VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 550-560 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Qualitative and Quantitative Phytochemical Screening and GC-MS Analysis of *Avicennia marina* Leaf Extracts

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Received: 24th June, 2023; **Accepted:** 25th July, 2023; **Published online:** 10th September, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.063

Abstract: Mangroves are widely used to extract natural compounds for use in traditional medicine and the pharmaceutical sector. In this study, the phytochemical analysis of *Avicennia marina* was examined. In the current study, ethanol, ethyl acetate, n-hexane and aqueous extracts were used. Terpenoids, flavonoids, phenols, proteins and coumarin were among the studied phytochemical components in *Avicennia marina* leaf. Quantitative phytochemical analysis of *A. marina* leaf extracts (total saponins, total tannins and total terpenoids) was performed. *Avicennia marina* demonstrated the presence of medicinally active ingredients by GC-MS. This study also helped identify the formula and structure of bimolecular therapy that can be used as a drug.

Keywords: *Avicennia marina*, Mangroves, Phytochemicals, Pharmaceutical

Citation: Vasantha Kumar K., Jayaseelan K., Murugesan R. and Dinesh Kumar G.: Qualitative and quantitative phytochemical screening and GC-MS analysis of *Avicennia marina* leaf extracts. Intern. J. Zool. Invest. 9(2): 550-560, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.063

Introduction

Phytochemicals are natural compounds made from plants and the phytomolecules hich are implicated in a wide range of human health benefits. Secondary metabolites are another name for chemical substances that are produced in plants during normal metabolic processes and are referred to as phytochemicals. Alkaloids, flavonoids, glycosides, coumarins, gums, phenols, polysaccharides, terpenes, and tannins are examples of secondary metabolites found in plants. To date, over 25,000 different phytochemicals have been identified, the vast majority of which are found in plant-based foods such as fruits, vegetables, nuts, legumes, and

whole grains.

Medicinal plants are plants in which at least one of their components (leaves, stems, bark or roots) is used for therapeutic purposes. Medicinal plants are found all over the world (Bruneton, 1993). In recent years, medicinal plants have gained increasing importance in the treatment of a variety of diseases and conditions, including diabetes, malaria and anemia (Fola, 1993). Compared to so-called modern medicines, medicinal plants are more attractive as potential treatments in sub-Saharan Africa because they are both readily available and very inexpensive (Agbor and Ngogang, 2005; Agbor *et al*., 2005). Due to the importance of medicinal plants and the contribution that phytomedicine makes to the health and well-being of a significant portion of the world's population, various areas of research has sparked interest in phytomedicine.

In addition to vitamins C, E and carotenoids, many medicinal plants have high concentrations of antioxidants (Javanmardi *et al*., 2003). Antioxidants are chemicals that, according to Vilioglu *et al*. (1998) can either delay or prevent the occurrence of an oxidative reaction. Free radicals are the catalysts for oxidative reactions. Above all, phenolic components such as flavonoids (Pietta, 1998), phenolic acids and phenolic diterpenes are responsible for this antioxidant effect (Shahidi *et al*., 1992). Antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) protect plants from oxidative attack (Dziezak, 1986) by binding to metal ions, scavenging free radicals, or breaking down peroxides. Although synthetic antioxidants are readily available, the current study focuses on identifying novel natural antioxidant molecules that have the potential to play a role in diseases caused by oxidative stress (Agbor *et al*., 2005). Epidemiological studies (Halliwell, 1994; Urquiaga and Leighton, 2000) have shown that fruit and vegetable consumption is associated with a reduction in the prevalence of chronic diseases in some communities. These results were found in specific populations.

Few studies have shown that the extract from the plant has a significant hepatoprotective effect (Miles *et al*., 1998). It has been strongly suggested to consider mangroves as a useful source of chemical compounds that have the potential for therapeutic and agricultural purposes (Miles *et al*., 1998). Although the chemical components of most mangrove plants are not fully understood, research to date has led to the identification of several new compounds that have the potential to be used medicinally in the mangrove plant to develop new chemotherapeutic agents. *Avicennia marina* (Forssk.) has been the subject of some research aimed at identifying the essential chemical components that make up the plant. Sutton *et al.* (1985) and Miles *et al.* (1998) contributed to the discovery of a naphthoforan compound with phytoalexin activity. Studies on fatty acids, sterols, and carbohydrates in eleven mangrove species including *A. marina* were performed to determine the chemotaxonomic importance of these compounds (Hogg and Gillan, 1984). According to Bousquet-Mlou and Fauvel (1998), iridoid glucoside 2-cinnamoyl-mussaenoside/acid can be used in the subspecific chemotaxonomy of *A. marina* extracts. This can be done by determining whether the acid is present. The current investigation focused on the characterization and analysis of phytochemicals of *A. marina* using GC-MS, which will provide more insight into the process of formulating a bimolecular treatment in pharmacology research. Both *Avicennia marina* and *Rhizophora mucronata* are common examples of mangrove plants found practically throughout the Egyptian coast bordering the Red Sea. It is a medicinal herb used in folklore primarily to treat rheumatism, paralysis, asthma, snakebite, skin diseases, and ulcers. Boils and tumors can be treated with Indian mangrove, a traditional medicine. A contraceptive can be derived from a resinous material isolated from the bark that is safe to take at any time of the year with no side effects (Thirunavukarasu *et al*., 2010).

The mangroves are the second most important marine ecosystem after the coral reefs. Mangroves

are deciduous trees that have evolved to thrive in the tidal wetlands of tropical and subtropical coastal areas. In addition, mangroves are widely used in the synthesis of bioactive substances used in traditional medicine. For example, mangrove extracts, whether derived from leaves, roots or other plant parts, have been shown to be effective against a wide range of diseases (Bandaranayake, 2002). *Avicennia officinalis* is a mangrove species that is known for its contained phytochemicals. These compounds are known to have antidiabetic and free radical scavenging effects. Raw leaf extracts of many mangrove species including *Avicennia marina* and *Rhizophora macromata* showed significant antibacterial and antioxidant activity (Gawali and Jadhav, 2011). In this study, the phytochemical analysis of *Avicennia marina* was examined. In the current study, ethanol, ethyl acetate, n-hexane and aqueous extracts were used.

Materials and Methods

Collection and Identification of Avicennia marina:

Fresh and Healthy leaves of *Avicennia marina* were collected from their natural habitat of Muthupet mangrove in Thiruvarur district, Tamil Nadu, India and authenticated by taxonomist in the Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The herbarium number of the plant is SK001.

Extraction of mangrove plant leaves (*Avicennia marina*):

After washing with distilled water, the leaves were dried in the shade, powdered, and extracted into ethanol separately. Plant powder (20 g) was removed, soaked in 100 ml of solvent, and kept in the shaker for 24 h. After centrifugation at 5000 rpm, the solvent phase was separated and evaporated. The crude oil was stored at 40 °C and used for further investigations.

Qualitative Phytochemical Analysis of Avicennia marina Leaf Extracts:

Test for terpenoids (*Salkowski Test*):

Mixed 2 ml extract, 2 ml of chloroform and 2 ml of concentrated sulfuric acid. The presence of terpenoids was confirmed by a red-brown coloration of the interface.

Test for flavanoids:

A few drops of 10% lead acetate solution was added to 2 ml extract. The presence of flavanoids is indicated by the yellow color.

Test for saponin (*Foam Test*):

Mixed 2 ml of extract with 2 ml of distilled water. The solution was then shaken vigorously and attention was paid to stable, persistent foam. Then the formation of an emulsion was observed, after vigorous shaking the mixture was foamed with 3 drops of olive oil.

Test for tannins (*Braymer's Test*):

To the 2 ml of extract added 2 ml of distilled water which is followed by a filtration. Then added a few drops of 0.1% ferric chloride to the filtrate. The formation of a green precipitate indicates the presence of tannins.

Test for alkaloids (*Hager's Test*):

Add a few drops of Hager's reagent to the 2ml of extract and shake gently to separate the alkaloid base. The appearance of a yellow precipitate indicates the presence of alkaloids.

Test for steroids (*Salkowski Test*):

Added 2 ml of extract, 2 ml of chloroform and a few drops of concentrated sulfuric acid. A reddish-brown ring indicates the presence of steroids.

Test for Glycosides (*Liebermann's Test*):

Added 2 ml of chloroform and 2 ml of acetic acid to the 2 ml of extract. Violet to blue or green colour indicates the presence of glycosides.

Test for phlobotannin:

Add a few drops of HCl to 2 ml extract. The presence of phlobotannins is confirmed by the formation of a red precipitate.

Test for proteins (*Xanthoprotein Test*):

Mixed 2 ml of extract with 2 ml of concentrated sulfuric acid and boil. The white precipitate indicates the presence of proteins.

Test for Coumarin:

Added few drops of NaOH to the 2 ml extract. The appearance of a yellow color confirms the presence of coumarin.

Test for Emodin:

2 ml extract was mixed with few drops of ammonium hydroxide followed by the addition of a few drops of benzene. The red precipitate indicates the presence of emodin.

Test for Anthroquinone:

Added few drops of benzene and a few drops of ammonia solution. The formation of a purple color indicates the presence of anthraquinone.

Test for Anthocyanin:

2 ml of extract was mixed with few drops of HCl and ammonia solution. The pink color confirms the presence of anthocyanins.

Test for Carbohydrate:

To the 2 ml extract added 2 ml distilled water, 2 drops ethanolic alpha-naphthol and then 2 ml concentrated sulfuric acid. The presence of carbohydrates is confirmed by the formation of a reddish-purple ring at the junction.

Test for Leucoanthocyanin:

Added few drops of isoamyl alcohol to the 2 ml extract. Red colour indicates the presence of leucoanthocyanin.

Test for Cardiacglycosides:

2 ml of extract was added with few drops of glacial acetic acid followed by ferric chloride and con.H_2SO_4.

Test for Xanthoproteins:

Added 2-3 drops of ferric chloride to 2 ml of extract. The blue color indicates the presence of xanthoproteins.

Test for Phenols:

Added few drops of ammonia solution to 2 ml of extract; the appearance of a reddish-orange precipitate confirms the presence of phenols.

***Quantitative Analysis of Secondary Metabolites*:**

Assessment of Flavanoids:

Added 2 ml of methanol to 0.5 g of sample. The solution was filtered through a filter paper. Then the filtrate was transferred to a crucible and evaporated to dryness over a water bath and weighed.

Assessment of Tannin:

0.5 g sample was mixed with 1 ml distilled water and then filtered. Added 1 drop of ferric chloride and potassium ferrocyanate to the filtrate. After evaporation, the sample was dried in a crucible to constant weight.

Assessment of Saponin:

Added 1 ml of diethyl ether to 0.5 g of sample and filtered after vigorous shaking, forming a double layer. Aspirated the top layer and added 1 drop of n-butanol and 1 drop of NaCl to it. Then heated in a water bath. After evaporation, the sample was dried in a crucible to constant weight.

Assessment of Alkaloids:

To 0.5 g sample add 10% acetic acid in ethanol. Then filtered and added a drop of ammonia solution to the filtrate. Then dried and weighed.

Assessment of Phenol:

Mix 0.5 g sample with 1 ml distilled water. Filtered it, added a drop of ammonia solution and isoamyl alcohol to it. After evaporation, the sample was dried in a crucible to constant weight.

Assessment of Terpenoids:

Added 1 ml petroleum ether to 0.5 g sample. Filtered it and put it in a crucible. Evaporated to dryness and weighed.

Gas Chromatography-Mass Spectrometry (*GC-MS*) *Analysis*:

For GC-MS analysis used Clarus 500 Perkin-Elmer (Auto System XL) gas chromatograph fitted and coupled to a Turbo Mass Gold Perking Elmer Turbomas 5.2 mass detector with an Elite-1 (100% dimethyl ply siloxane), 300m x 0.25mm x 1 MDF mass detector -Capillary column. First, the

device was set to a temperature of 110 °C and then held at this temperature for 2 min. At the end of this period, the oven temperature was increased to 280°C at a rate of 5°C per min and held for 9 min. The temperature of the injection port was secured at 250°C and the flow rate of helium at 1 ml/min. The ionization voltage was 70 eV. Samples were injected incrementally in a 10:1 split mode. The range of the mass spectrum was adjusted to 45-450 (MHz). The chemical components were identified by GC-MS. The fragmentation patterns of the mass spectra were compared to those in the spectrometer database using the National Institute of Standards and Technology (NIST-MS) mass spectral database. The percentage of each component was calculated from the relative peak area of each component in the chromatogram.

Identification of Compounds:

GC-MS mass spectrum interpretation was performed using the National Institute Standard and Technology (NIST) database of more than 62,000 samples. The spectrum of the unknown component was compared to the spectrum of the known components stored in the NIST library. The structure, name and molecular weight of the components of the test materials were determined.

Results and Discussion

Qualitative and Quantitative Phytochemical Analysis:

Avicennia marina leaf extract was analyzed for qualitative phytochemical screening. In the current study, ethanol, ethyl acetate, n-hexane and aqueous extracts were used. Terpenoids, flavanoids, phenols, proteins and coumarin were among the studied phytochemical components in *Avicennia marina* leaf. The presence of phytocompounds is listed in Table 1.

In the quantitative analysis of the hexane extract of *A. marina*, TSC was present at 18.60 mg/g, TTC at 18.40 mg/g and TTeC at 18.40 mg/g. In ethyl acetate extract 18.28 mg/g TSC, 20.83 mg/g TTC and 18.12 mg/g TTeC was present. Ethanol extract showed presence of TSC at 19.90 mg/g, TTC at 20.28 mg/g and TTeC at 18.49 mg/g. In aqueous extracts TSC at 19.90 mg/g, TTC at 20.28 and TTeC at 18.49 mg/g were observed in *A. marina* (Table 2).

GC-MS Analysis of A. marina Leaf Extracts:

Marine environments offer a fascinating opportunity for the discovery of new bioactive chemicals. According to a study by Saleh Amer *et al*. (2020), mangroves are extremely unique plants native to tropical and subtropical coastal regions. For this reason, mangroves develop a very specific ecosystem of bacteria, fungi and endophytic microorganisms responsible for the production of novel compounds with different biological activities.

Most therapeutic chemicals used to treat human diseases come from medicinal plants. Recent studies on medicinal and aromatic plants have led to an improvement in health care for all of mankind. The extensive floral resources of the mangrove forests are best known for the medicinal properties of the plants they contain. Because of the significant potential medicinal applications, mangrove forest plants and the bioactive substances they contain have been the subject of extensive research in recent years. Since the beginning of recorded history, herbal extracts from mangrove trees have been used as a standard therapy for a variety of diseases and ailments. The naturally occurring bioactive chemicals found in mangrove plants have their own functions.

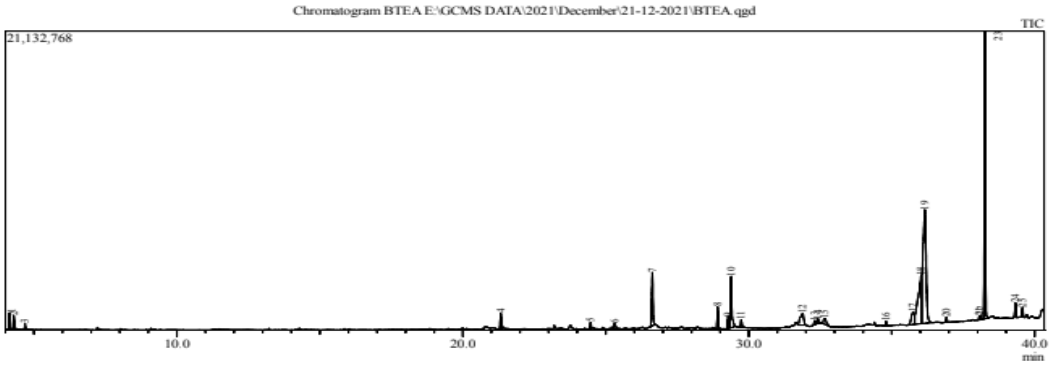
In addition, it has been discovered that the leaves of *A. marina* contain a significant amount of phytol, a potent bioactive chemical that can be used in the manufacture of medicinal and agricultural goods. The elimination of hydroxyl radicals and nitric oxide and the prevention of the synthesis of thiobarbituric acid-reactive compounds were the mechanisms used by Phytol to demonstrate its potent antioxidant activity in vitro. It is an essential acyclic diterpene alcohol that acts as a precursor to both vitamin K1 and vitamin E. As a candy hardener, it is commonly

Table 1: Preliminary Qualitative Screening of *Avicennia marina* leaf extracts

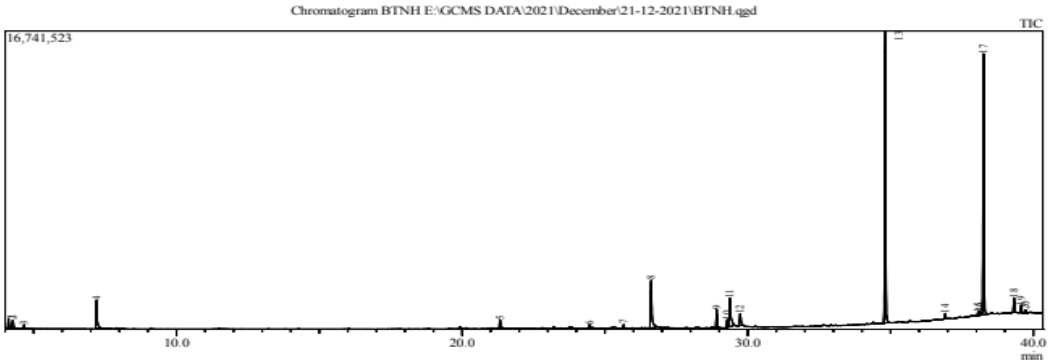
S. No.	Phytochemicals	Ethanol Extract	Ethyl acetate Extract	n- Hexane Extract	Aqueous Extract
1.	Terpenoids	+++	+++	+++	++
2.	Flavanoids	+++	+++	+++	++
3.	Saponin	+++	+	+++	+++
4.	Tannins	+++	++	+++	+++
5.	Alkaloids	+++	+++	++	+++
6.	Steroids	+++	+++	+++	+++
7.	Glycosides	+++	+++	+++	+++
8.	Phlobotannin	+++	+++	+++	Absent
9.	Proteins	+++	+++	+++	+++
10.	Coumarin	+++	+++	++	+++
11.	Emodin	+++	+	+++	Absent
12.	Anthroquinone	+++	+	+++	Absent
13.	Anthocyanin	+++	+++	+++	++
14.	Carbohydrate	+++	+++	+++	+++
15.	Leucoanthocyanin	+++	+++	+++	+++
16.	Cardiacglycosides	+++	+	++	+++
17.	Xanthoproteins	+++	+++	+++	++
18.	Phenols	+++	+	+++	++

Table 2: Quantitative phytochemical analysis of different leaf extract of *A. marina*

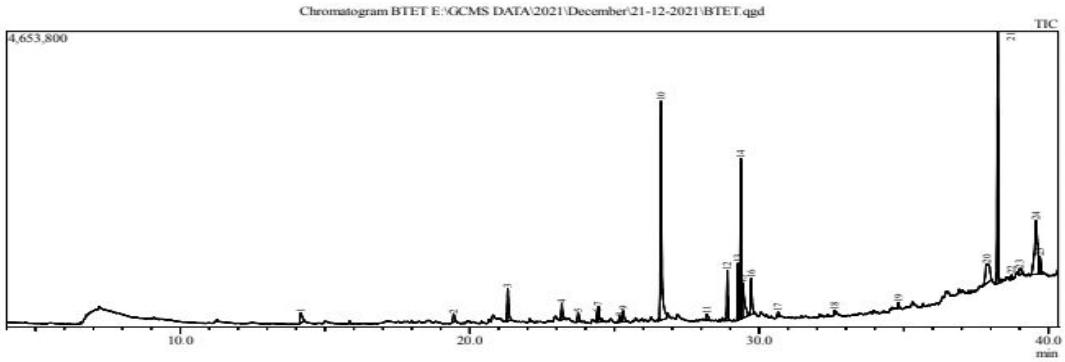
S. No.	Plant Extract	Total Saponins Content (TSC) (Galic acid Equivalent mg/g)	Total Tannins Content (TTC) (Qurcetin Equivalent mg/g)	Total Terpenoids Content (TTeC) (Galic acid Equivalent mg/g)
1.	Hexane	18.60 ± 0.013	18.40 ± 0.024	18.40 ± 0.005
2.	Ethyl acetate	18.28 ± 0.012	20.83 ± 0.018	18.12 ± 0.005
3.	Ethanol	19.90 ± 0.002	20.28 ± 0.005	18.49 ± 0.005
4.	Aqueous	19.90 ± 0.022	20.28 ± 0.005	18.49 ± 0.005



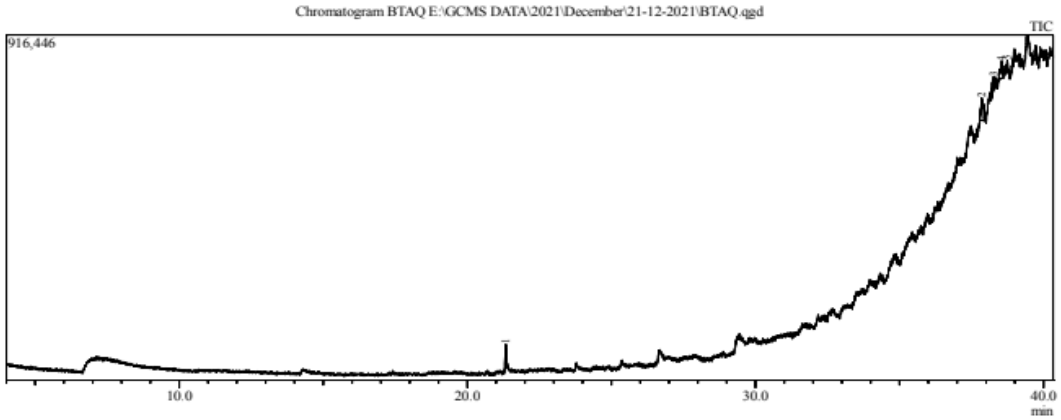
Hexane Extract of *A. marina*



Ethyl acetate Extract of *A. marina*



Ethanol Extract of *A. marina*



Aqueous Extract of *A. marina*

Fig. 1: Chromatogram of different leaf extracts of *A. marina.*

Table 3: Chemicals present in aqueous, n-hexane, ethyl acetate and ethanol leaf extract

S.No	Name of the Compound	Reduction Time	Area %			
			Hexane	Ethyl Acetate	Ethanol	Aqueous
1.	2-Methoxy-4-Vinylphenol	14.165	_	1.49	_	_
2.	3',5'-Dimethoxyacetophenone	19.445	-	0.86	-	-
3.	3'-BUTEN-2-OL, 4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)	21.328	1.33	3.01	1.63	-
4.	Tetradecanoic acid	23.181	-	1.5	-	-
5.	PLUCHIDIUL	23.745	-	0.99	-	-
6.	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL [R-[R*,R-(E)]]-	24.405	-	0.77	-	-
7.	Neophytadiene	24.468	0.43	1.25	0.49	-
8.	3,7,11,5-Tetramethyl-2-hexadecen-1-01	25.192	-	0.63	-	-
9.	4, Xi-Germacr-9-en-12-oic acid, 6.alpha.-hydroxy-1-OXO-, gamma, -lactone, (11S)-	25.305	-	1.26	-	-
10.	n-Hexadecanoic acid	26.629	7.84	20.25	5.21	-
11.	HEPTADECANOIC ACID	28.189	-	0.64	-	-
12.	Phytol	28.912	2.27	3.33	1.77	-
13.	9-12-octadecadienoic acid (Z,Z)-	29.282	1.16	4.89	1.22	-
14.	7-Tetradecenal, (Z)-	29.383	5.52	13.53	5.57	-
15.	9-OCTADECENOIC ACID (Z)	29.435	-	4.93	-	-
16.	Octadecanoic acid	29.736	1.74	3.27	0.69	-
17.	ICOSANOIC ACID	32.614	-	0.28	-	-
18.	GLYCINE,N-[N-(2-HYDROXYBENZOYL)-, BETA, - ALANYL]-, METHYL ESTER	34.814	-	0.52	-	-
19.	2-[12-(2-OXIRANYL) DODECYL] OXIRANE	37.879	-	5.45	-	-
20.	Squalene	38.262	32.04	16.34	23.73	-
21.	1H-PURIN-6-AMINE, [(2-FLUORDPHENYL) METHYL	38.725	-	0.56	-	-
22.	Phyty1 decanoate	39.011	-	0.99	-	-
23.	Olean-12-en-28-al	39.565	-	10.97	-	-
24.	SOLANESOL	39.705	1.08	1.87	0.98	-
25.	BENZENE, ETHYL-	4.144	0.99	-	1.06	-
26.	BENZENE, 1,3-dImethyl-	4.293	1.11	-	1.28	-
27.	O-Xylene	4.689	1.11	-	0.38	-
28.	14-.BETA. –H-PREGNA	25.308	-	-	0.68	-

29.	Hexatriacontane	31.875	-	-	3.39	-
30.	.beta.-Amyrin	32.657	-	-	2.51	-
31	1,2-BENZENEDICIRBOXOLIC ACID	34.816	35.54	-	0.4	-
32.	.alpha, -Amyrin	35.743	-	-	3.05	-
33.	METHYL COMMATED	36.025	-	-	13.23	-
34.	Lupeol	36.158	-	-	30.36	-
35.	Octacosane	39.326	-	-	1.74	-
36.	1-Hexacosanol	38.06	-	-	0.34	-
37.	Tetracosane	38.111	0.54	0.29	-	-
38.	Benzene, 1,3- dichloro	7.196	3.91	-	-	-
39.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	25.657	0.39	-	-	-
40.	TRIACONTANE	36.912	0.62	-	-	-
41.	Eicosyl trifluoroacetate	38.064	0.48	-	-	-
42.	CELIDONIOL, DEOXY-	39.322	2.06	-	-	-
43.	(6E,10E,14E,18E)-2,6,10,15,19,23-HEXAMETHYL-1,6,10,14,18,22-TETRACOSAHEXAEN-3-OL #	39.723	0.59	-	-	-
44.	2-Butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	21.338	-	-	-	19.31
45.	Sulfurous acid, octadecyl 2-propyl ester	37.854	-	-	-	19.31
46.	2-Bromolauric acid	38.268	-	-	-	16.86
47.	3-BROMO-2-PROPYNYL PALMITATE #	38.54	-	-	-	25.41
48.	5H-Dibenzo[d,f][1,3]diazepine, 6-ethyl-6,7-dihydro-5,7-dihydroxy-	38.741	-	-	-	6.77

used with either plain syrup or corn syrup. Erythritol is a sugar alcohol used as a food additive in the United States. It is also known as 2R, 3S-butane-1, 2, 3, 4-tetraol and is found in the leaves of *A. marina*. Since 1990, it has been used in the Japanese food industry as an ingredient in a variety of products including chocolate, candy, sugar substitutes, soft drinks, chewing gum, jellies, jams and yogurt.

Erythritol is safely used as a non-cariogenic sweetener in many countries due to the inability of the cariogenic organisms found in such areas to metabolize erythritol. In living organisms, erythritol has antioxidant effects and may help protect blood vessels from the damage that high blood sugar can cause. A saturated fatty acid called

la uric acid, also known as dodecanoic acid, is a component of triglycerides and has been shown to be found in *A. marina* leaf extract. Researchers have performed *in vitro* tests and found that lauric acid may be effective as an acne therapy. Compared to many other fatty acids, lauric acid has been shown to increase HDL, the good cholesterol in the blood. As a result, lauric acid has a more positive impact on total HDL cholesterol than any other fatty acid tested, whether saturated or unsaturated.

A total of 48 chemicals were identified using GCMS analysis of *A. marina* leaf extracts. 05, 20, 24 and 20 chemicals were present in aqueous, n-hexane, ethyl acetate and ethanol leaf extract (Fig. 1; Table 3).

Conclusion

Compared to the contaminated site, the *Avicennia marina* facility demonstrated the presence of multiple bioactive chemicals and significant bacterial activity at the uncontaminated site. It is important to understand the therapeutic benefits of mangroves to combat the harmful bacteria that cause infectious diseases in humans, animals and plants. These medicinal properties can be of great importance in therapeutic procedures. *Avicennia marina* demonstrated the presence of medicinally active ingredients by GC-MS. Their medical benefits are based on the presence of secondary plant substances.

Acknowledgements

The authors express their gratitude to their institutions for providing the help during preparation of this manuscript. Vasantha Kumar expresses his thanks to the management of A. V. V. M. Sri Pushpam College (Autonomous) and Annai Vailankanni Arts and Science College, Thanjavur for the help during this study.

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