INNOVARE JOURNAL OF SCIENCES

Vol 4, Issue 4, 2016

ISSN - 2321-5496

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BIOACTIVE COMPONENTS FROM THE ETHANOL EXTRACT OF *AVICENNIA MARINA* LEAVES

DINESH KUMAR G*, RAJAKUMAR R

Department of Zoology and Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India. Email: dineshkumar.june28@gmail.com

Received: 09 June 2016, Revised and Accepted: 14 June 2016

ABSTRACT

Objectives: To investigate the phytoconstituent of ethanol extract of *Avicennia marina* using gas chromatography-mass spectrometry (GC-MS).

Methods: GC-MS analysis of the leaves extract were performed using a Clarus 500 Perkin-Elmer (Auto System XL) GC equipped and coupled to a mass detector turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% dimethyl poly siloxane), 300 m × 0.25 mm × 1 μm df capillary column.

Results: The result of the GC-MS analysis confirmed the presence of 12 compounds. The most of the prevailing compounds are squalene; 2R, 3S-butane-1,2,3,4-tetraol; palmitic acid; hexadecanoic acid, ethyl ester; 3, 7, 11, 15-tetramethyl-2-hexadecen-1ol; phytol; (E)-9-octadecenoic acid ethyl ester; dodecanoic acid; octadecanoic acid, 2-methyl-,methyl ester; 3Cyclohexen-1 carboxaldehyde, 3-methyl; cis-9-hexadecenal; 3, 7-dimethyl-2, 6-octadienyl ester, etc.

Conclusion: This study concluded that the plant leaves contains rich amount of phytoconstituents. These bioactive components are very useful to cure many diseases and also used in many pharmaceutical industries.

Keywords: *Avicennia marina,* Gas chromatography-mass spectrometry, Bioactive compounds.

INTRODUCTION

Plants are human friendly, which give food, fuel, and medicine from the days beyond dawn of civilization. Due to the health promoting effects, mangrove plants have been used for the medicinal rationale for many centuries and recent research has been focused on the plant-derived bioactive compounds. The herbal extract has potential inhibitory effects against human, animal, and plant pathogens. Today, herbal medicines occupied worldwide and used as home remedies and also in different health-care systems. In some developing countries, society relies profoundly on traditional health practitioners and medicinal plants to meet their vital health-care requirements. These herbal medicines are gaining popularity among people in many developed countries as complementary and alternative therapies [1].

During the last decade, traditional medicine has been used globally and gained attractiveness. Since the traditional medicines are safe, efficient, and of best quality, they become important criteria for both public and health authorities [2]. The mangrove plants constitute a source of novel potential chemical compounds which are used in medicine and other applications. These novel phytoconstituents are analyzed by gas chromatography-mass spectrometry (GC-MS). In current scenario, GC-MS techniques have proved to be a valuable method for the analysis of bioactive compounds such as fatty acids, lipids, alkaloids [3,4], and volatile essential oil in medicinal plants.

Avicennia marina is commonly known as gray mangrove tree classified in the plant family Acanthaceae and is commonly used for the treatment of ulcers [5], rheumatism, smallpox, and other ailments [6]. Some studies were already reported for *A. marina* against parasites, fungi, and bacteria. Hence, the present attempt is to investigate the bioactive compounds of *A. marina* leaves using GC-MS technique.

METHODS

Collection and authentication of experimental plant

Fresh, healthy, and young leaves of *A. marina* (Forssk) Vierh. were collected from their natural habitat of Muthupet mangrove in Thiruvarur district, Tamil Nadu, India, and authenticated by professionals in the Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The herbarium number of the plant is GD001.

Preparation of extract

The dried and powdered leaves of *A. marina* (500 g) were extracted using Soxhlet extractor by evaporating with 75% ethanol. The Soxhlet extraction was carried out for 3 days, and the extract was collected. The excess ethanol was evaporated using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55°C.

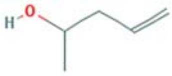
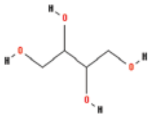
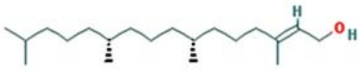
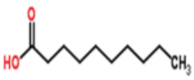
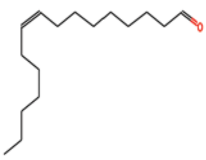
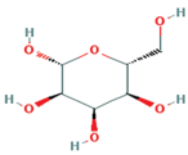
GC-MS analysis

Clarus 500 Perkin-Elmer (Auto System XL) was used to carry out GC-MS analysis. GC equipped and coupled to a mass detector turbo mass gold - Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% dimethyl poly siloxane), 300 m × 0.25 mm × 1 μm df capillary column. Initially, the instrument was maintained at temperature of 110°C for 2 minutes. The temperature was raised up to 280°C at the rate of 5°C/min and maintained for 9 minutes during the end of this period. Injection port temperature and helium flow rate were ensured as 250°C and 1 ml/min, respectively. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. MS scan range was set at 45-450 (mHz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology MS database. The percentage of each component was calculated from relative peak area of each component in the chromatogram.

RESULTS

GC-MS analysis of ethanolic leaves extract of *A. marina* that revealed the presence of 12 compounds, which are presented in Table 1. The GC-MS chromatogram of the 12 peaks of the compounds detected was shown in Fig. 1, and the components corresponding to the peaks were determined

Table 1: Phytochemical compounds and its activity of *A. marina* leaves

S. No.	Name of the compound	Nature of the compound	Structure	Retention time	Molecular weight	Activity
1.	Squalene	Triterpene		41.30	410	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide
2.	2(R),3(S)-1,2,3,4-Butane tetrol	Polyol		14.88	122	Antioxidant, antihyperglycemic
3.	n-Hexadecanoic acid	Palmitic acid		24.88	256	Anti-inflammatory, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant, hypocholesterolemic
4.	Hexadecanoic acid, ethyl ester	Fatty acid ester		25.06	284	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor
5.	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Terpene alcohol		22.18	296	Antimicrobial and anti-inflammatory
6.	Phytol	Diterpene		28.03	296	Antimicrobial, anti-inflammatory, anticancer, diuretic
7.	(E)-9-Octadecenoic acid ethyl ester	Fatty acid		29.04	310	Antioxidant, anti-inflammatory
8.	Dodecanoic acid	Saturated fatty acid		18.55	200	Antimicrobial, anti-inflammatory
9.	Octadecanoic acid, 2-methyl-, methyl ester	Fatty acid		29.50	294	Potent antifungal, antimicrobial, antibacterial
10.	cis-9-Hexadecenal	Aldehyde		32.96	238	Antimicrobial
11.	D-Allose	Aldohexose sugar		18.33	180	Antioxidative activity
12.	Nonanoic acid	Fatty acid		13.75	158	Anti-seizures

Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (online database). *A. marina*: *Avicennia marina*

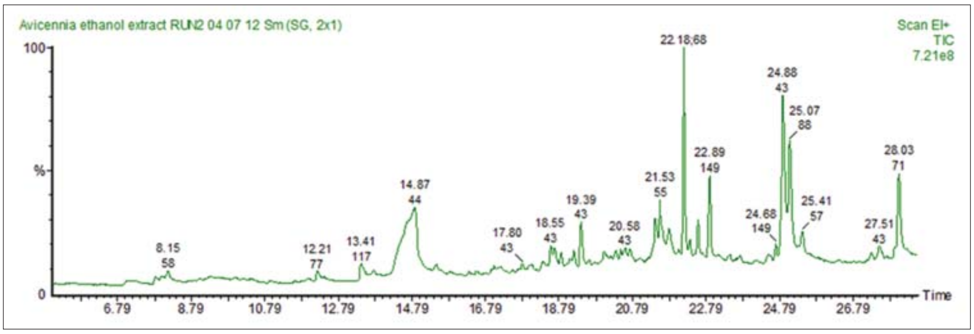


Fig. 1: Gas chromatography-mass spectrometry chromatogram of *Avicennia marina* leaves

as follows: Squalene (25.0282%), 2R, 3S-butane-1, 2, 3, 4-tetraol (21.3770%), palmitic acid (11.1822%), hexadecanoic acid, ethyl ester (8.6922%), 3, 7, 11, 15-tetramethyl-2-hexadecen-1ol (6.7546%), phytol (4.1372%), (E)-9-octadecenoic acid ethyl ester (4.6300%), dodecanoic acid (2.1702%), octadecanoic acid, 2-methyl-,methyl ester (1.8503%), 3Cyclohexen-1carboxaldehyde, 3-methyl- (1.6602%), cis-9-hexadecenal (1.4864%), and 3, 7-dimethyl-2, 6-octadienyl ester (1.4204%).

DISCUSSION

Medicinal plants are the major source of therapeutic agents to cure human diseases. Recent researches in medicinal and aromatic plants made the health-care enhancement for the purpose of humankind. The vast floral resources of mangrove forest are best known for their medicinal properties. Vast studies have been made on mangrove forest plants and their bioactive compounds during these days due to the medical importance. The mangrove herbal extracts have been practiced as a common method for the treatment of health disorders for many centuries. The bioactive compounds of mangrove plants are unique in their actions. Since they possess competence in many bioactive principles against disease producing microbial organisms [7], secondary metabolites such as alkaloids, steroids, phenols, and terpenoids have been chemically characterized from mangroves which have toxicological, pharmacological, and ecological importance [8].

In the present study, the leaves of *A. marina* contain more amount of squalene (25.0282%). Squalene is a polyunsaturated hydrocarbon and triterpene compound that is widely distributed in nature and primarily known for its key role as an intermediate in cholesterol synthesis. In our body, squalene is converted into useful high-density lipoprotein (HDL)-cholesterol, whereby the level of good cholesterol increase and the level of bad low-density lipoprotein-cholesterol become reduced [9]. It also has some antifungal properties which make it effective in candidiasis and has been shown to enhance the effects of amphotericin-B (fungizone) against a variety of *Candida* species. Squalene, by enhancing drug elimination from the body, might be a good candidate as an antidote to reduce the toxicity of accidentally ingested drugs [10]. *In vitro* experimental evidence indicates squalene is a highly effective oxygen scavenging agent [11].

N-hexadecanoic acid (palmitic acid, 11.1822%) which is observed in *A. marina* extracts have antioxidants, hypochloresterolenic, and hemolytic properties. This phytoconstituents also act as an anticancer drug and have antioxidant and antimicrobial properties [12,13]. Similarly, N-hexadecanoic acid reported to act as a pesticide, nematicide, antiandrogenic flavor, and 5-alpha reductase inhibitor [14].

A rich amount of phytol is also found to be present in leaves of *A. marina* which is used as an effective bioactive compound in the preparation of medicinal and agricultural products. Phytol demonstrated a strong antioxidant effect *in-vitro* by removing hydroxyl radicals and nitric oxide and also to prevent the formation of thiobarbituric acid reactive substances [15]. It is a key acyclic diterpene alcohol which is a precursor for vitamin K1 and E. It is used along with simple or corn syrup as a hardener in candies [16].

Erythritol or 2R, 3S-butane-1, 2, 3, 4-tetraol in *A. marina* leaves is a sugar alcohol that has been used as a food additive in the United States. Since 1990, it has been used in Japan as a component of chocolates, candies, sugar substitutes, soft drinks, chewing gum, jellies, jams, and yoghurt [17]. Erythritol has been used safely as a non-cariogenic sweetener in many countries, where the cariogenic organisms lack erythritol metabolism [18]. Erythritol acts as an antioxidant *in vivo* and may help to prevent vascular damage caused by hyperglycemia [19].

Lauric acid or dodecanoic acid is a saturated fatty acid, a component of triglycerides found to be present in leaves extract of *A. marina*. *In vitro* experiments have suggested that lauric acid can be used in treatment for acne [20,21]. Lauric acid increases total serum cholesterol when compared to many other fatty acids. It results in an increase in HDL (the “good” blood cholesterol). As a result, lauric acid has “a more favorable effect on total HDL cholesterol than any other examined fatty acid, either saturated or unsaturated” [22]. In general, lower HDL cholesterol correlates with a decrease in atherosclerotic risk [23].

CONCLUSION

Phytochemical study showed the presence of phytoconstituents such as alkaloids, flavonoids, saponin, and phytosterols in *A. marina* which might be responsible for their therapeutic effects. In conclusion, the leaf extract of *A. marina* possesses a wide spectrum of bioactivity against a group of microbes responsible for the most common pathogenic diseases. The results obtained from the phytochemical screening and GC-MS analysis suggested to do further investigations which may lead to the development of drug formulation.

ACKNOWLEDGMENT

The author is very grateful to the Secretary, Correspondent, and the Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur (Dt.) and also thankful to Mr. R.J. Antony Raj, Chairman, SIMPRA Research Institute, for providing the excellent infrastructure and necessary facilities. The author is also very grateful to University Grants Commission, Government of India, New Delhi, for providing financial assistance in the form of Rajiv Gandhi National Fellowship which buttressed me to perform my work comfortably.

REFERENCES

1. Lalitharani S, Mohan VR, Regini GS, Kalidass C. GC-MS analysis of ethanolic extract of *Pathos scandens* L. leaf. J Herb Med Toxicol 2009;3:159-60.
2. Dineshkumar G, Rajakumar R. GC-MS evaluation of bioactive molecules from the methanolic leaf extract of *Azadirachta indica* (A. Juss). Asian J Pharm Sci Technol 2015;5:64-9.
3. Jie MS, Choi CY. MS characterization of picolinyl and methyl ester derivatives of isomeric thia fatty acids. J Int Fed Clin Chem 1991;3:122.
4. Betz JM, Gay ML, Mossoba MM, Adams S, Portz BS. Chiral gas

chromatographic determination of ephedrine - type alkaloids in dietary supplements containing Má Huáng. J AOAC Int 1997;80(2):303-15.
5. Bandaranyake WM. Survey of mangrove plants from Northern Australia for phytochemical constituents and UV-absorbing compounds. Curr Top Phytochem Life Sci Adv 1995;(14):69-78.
6. Subashree M, Mala P, Umamaheswari M, Jayakumari M, Maheswari K, Sevanthi T, *et al*. Screening of the antibacterial properties of *Avicennia marina* from Pichavaram mangrove. Int J Curr Res 2010;1:16-9.
7. Piyusha G, Shelar S, Reddy VK, Shelar GS, Reddy V. Medicinal value of mangroves and its antimicrobial properties - A review. Cont J Fish Aquat Sci 2012;6(1):26-37.
8. Philip K, Sinniah SK, Muniandy S. Antimicrobial peptides in aqueous and ethanolic extracts from microbial, plant and fermented sources. Biotechnology 2009;8(2):248-53.
9. Kelly GS. Squalene and its potential clinical uses. Altern Med Rev 1999;4(1):29-36.
10. Kamimura H, Koga N, Oguri K, Yoshimura H. Enhanced elimination of theophylline, phenobarbital and strychnine from the bodies of rats and mice by squalane treatment. J Pharmacobiodyn 1992;15(5):215-21.
11. Saint-Leger D, Bague A, Cohen E, Chivot M. A possible role for squalene in the pathogenesis of acne. I. *In vitro* study of squalene oxidation. Br J Dermatol 1986;114(5):535-42.
12. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res 2002;22(5):2587-90.
13. Graikou K, Kapeta S, Aligiannis N, Sotiroudis G, Chondrogianni N, Gonos E, *et al.* Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation properties. Chem Cent J 2011;5(1):33.
14. Praveenkumar P, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Res 2010;4(7):191-5.
15. Santos CC, Salvadori MS, Mota VG, Costa LM, de Almeida AA, de Oliveira GA, *et al.* Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci J 2013;2013:949452.
16. Ogunlesi M, Okiei W, Osibote AE. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn. a potential medication for asthma. Afr J Biotechnl 2009;8:7042-50.
17. Shindou T, Sasaki Y, Miki H, Eguchi T, Hagiwara K, Ichikawa T. Determination of erythritol in fermented foods by high-performance liquid chromatography. J Food Hyg Soc Jpn 1988;29:419-22.
18. Mäkinen KK, Isotupa KP, Kivilompolo T, Mäkinen PL, Toivanen J, Söderling E. Comparison of erythritol and xylitol saliva stimulants in the control of dental plaque and mutans streptococci. Caries Res 2001;35(2):129-35.
19. Den Hartog G, Boots A, Adam-Perrot A, Brouns F, Verkooijen I, Weseler A, *et al*. Erythritol is a sweet antioxidant. Nutrition 2009;5:4-10.
20. Nakatsuji T, Kao MC, Fang JY, Zouboulis CC, Zhang L, Gallo RL, *et al.* Antimicrobial property of lauric acid against *Propionibacterium* acnes: Its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 2009;129(10):2480-8.
21. Yang D, Pornpattananangkul D, Nakatsuji T, Chan M, Carson D, Huang CM, *et al.* The antimicrobial activity of liposomal lauric acids against *Propionibacterium* acnes. Biomaterials 2009;30(30):6035-40.
22. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77(5):1146-55.
23. Thijssen MA, Mensink RP. Fatty acids and atherosclerotic risk. In: Eckardstein AV, editor. Atherosclerosis: Diet and Drugs. Germany: Springer; 2005. p. 171-2.