Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 5 (1): 60-65, 2011
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2011.60.65

Phytochemical Analysis of *Acanthus ilicifolius* and *Avicennia officinalis* by GC-MS

S. Ganesh and J. Jannet Vennila
Department of Bioinformatics, Karunya University, Coimbatore-641114, Tamilnadu, India

Corresponding Author: S. Ganesh, Department of Bioinformatics, Karunya University, Coimbatore-641114, Tamilnadu, India

ABSTRACT

In this study, the phytochemical analysis of *Acanthus ilicifolius* and *Avicennia officinalis* were studied. These created an interest to test the possible phytochemical activity of the leaves of these plants, which has not been reported. *Acanthus ilicifolius* and *Avicennia officinalis* leaves indicate the presence of Protein, Resin, Steroids, Tannins, Glycosides, Reducing sugar, Carbohydrates Saponnins, Sterols, Terpenoids, Phenol, Cardioglycosides and Catachol. However acidic compounds are absent in this plant. In the GC-MS analysis, 7 bioactive phytochemical compounds were identified in the methanol extract of *Acanthus ilicifolius* leaves and 3 bioactive phytochemical compounds were in the methanol extract of *Avicennia officinalis*. The *Acanthus ilicifolius* and *Avicennia officinalis* revealed the presence of medicinal active constituents by GC-MS. The functional group present in these compounds was identified by IR spectral analysis. This study also helped to identify the formula and structure of biomolecules which can be used as drugs.

Key words: *Acanthus ilicifolius*, *Avicennia officinalis*, phytochemical activity, functional group, IR, GC-MS

INTRODUCTION

Mangroves are trees and shrubs that grow in saline coastal habitats in the tropics and subtropics-mainly between latitudes 25° N and 25° S (Saenger, 2002). They have recognized 65 mangrove species in 22 genera and 16 families (Kathiresan and Bingham, 2001). *Acanthus ilicifolius* (sea holly) occurs in tropical Asia and Africa, through Malaya to Polynesia. It is a tiny shrub or tall herb, upto 1.5 m high, scarcely woody, bushy, with very dense growth. Shallow tap roots, but occasionally stilt roots are conspicuous. Leaf simple, opposite, decussate, cauline, exstipulate, petiole short, flattened, glabrous, pulvinous to sheathing base. Flower bisexual, typically zygomorphic, complete, erect, sessile, hypogynous. Fruit 1 cm green and 2.5-2.0 cm long, kidney shaped 4 seed drupe, Seed 0.5-1.0 cm long (Xie *et al*., 2005). Malaysia: The leaves of *A. ilicifolius* are used to treat rheumatism, neuralgia and poison arrow wounds. It is widely believed among mangrove dwellers that chewing the leaves will protect against snake bite. Malay: The pounded seeds of *A. ilicifolius* and *A. ebracteatus* are used to treat boils and the juice of leaves to prevent alopecia. Both species are also used to treat urolithiasis. India: In Ayurveda, the plant is known as Sahachara. The drug is astringent and makes a good nervine tonic, expectorant and stimulant. It is used in coughs and asthma. The root, boiled in milk, is largely used in leucorrhoea and general debility. The Siamese and Indo-Chinese consider the roots to be cordial and attenuant

and useful in paralysis and asthma. The tender shoots and leaves are used in India for bite. In Goa, the leaves, which abound in mucilage, are used as an emollient fomentation in rheumatism and neuralgia. Thailand: Water extracted from the bark is used to treat colds and dermatitis. Ground fresh bark is used as an antiseptic. Tea brewed from the leaves relieves pain and purifies the blood (Singh *et al.*, 2009). The ethanol extract of the plant was found to scavenge superoxide and hydroxyl radicals. The extract was also found to inhibit the generation of nitric oxide radical and lipid peroxides. Recent, studies have shown that the plant extract has a remarkable hepatoprotective effect. The flavonoids present in the plant were found to have hepatoprotective and antioxidant activities (Babu *et al.*, 2001). The present study focus to characterize and analyze the phytochemicals by GC-MS, which will throw more insight into identifying the formula of bimolecular therapy in drug studies. *Avicennia officinalis* is a commonly available as white mangrove plant in almost all the coastal states of India. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer. A decoction of the plant with sugar candy and cumin is used in dyspepsia with acid eructations. The fruits are plastered onto tumors in India. Indian mangrove is a folk remedy for boils and tumors. A resinous substance exuded from the bark acts as a contraceptive and apparently can be taken all year long without ill effects (Thirunavukkarasu *et al.*, 2010). The plant ranges from tropical Moist to Wet through Subtropical Moist to Wet Forest Life Zones, Indian mangrove is estimated to tolerate annual precipitation of 10 to 45 dm, annual temperature of 20 to 26°C and pH of 6 to 8.5. The present study focus to characterize and analyze the phytochemicals by GC-MS, which will throw more insight into identifying the formula of bimolecular therapy in drug studies.

MATERIALS AND METHODS

Collection of plant: Fresh leaves of *Acanthus ilicifolius* and *Avicennia officinalis* were collected from Parankipettai, cuddalore district, Tamil nadu, in the month of February to March (2010) and 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 the leaf were air-dried, coarsely powdered and were subjected to extraction.

Preparation of the extracts: The fresh leaves of *Acanthus ilicifolius* and *Avicennia officinalis* were washed with tap water and shade dried at room temperature (28±2°C). The dried leaves were powdered by electrical blender. Methanol was used for the extraction of 25.0 g in the Soxhlet apparatus followed by the standard procedure (Duke and Wain, 1981). The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing methanol. The solvent was boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 h) and the solvent was removed at the reduced pressure with the help of rotary vaccum evaporator to yield a viscous dark green residue of methanol leaf extracts. The phytochemical, GC-MS and IR Spectrum analysis of *Acanthus ilicifolius* and *Avicennia officinalis* plant extract was investigated.

Phytochemical screening: Phytochemical screenings were performed using standard procedures (Sofowora, 1931; Trease and Evans, 1989).

- **Tests for proteins-xanthoprotein test:** To 1 mL of extract, few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow color

- **Tests for resins:** Five milliliter of distilled water was added to the extract and observed for turbidity
- **Tests for steroids:** Two milliliter of acetic anhydride was added to 0.5 g of extract and 2 mL of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green
- **Tests for tannins:** About 0.5 g of the each extract was taken in a boiling tube and boiled with 20 mL distilled water and then filtered added few drops of 0.1% ferric chloride was added mixed well and allowed to stand some time. Observed for brownish green or a blue-black coloration
- **Tests for glycosides-keller-killani test:** About 0.5 mL of alcoholic extracts was taken and subjected to the following test, 1 mL of glacial acetic acid containing traces of ferric chloride and 1 mL of conc. Sulphuric acid was added to extract and observed for the formation of reddish brown color at the junction of two layers and the upper layer turned bluish green in the presence of glycosides
- **Tests for reducing sugar-fehling's reagent:** Few drops of Fehling's solution A and B in equal volume were added in dilute extracts and heated for 30 min and observed for the formation of brick red colored precipitate
- **Tests for carbohydrates-molisch test:** Small quantities of alcoholic and aqueous extracts was dissolved in 5 mL of distilled water and filtered. To this solution 2-3 drops of α-naphthol was added and 1 mL of concentrated sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet coloured ring at the interface to detect the presence carbohydrates
- **Tests for saponins:** To 0.5 g of extracts was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion
- **Tests for sterols-liebermann-buchard test:** The insoluble residue was dissolved in chloroform and few drops of acetic anhydride were added along with a few drops of conc. Sulphuric acid from the sides of the test tube and observed for the formation of blue to blood red color
- **Tests for terpenoids-salkowski test:** To 0.5 g of the extract, 2 mL of chloroform was added; Conc. H_2So_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids
- **Tests for acidic compounds:** To the alcoholic extract sodium bicarbonate solution was added and observed for the production of effervescences
- **Tests for phenols:** The extracts were taken in water and warmed. To this 2 mL of ferric chloride solution was added and observed for formation of green or blue colour
- **Test for cardiac glycosides (Keller Killiani's):** Among 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was then underlayer with 1 mL of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar charactersitcs of cardenolides
- **Test for catachol:** To 2 mL of test solution alcohol is added and erlich's reagent and few drops of conc.hydrochloric acid was added. The result was obtained

GC-MS analysis for organic constituents of leaves: The GC-MS analysis of *Acanthus ilicifolius* and *Avicennia officinalis* was performed using a fison 800 top gas chromatography

equipped with a Ab 35 ms capillary coloum (10 m×0.25 mm×0.025 μm) and mass detector fision MS-800 md. Helium was the carries gas at flow rate of 1 mL min^{-1}. The column temperature 100 to 250°C and the oven temperature was programmed as follows; 250°C for 10 min. The MS operating parameters TIC (Total Ion Current) 70 Ev scat. The identification compound based on comparison of their mass spectra with those of NIST and Wiley Libraries (Dool and Kratz, 1963).

RESULTS

The phytochemical active compounds of *Acanthus ilicifolius and Avicennia officinalis* were qualitatively analyzed and the results are presented in Table 1. Table I indicates that the methanol extraction of *Acanthus ilicifolius* leaf showed the presence of phytochemical active compounds such as Protein, Resin, Steroids, Tannins, Glycosides, Reducing sugar, Carbohydrates Saponins, Sterols, terpenoids, phenol, Cardioglycosides and Catachol. But acidic compounds were absent. In the same way, methanolic extraction of *Avicennia officinalis* leaf showed the presence of phytochemical active compounds such as Protein, Resin, Steroids, Tannins, Glycosides, Reducing sugar, Carbohydrates Saponins, Sterols, terpenoids, phenol, Cardioglycosides and Catachol. But acidic compounds showed negative result.

Table 2 represents the GC-MS analysis of methanohc leaf extraction of *Acanthus ilicifolius* and *Avicennia officinalis*. The identified compounds of *Acanthus ilicifolius* are Cyano colchicines (RT = 6.06), 26,27-di (nor)-cholest-5,7,23-trien-22-ol,3-methoxymethoxy (RT = 12.31), 9H-purin-6-amine,N,9-bis(trimethylsilyl)-8-((trimethylsilyl)oxy)(RT = 14.09), Glycine, N-((3a, 5a, 12a)-24-oxo-3,12-bis((trimethylsilyl)oxy) cholan-24-yl)-methyl ester (RT = 15.68)) and 4,7-Methano-1H-inden-1-one,3,4,5,6,7,8,8-heptacholro-3a,4,5,6,7,7a-hexahydro (RT = 17.14). The identified compounds of *Avicennia officinalis* are 3a,6-Methano-3ah-inden-5-ol, octahydro (RT = 9.28) and 26,27-Di(nor)-cholest-5,7,23-trien-22-ol,3-methoxy (RT = 12.28).

Furthermore analysis was done with Infrared spectroscopy to identify the functional group present in the above listed compounds for *Acanthus ilicifolius* and *Avicennia officinalis*. The IR spectral analysis showed more or less the same number of peaks for both the plants. Alcohol, phenol, Alkane and Sulphate groups are present in these compounds.

Table 1: Qualitative analysis of phytochemical components

	Methanol leaf	
Phytochemical components of qualitative analysis	*Acanthus ilicifolius*	*Avicennia officinlis*
Protein	+	+
Resin	+	+
Steroids	+	+
Tannins	+	+
Glycosides	+	+
Reducing sugar	+	+
Carbohydrates	+	+
Saponnins	+	+
Sterols	+	+
Terpenoids	+	+
Acidic compounds	+	+
Phenol	+	+
Cardio glycosids	+	+
Catachol	+	+

Table 2: GC-MS analysis

Name of the compound	RT
***Acanthus ilicifolius* Methanol Leaves**	
Cyano colchicine	6.06
26,27-di(nor)-cholest-5,7,23-trien-22-ol,3-methoxymethoxy	12.31
9H-purin-6-amine,N,9-bis(trimethylsilyl)-8-((trimethylsilyl)oxy)	14.09
Glycine, N-((3a, 5a, 12a)-24-oxo-3,12-bis((trimethylsilyl)oxy)	15.68
cholan-24-yl)-methyl ester	
4,7-Methano-1H-inden-1-one,3,4,5,6,7,8,8-heptacholro-3a,4,	17.14
5,6,7,7a-hexahydro	
***Avicennia officinalis* Methanol leaves**	
3a,6-Methano-3ah-inden-5-ol, octahydro	9.24
26,27-Di(nor)-cholest-5,7,23-trien-22-ol,3-methoxy	12.28

RT: Retention time

DISCUSSION

Wahidulla and Bhattacharjee, 2001 identified (2*R*)-2-b-D-glucopyranosyloxy-2*H*-1,4-benzoxazine-3(4*H*)-one(GHBOA, blepharin) (1) and (2*R*)-2-b-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazine-3-one (GDIBOA) (2) from the pods of a mangrove *Acanthus illicifolius*. Amer *et al* 2004 reported three novel alkaloids namely 6-hydroxy-benzoxazolinone, 4-hydroxyacanthamine and acanthaminoside. Patra *et al*. (2009) identified compounds in *H. spinosa (*Acanthaceae) are lupeol, lupenone, 25-oxo-hentriacontanyl acetate, stigmasterol, betulin, β-carotene, hentriacontane, apigenin-7-O-glucuronide, apigenin-7-O-glucoside, 3-methylnonacosane, 23-ethylcholesta-11(12), 23(24)-dien-3β-ol, luteolin, asteracanthine, asteracanthicine, luteolin-7-rutinoside, methyl-8-n-hexyltetracosanoate, β-sitosterol, histidine, phenylalanine, lysine, ascorbic acid, nicotinic acid, n-triacontane, glucose, mannose, rhamnose, arabinose, xylose, maltose, myristic acid, oleic acid, palmitic acid, stearic acid, linoleic acid etc. Antibacterial activity was exhibited by the chloroform and methanol extract of the whole plant and methanolic extract of the leaves. In this study, the methanolic extract of the leaves of *Acanthus ilicifolius* resulted in the isolation of 5 compounds namely Cyano colchicines, 26,27-di(nor)-cholest-5,7,23-trien-22-ol,3-methoxymethoxy, 9H-purin-6-amine,N,9-bis(trimethylsilyl)-8-((trimethylsilyl)oxy), Glycine, N-((3a, 5a, 12a)-24-oxo-3,12-bis((trimethylsilyl)oxy) cholan-24-yl)-methyl ester and 4,7-Methano-1H-inden-1-one,3,4,5,6,7,8,8-heptacholro-3a,4,5,6,7,7a-hexahydro. In the same way, the methanolic extract of the leaves of *Avicennia officinalis* resulted in the isolation of 2 compounds namely 3a,6-Methano-3ah-inden-5-ol, octahydro and 26,27-Di(nor)-cholest-5,7,23-trien-22-ol,3-methoxy. The compounds that are obtained by GC-MS are similar with other reviewers but a new sulphonyl chloride compound has been obtained through IR spectral analysis.

The IR spectral analysis are not done in these plants. It showed the presence of alcohols and phenols in the O-H region at 3389 cm^{-1}. It indicates that an intermolecularly hydrogen bonded polymeric association compound is present. The alcohols and phenols are in low concentration and the peak is broad. The C-H stretch at 2924 cm^{-1} showed the presence of octane group and the peak is medium to strong. The peak at 1384 cm^{-1} indicates the presence of alcohol, phenol, alkane or S = O sulfate group. Sulfonyl chloride compound may be present and the band is strong.

CONCLUSION

The present study carried out on the *Acanthus ilicifolius* and *Avicennia officinalis* revealed the presence of medicinal active constituents by GC-MS. This study also helped to identify the

formula and structure of biomolecules which can be used as drugs. In the screening process of Acanthus ilicifolius and Avicennia officinais of leaves indicate the presence of protein, resin, steroids, tannins, glycosides, reducing sugar, carbohydrates and saponnins, sterols, terpenoids, phenol, cardioglycosides and catachol. However Acidic compounds are absent in these plants. The result obtained from the phytochemical in GC-MS analysis IR spectral study of these plants, revealed that further investigations may lead to the development of drug formulation.

ACKNOWLEDGMENT

We thank Dr. D. Jaya Sundra Sharmila for her keen interest in the work and constant encouragement.

REFERENCES

Amer, M.E., M.I. Abou-Shoer, M.S. Abdel-Kader, A.M.S. El-Shaibany and N.A. Abdel-Salam, 2004. Alkaloids and flavone acyl glycosides from *Acanthus arboreus*. J. Brazilian Chem. Soc., 15: 262-266.

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

Dool, H.V.D. and P.D. Kratz, 1963. A generalization of the retention index system inclg linear temperature programmed gas-liquid partition chromatography. J. Chromatog. A, 11: 463-471.

Duke, J.A. and K.K. Wain, 1981. Medicinal Plants of the World: Computer Index with More Than 85,000 Entries. Vol. 3, Longman Group Ltd., UK.

Kathiresan, K. and B.L. Bingham, 2001. Biology of mangrove and mangrove ecosystems. Adv. Mar. Biol., 40: 81-251.

Patra, A., S. Jha and P.N. Murthy, 2009. Phytochemical and pharmacological potential of *Hygrophila spinosa* T. anders. Pharmacognosy Rev., 3: 330-341.

Saenger, P., 2002. Mangrove Ecology, Silviculture and Conservation. Kluwer Academic Publishers, Dordrecht.

Singh, A., S. Duggal and A. Suttee, 2009. *Acanthus ilicifolius* linn.-lesser known medicinal plants with significant pharmacological activities. Int. J. Phytomed., 1: 1-3.

Sofowora, A., 1931. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books, Ibadan.

Thirunavukkarasu, P., T. Ramanathan, L. Ramkumar and R. Shanmugapriya, 2010. Anti ulcer effect of *Avicenia officinalis* leaves in albino rats. World Applied Sci. J., 9: 55-58.

Trease, G.E. and W.C. Evans, 1989. Trease and Evans: Pharmacognosy. 13th Edn., Bailliere Tindale, London.

Wahidulla, S. and J.J. Bhattacharjee, 2001. Benzoxazinoids from *Acanthus illicifolius*. J. Indian Inst. Sci., 81: 485-490.

Xie, L.S., Y.K. Liao, Q.F. Huang and M.C. Huang, 2005. Pharmacognostic studies on mangrove *Acanthus ilicifolius*. Zhongguo Zhong Yao Za Zhi, 30: 1501-1503.