and *E.coli* respectively. Gentamycin showed 1.042 mg/ml MIC value against *P.aeruginosa*. For *C.albicans*, positive control, fluconazole showed the MIC value as 0.521 mg/ml. Clotrimazole was used for all the other pathogens. The least MIC value was 0.26 mg/ml against *R.biflorus*, 0.521 mg/ml against *B. subtilis* 0.938 mg/ml against *K pneuconiae* and *A.flavus* and 1.042 mg/ml against *S. aureus*. Chloroform extract showed comparatively poor result in the sample.

In bacteria, the leaf sample showed least MIC value in *S. aureus*and*K. pneumonia* (0.469mg/ml and 0.521 mg/ml) and in fungus, it was reported in *C.albicans* and *P.notatum*(0.416 mg/ml).The results obtained in REMA are tabulated in Table-5 and the images are given in Figures-5-10. The overall observation of the MIC results concluded that methanol extracts from the studied plant showed broad range of activity and is a potential source of antimicrobial compounds. The methanol and ethanol extract of leaf, stem and root of *A. ilicifolius,* L.showed considerable inhibitory activity against *E. coli*[38]. *A.ilicifolius,* L.possesses bioactive compounds that have antibacterial potential[39]. Thealcoholic and chloroform extracts of *A.ilicifolius,* L.leaves exhibited strong inhibitory action against *B. subtilis, S.aureus, C.albicans, A.fumigatus* and *A.niger*and moderate inhibitory action against *P.aeruginosa* and *P.vulgaris.* The organisms tested were most susceptible to methanol extract[40].

In this study, it was observed that methanol was the best solvent for extracting the effective antimicrobial compounds from *A.ilicifolius,* L. when this extract was tested against the selected strains of microbes compared to ethanol, ethyl acetate and chloroform extracts.

Table-4: Correlation analysis of Antioxidant assays and Phenolic compounds in methanol extract.

	Phenol	Flavanoid	Tannin	DPPH	SOD	NO	ABTS
Phenol	1						
Flavanoid	0.82374	1					
Tannin	0.99939	0.84307	1				
DPPH	0.87766	0.45124	0.86037	1			
SOD	0.03826	-0.535	0.00329	0.51251	1		
NO	-0.5581	0.01072	-0.5287	0.8875	0.8505	1	
ABTS	0.9903	-0.7369	0.9848	0.9358	-0.1768	0.66802	1

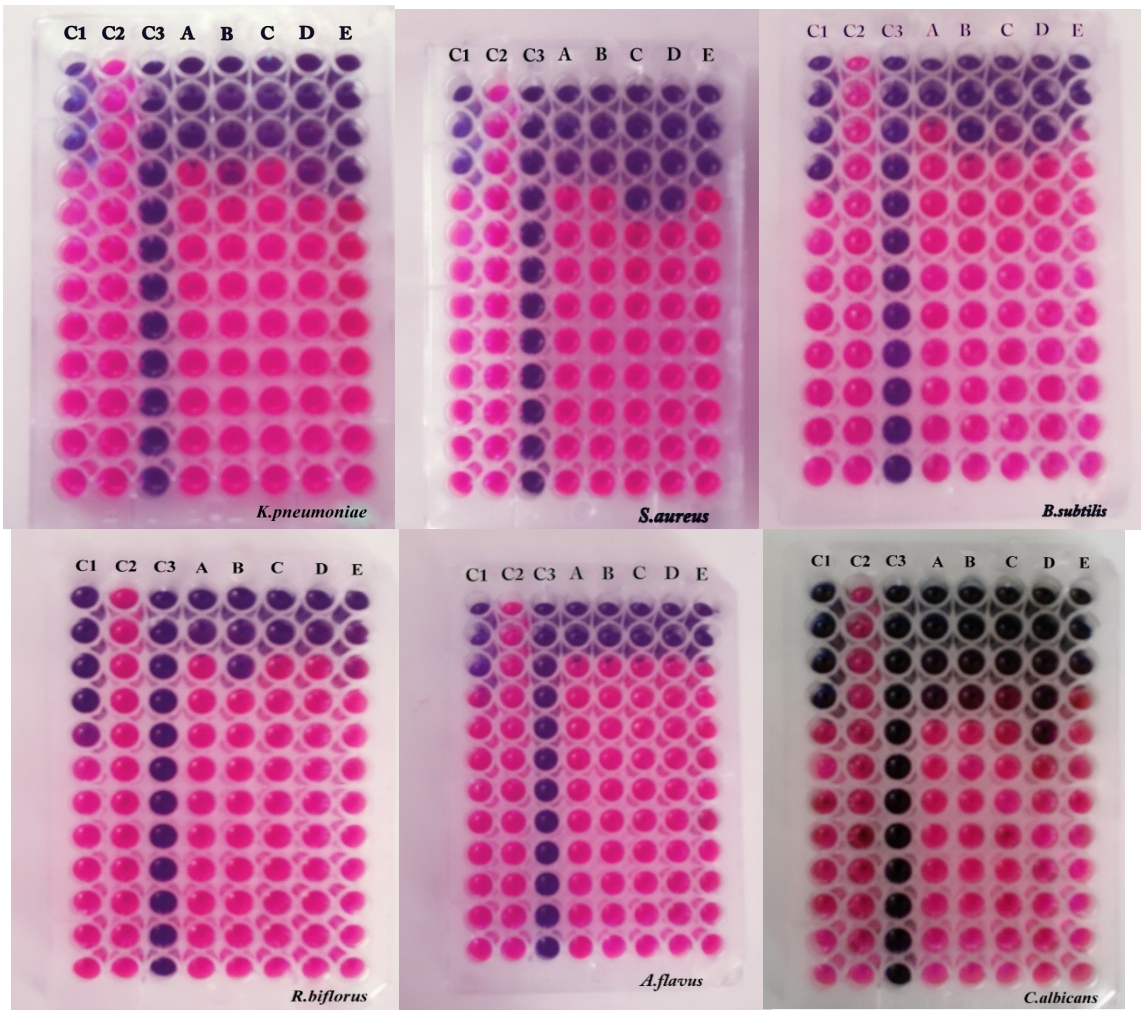

Figure-5-10: MIC of different extracts of *A.ilicifolius,* L.against selected pathogens.

Table-5: MIC values of *A.ilicifolius,* L.againstselected pathogens in different extracts (mg/ml)**.**

	Ethanol	Methanol	Ethyl acetate	Chloroform	Water	Control (+ve)
P.aeruginosa	0.521±0.104	1.042±0.208	0.938±0.312	1.042±0.208	2.08±0.417	1.042±0.208
B.subtilis	1.042±0.208	1.875±0.625	2.08±0.417	2.08±0.417	1.8750.625	0.521±0.104
K.pneumoniae	1.042±0.208	0.521±0.104	0.625±0.000	1.25±0.000	0.833±0.208	0.938±0.312
S.aureus	0.521±0.104	0.469±0.156	1.25±0.000	1.042±0.208	1.25±0.000	1.042±0.208
E.coli	1.042±0.208	2.08±0.417	1.042±0.208	2.08±0.417	1.875±0.625	0.938±0.312
R.biflorus	2.08±0.417	1.875±0.625	1.042±0.208	2.08±0.417	1.875±0.625	0.26±0.052
A.flavus	2.08±0.417	1.875±0.625	2.08±0.417	1.875±0.625	2.08±0.417	0.938±0.312
P.notatum	2.5±0.000	0.416±0.104	2.08±0.417	1.25±0.000	1.042±0.208	0.833±0.208
A.niger	1.25±0.000	0.625±0.000	1.67±0.417	1.25±0.000	1.042±0.208	0.416±0.104
C.albicans	0.625±0.000	0.416±0.104	0.833±0.208	0.625±0.000	1.25±0.000	0.521±0.104

Each value is expressed as mean± standard error done in triplicates. Data were analysed by ANOVA SPSS version 20.0 for windows followed by Duncan Multiple Range Test (DMRT) for comparison at P= 0.05 level of significance.

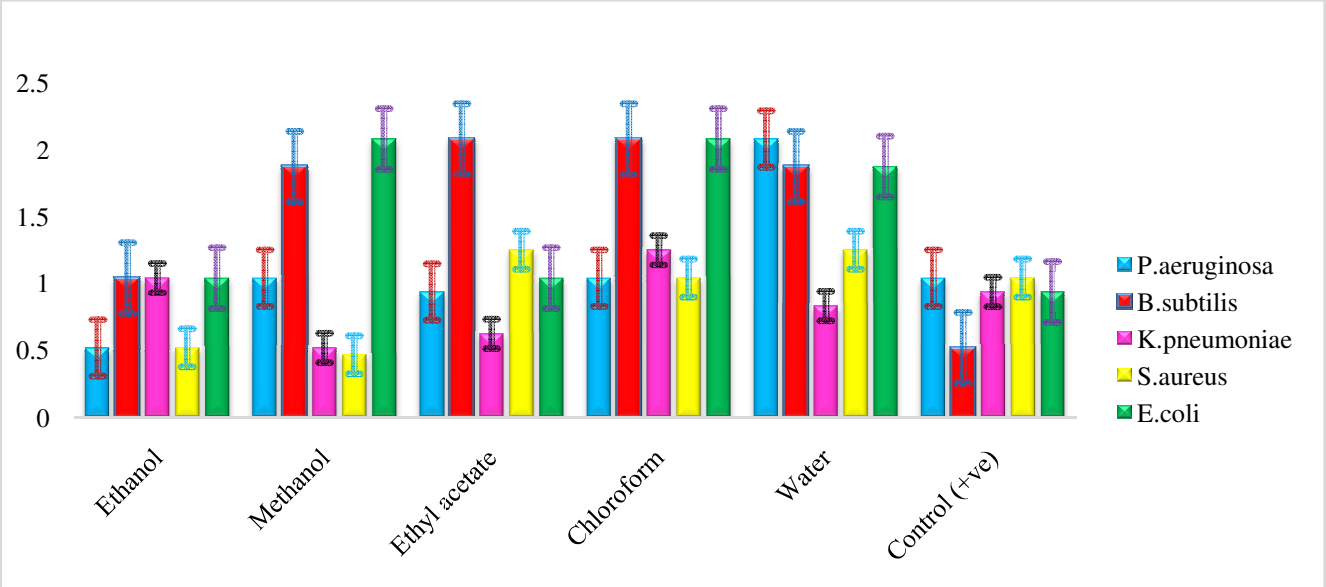

Figure-11: MIC values of *A. ilicifolius,* L.against selected bacteria in different extracts (mg/ml).

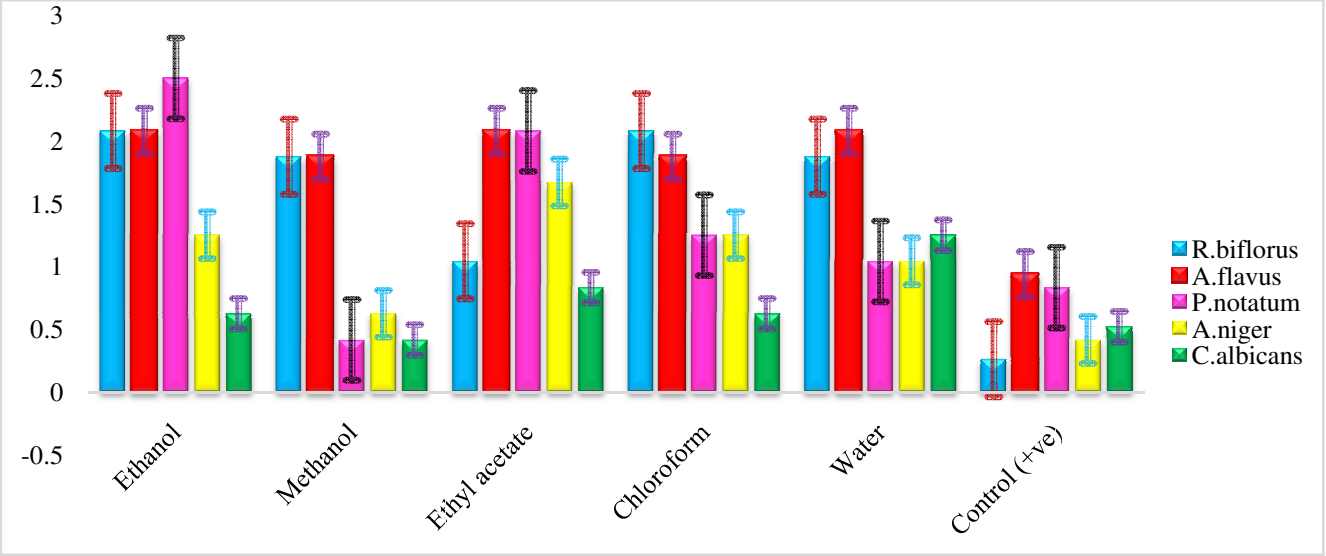

Figure-12: MIC values of *A. ilicifolius,* L.against selected fungus in different extracts (mg/ml).

GC-MS analysis: In nature mangroves are one of the best source of bioactive compounds[60-62]. A large number of active compounds have been structurally elucidated from the aerial part of *A. ilicifolius,* L[63]. The plant contained alkaloids like acanthicifoline and benzoxazinium compounds, phenolic compounds-acanfolioside, ilicifolioside, acteoside, verbascoside and apigenin derivatives. In the leaf of *A. ilicifolius,* L.steroids detected in the form of stigmasterol, campesterol, and sitosterol[64,65]. In the phytochemical screening, secondary metabolites like, proteins, resins, steroids, tannins, glycosides, sugars, carbohydrates, saponins, sterols, terpenoids, phenol, alkaloids, cardiac glycosides and catechol are isolated[66]. Two new cyclolignan glycosides, (+)-lyoniresinol 3a-O-β-D-galactopyranosyl -(1→6)--D glucopyranoside and (+)-lyoniresinol 2a-O-β-D- galactopyranosyl -3a-O--D-glucopyranoside have been reported from aerial parts of *A. ilicifolius,* L.[67]. Methanol extract of *A. ilicifolius,* L. exhibited antioxidant[68-70] and anti-tumour activities[7], anti-inflammatory actions[1,71] (Figure 13).

A phenyl ethanoid glycoside (ilicifolioside A) and an aliphatic alcohol glycoside (ilicifolioside B) have been isolated. Two lignanglucosides, (+)-lyoniresinol 3a-[2-(3, 5-dimethoxy-4-hydroxy)-benzoyl]-O-beta-glucopyranoside, and dihydroxymethyl-bis (3, 5-dimethoxy-4-hydroxyphenyl) tetrahydrofuran-9(or 9')-O-betaglucopyranoside have been isolated from the aerial parts. A new coumaric acid derivative acancifoliuside, acteoside, isoacteoside, acanthaminoside, (+)-lyoniresinol 3a-O-betaglucopyranoside, (-)-lyoniresinol, and alpha-amyrin , have been isolated from the methanolic extract of the leaves of *A. ilicifolius,* L.[72,73].

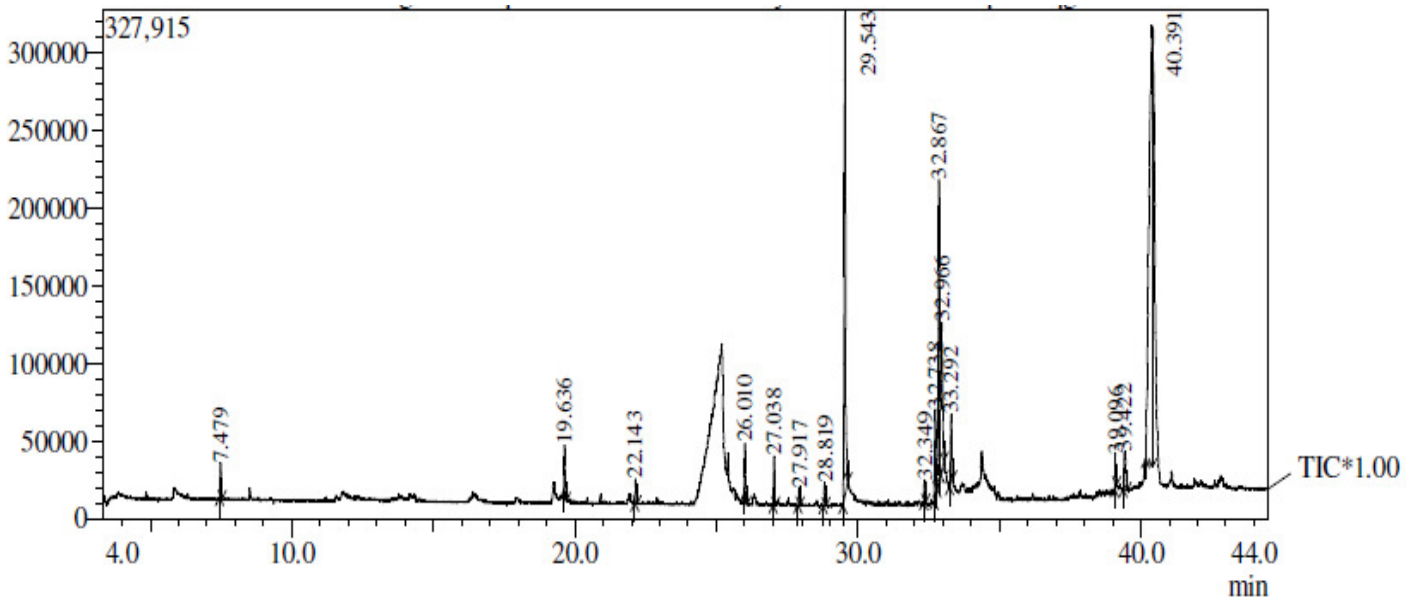

Figure-13: GC-MS Chromatogram of the methanol extract of of *A.ilicifolius,* L. *leaf.*

Table-6: Phyto-components identified in the methanol extract of the leaf of *Acanthusilicifolius,* L. by GC-MS.

Peak	Name	Retention Time	Activity
1	2-Octanone	7.479	Anticancer, Antioxidant, Ant allergic, Antiulcer and Antimicrobial [74, 75]
2	Phenol, 3,5-Bis(1,1-Dimethylethyl)-	19.636	Antimicrobial and Antiulcer [75]
3	Ethanone, 1-(2-Furanyl)-	22.143	Antioxidant and Antimicrobial [76]
4	3-Butylindolizidine	26.010	Anti-inflammatory and Antioxidant [77]
5	Tetradecanal	27.038	Anticancer and antioxidant [75, 78]
6	2-Decen-1-Ol	27.917	Antiulcer, Antimicrobial and Antioxidant [75,76]
7	Tridecanoic Acid, Methyl Ester	28.819	Anticancer and Antioxidant [76,79]
8	Hexadecanoic Acid	29.543	Antimicrobial and Ant allergic [80]
9	6-Octen-1-Ol, 3,7-Dimethyl-, Propanoate	32.349	Anticancer and Anti-inflammatory [81]
10	5-Decen-1-Ol, (Z)-	32.738	Antioxidant and Antimicrobial [74,76]
11	13-Tetradecenal	32.867	Antipyretic and Antioxidant [75,76]
12	9,12-Octadecadienoic Acid (Z,Z)-	32.966	Antiulcer, Antimicrobial and Anthelmintic [82]
13	9-Octadecenoic Acid (Z)-	33.292	Antioxidant and Ant allergic [76,79]
14	1-Hexanol, 5-Methyl-2-(1-Methylethyl)-	39.096	Ant rheumatic and Antidermatogenic [80]
15	1,2-Benzenedicarboxylic Acid, Di octyl Ester	39.422	Antimicrobial and Antipyretic [74]
16	Lupeol	40.391	Anticancer , Anti-inflammatory and Antioxidant [83]

Conclusion

The active extracts of *A. ilicifolius,* L. serve as a reservoir of potential bioactive compounds. The isolation of bioactive components from this readily available natural resource and their utilization as potential natural antibacterial agents could be of high economic value. The potential of plant extracts are due to presence of secondary metabolites and the extract were proven in containing various beneficial compounds for antioxidant and anti-inflammatory effects. The antimicrobial activity of *A. ilicifolius,* L .against the skin infecting bacterial and fungal pathogens. Free radical scavenging property of medicinal plants have shown light to pharmaceutical drug industry to produce anticancerous medicinal formulation. The results indicated the presence of different chemical constituents in the leaf sample of *A.ilicifolius,* L.. A particular plant can be identified on the basis of its GC–MS chemical profile of different extracts. The GC–MS of *A.ilicifolius,* L. can be a significant fingerprint chemotaxonomic marker for the identitification.

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