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IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM *RHIZOPHORA MUCRONATA* METHANOLIC LEAF EXTRACT BY GC-MS ANALYSIS

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ABSTRACT: Herbal medicines play an important role in cancer prevention. Mangroves have been used worldwide due to the presence of various bioactive metabolites in it. The specific medicinal properties of *Rhizophora mucronata* have been an interest in preventing breast cancer. Mangroves *Rhizophora mucronata* belongs to family Rhizophoraceae. The leaf extract reveals various roles in folk remedies to treat various diseases. This analytical study aims to find out various phytochemical and components present in the leaf extract by the GC-MS technique. The methanol leaf extract revealed the presence of phytochemicals like saponins, flavonoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, and steroids. The GC-MS analysis provided nine different compounds like Phenol, 2, 4-bis (1,1-dimethyl ethyl)- (28.2%), Flavone- (66.9%), 4H-1-benz0pyran-4-one,7-hydroxy-2phenyl (39.8%), Chrysin (100%), 4H-1 Benzopyram-4-one, 7-hydroxy-2-phenyl-(100%), 5,8 Octadecadienoicacid, methyl ester-(55.8%), Phytol- (31.8%), Coumarine, 3-(2,4-dinitrophenyl)-(31.3%), Elaidic acid, isopropyl ester-(9.2%). Further purified compounds are used in the pharmacological industry against breast cancer and reduce the side effects and pains during the treatment of cancer.

INTRODUCTION: Cancer is a term to describe with distinguishing characteristics, uncontrolled cell proliferation, invasion of abnormal cells to healthy cells, and their spread towards vital organs *via* bloodstream [1]. Most occurring cancer around the world that causes death are lung cancer, blood cancer, prostate cancer, breast cancer, cervix cancer, and bone cancer [2]. Breast cancer is becoming a leading cause of mortality in women, and new therapeutic compounds are constantly worked out [3].

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The traditional use of Medicinal plants has no side effects, toxicity with improved efficacy and safety. Antioxidants, anti-inflammatory, anti-diabetic, and anti-viral are the important biological activities that are studied in medicinal plant extracts [4, 5, 6].

Herbal medicine plays a specific role, through its vitamins and selenium, in the prevention of breast cancer has not been established; some anticancer activities have been shown *in-vitro*. Mangroves are known for their medicinal wealth. Various parts of *Rhizophora mucronata* is used to treat various diseases. Mangroves are salt-tolerant plants of tropical and subtropical intertidal parts of the world. Despite the commercial properties, mangroves are used in folklore medicine. The plant has an acceptable level of the antioxidant and thrombolytic property along with the moderate level of the cytotoxic property [7].

In-vitro antioxidant activity of bark extracts of *Rhizophora mucronata*, science, technology and arts research journal, stem bark 95% ethanol, water by cold extraction methods using antioxidant activity [8]. *Rhizophora mucronata* shows abundant phytochemicals like triterpenoids, lipids, alkaloids, and tannins [9]. It can also affect cell cycle distribution and significantly decrease cyclin D1 expression [10].

There are more than 4000 different phytochemicals that have the potential to cure diseases like cancer, stroke, and other metabolic syndromes [11]. Oxidative stress is a significant risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses of various diseases. Antioxidants are regarded as a possible protection agent for subduing oxidative of the human body from the ROS and impede the progress of several chronic diseases as well as lipid peroxidation.

Rhizophora mucronata leaf extracts exhibit best anti-diarrhea natural agent. The natural compounds used in both traditional and modern therapies for human health improvement with fewer side effects. Leaf extracts show novel drug molecules to combat the threat of human diseases [12, 13, 14]. The screening of plant extracts is an innovative method to find therapeutically active compounds in many plant species. In the last few years, GC-MS has become well developed technological screening for secondary metabolite profiling of plants [15]. Since GC-MS detection techniques have become a sophisticated means for analysis of various compounds. A literature survey revealed that no work had been reported on GC-MS analysis of methanol extract of *Rhizophora mucronata* leaves to date. Therefore, in our present study, isolation, and characterization of the bioactive phytochemical compounds were analyzed from the methanol extract of the plant with the help of the GC-MS technique.

MATERIALS AND METHODS:

Plant Collection: The plant sample of the *Rhizophora mucronata* leaf was collected from Annamalai University, Parangipettai. The leaves of *R. mucronata* were air-dried within the laboratory and grained into a fine powder.

Preparation of Plant Extraction: The dried material was mixed with Methanol for seven days. Then the extract was filtered through Whatman no. 1 paper, and therefore the extract was dried employing a rotor evaporator to get rid of the solvents and to get the concentrated jelly extract. The extract was later kept at -20 °C in a sterile bottle.

Phytochemical Screening: The phytochemical screening of leaf extract was assessed by standard methods given by Trease *et al*., Phytochemical screening was disbursed for the presence of carbo-hydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, phenols, terpenoids, cardiac glycosides, coumarins, steroids, phytosterols, phlobatannins, and anthraquinones.

Test for Carbohydrates: To 2 ml of plant extract, 1ml of Molisch's reagent and few drops of sulphuric acid was added. The formation of a purple or reddish color indicates the presence of carbohydrates.

Test for Tannins: To 1 ml of plant extract, 2 ml of ferric chloride (5%) was added. The formation of navy blue or greenish-black indicates the presence of tannins.

Test for Saponins: To 2 ml of plant extract, 2 ml of water was added and shaken. After 15 min, the formation of a 1 cm layer of the form indicates the presence of saponins.

Test for Flavonoids: To 2 ml of plant extract, 1 ml of sodium hydroxide was added. The presence of the yellow color indicates the presence of flavonoids.

Test for Alkaloids: To 2 ml of plant extract, 2 ml of hydrochloric acid was added. Then few drops of Mayer's reagent were added. The presence of green color white precipitated indicates the presence of alkaloids.

Test for Quinines: To 2 ml of plant extract, 1 ml of sulphuric acid was added. The formation of red color indicates the presence of quinones.

Test for Glycosides: To 2 ml of plant extract, about 3 ml of chloroform and ammonia solution (10%) was added. The formation of light to dark pink color indicates the presence of glycosides.

Test for Cardiac Glycosides: To 0.5 ml of plant extract, 2 ml of glacial acetic acid, and few drops of ferric chloride (5%) were added. This was under layered with 1ml of sulphuric acid. The formation of the brown ring at the interface indicates the presence of cardiac glycosides.

Test for Terpenoids: To 0.5 ml of extract, 2 ml of chloroform was added, and sulphuric acid was added carefully. The formation of red-brown color at the interface indicates the presence of terpenoids.

Test for Phenols: To 1 ml of the extract, 2 ml of water and some drops of 10% ferric chloride was added. The formation of a bluish-green color indicates the presence of phenols.

Test for Coumarins: To 1 ml of plant extract, 1 ml of sodium hydroxide (10%) was added. The formation of a deep yellow color indicates the presence of coumarins.

Steroids and Phytosteroids: To 1 ml of plant extract, an equal volume of chloroform is added and subjected with few drops of the sulphuric acid were added. The appearance of the brown ring indicates the presence of steroids. Following the appearance of the bluish brown ring indicates the presence of phytosterols.

Phlobatannins: To 1 ml of plant extract, few drops of Hydrochloric acid (2%) were added. The appearance of a red color precipitate indicates the presence of phlobatannins.

Anthraquinones: To 1 ml of plant extract a few drops of 10% ammonia solution were added, the appearance of a pink color precipitate indicates the presence of anthraquinones.

GC-MS Analysis: By using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped with matched mass detector (Turbo mass gold Perkin Elmer Turbomass 5.1spectrometer) the GC-MS analysis was performed with an Elite - 1 provided with 100% Dimethylpolysiloxane, and 30 m × 0.25 mm ID × 1 µm of the capillary column. The GC-MS was set to an initial temperature of about 70 °C and maintained at the same for 3 min. At the top of this era, the oven temperature was rose to 300 °C, at the speed of a rise of 10 °C/min, and maintained for 9 min. Injection port temperature was ensured at 250 °C and Helium rate at 1.5 ml/min. The ionization voltage was 70 eV. The extract was applied in split mode as 10:1. The mass spectral scan range was set at 40-700 (m/z). The ion source temperature was maintained at 230 °C, and therefore the Interface temperature was at 240 °C. The MS start time was 3 min, and therefore the end time was 35 min with solvent cut time was 3 min. Using computer searches on a NIST Ver. 11 MS data library and comparing the spectrum obtained through GC-MS, compounds present within the plant samples were identified.

RESULTS:

Sample Preparation: The *Rhizophora mucronata* leaf extract was powdered, and the powdered material was extracted using methanol solvent. The resultant extract was stored in an airtight container **Fig. 1**.

FIG. 1: *RHIZOPHORA MUCRONATA* METHANOL LEAF EXTRACT

Phytochemical Screening: The preliminary Phytochemical analysis for the Methanol leaf extract was done based on the method described by Trease *et al*., 1989. The methanolic extract of *R. mucronata* was tabulated and shown in **Table 1**.

TABLE 1: SHOWING THE VARIOUS PHYTO-CHEMICALS PRESENT IN *R. MUCRONATA*

Phytochemicals	Result
Carbohydrate	-
Tannins	-
Saponins	+
Flavonoids	+
Alkaloids	+
Quinones	+
Glycosides	+
Cardiac glycosides	+
Terpenoids	+
Phenols	+
Coumarins	+
Steroids	+
Phlobatannins	-
Anthraquinones	-

Where +: Denotes presence; - : Denotes absence

GC-MS Analysis: GC-MS (Gas chromatography and Mass spectroscopy) spectrum analysis of *Rhizophora mucronata* leaf of methanol extract revealed the presence of nine compounds. These compounds were identified based on their retention time (RT), molecular formula, molecular weight and concentration (peak area %).

Phenol, 2, 4- bis (1, 1-dimethyiethyl) - (28.2%), Flavone- (66.9%), 4H- 1- benz0pyran- 4- one, 7-hydroxy-2phenyl (39.8%), Chrysin (100%), 4H-1 Benzopyram- 4- one, 7- hydroxy- 2-phenyl-(100%) 5,8 Octadecadienoic acid, methylester- (55.8%), Phytol- (31.8%), Coumarine, 3-(2,4-dinitrophenyl)-(31.3%), Elaidic acid, isopropyl ester-(9.2%) with retention time; 16.15, 17.12, 17.78, 18.8, 19.43, 20.83, 21.85, 22.82 and 23.63. The chemical compounds were given in **Table 2** and **Fig. 2** showing the GC-MS spectrum analysis of *R. mucronata.*

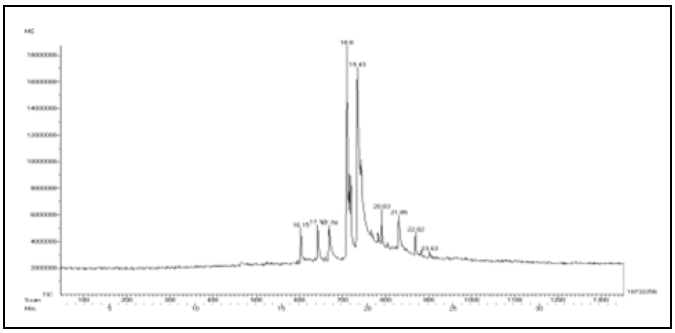

FIG. 2: COMPOUNDS IDENTIFIED IN GC-MS ANALYSIS: (A) PHENOL, 2, 4-BIS(1,1-DIMETHYLETHYL)-, (B) FLAVONE, (C) 4H-1-BENZOPYRAN-4-ONE, 7-HYDROXY-2-PHENYL-, (D) CHRYSIN, (E) 4H-1-BENZOPYRAN-4-ONE, 3-HYDROXY-7-METHOXY-2-PHENYL-, (F) 5,8-OCTADECA-DIENOICACID, METHYL ETHER, (G) PHYTOL, (H) COUMARINE, 3-(2,4-DINITROPHENYL)-, (I) ELAIDIC ACID, ISOPROPYL ESTER

TABLE 2: GC-MS ANALYSIS OF *R. MUCRONATA* LEAF EXTRACT

Peak	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	16.15	Phenol,2,4-bis(1,1-dimethyl ethyl)-	$C_{14}H_{22}O$	209.3329	28.2%
2	17.12	Flavone	$C_{15}H_{10}O_2$	222.243	66.9%
3	17.78	4H-1-Benzopyran-4-one,7-hydroxy-2-phenyl-	$C_{15}H_{10}O_3$	238.24	39.8%
4	18.8	Chrysin	$C_{15}H_{10}O_4$	254.241	100%
5	19.43	4H-1-Benzopyran-4-one, 3-hydroxy-7-methoxy-2-phenyl-	$C_{16}H_{12}O_5$	284.267	100%
6	20.83	5,8-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.479	55.8%
7	21.85	Phytol	$C_{20}H_{40}O$	296.539	31.8%
8	22.82	Coumarine, 3-(2,4-dinitrophenyl)-	$C_{15}H_7N_3O_8$	357.23138	31.3%
9	23.63	Elaidic acid, isopropyl ester	$C_{21}H_{40}O_2$	324.549	9.2%

DISCUSSION: Phytochemical analysis of the present study made a clear idea that *R. mucronata* has various significant secondary metabolites for structure elucidated. The presence of Phytol in the *Rhizophora mucronata* leaf extracts was determined by Joel and Bhimba in 2010. The presence of phytochemical compounds like saponins, flavonoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, and steroids. The structure of the compounds present in the leaf extract is identified by GC-MS studies. The present study reveals nine compounds, and these might have an important functional role in anticancer activity. The biological activities present in these compounds predict the valuable role in cancer studies.

CONCLUSION: In conclusion, the obtained result established the preliminary phytochemical analysis of *Rhizophora mucronata* leaf. The outcome of the existing study encourages carrying out further compounds identification present in the extract. Large screening is required for the evaluation of natural products. This provides a way for the future pipeline in drug discovery. Thus, *Rhizophora mucronata* possesses various pharmaceutical properties in the field of herbal medicine.

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CONFLICTS OF INTEREST: Nil

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