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PHYTOCHEMICAL ANALYSIS OF MANGROVE DERIVED CRUDE PLANT EXTRACT- *RHIZOPHORA MUCRONATA*

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ABSTRACT

The preliminary phytochemical analysis of *R.mucronata* using three different solvent (methanol, ethanol and chloroform) among them methanol extract has potential activity. Protein, phenols, flavonoids, saponins, glycosides, terpenoids and tannins were present in all the three solvent extracts. Whereas, the steroids were deficient in chloroform and methanol extracts. Alkaloids were absent only in ethanol extract. The total phenolic, total flavonoid and total terpenoids content were analyzed in the three different solvent, from the observed result methanol extract has higher percentage of phenolic, flavonoid, terpenoids and total antioxidant content. Therefore the phytoconstituents in *R.mucronata* provides scientists with insight into how effective plants are medicinally, and understanding how and why they are effective can lead to the development of gastric cancer drugs.

INTRODUCTION:

In general, medicinal plants are of great importance to the health of individuals and communities. The medicinal values of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bio-active constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. [1,2]. In addition, the uses of herbal medicine for the treatment of diseases and infections are as old as mankind. The World Health Organization supports the use of traditional medicine, provided they are proven to be efficacious and safe [3]. In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for safe water and medicine. They have no alternative for primary health care [4]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain variety of novel drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [5]. About 80% of individual compounds have been obtained from the medicinal plants [6].Drugs from the plants are easily available, less expensive, safe, and efficient and have less side effects. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [7] Mangrove is an ecological term referring to a taxonomically diverse assemblage of trees and shrubs that appearance is dominant among the plant

communities in tidal, saline wetlands along sheltered tropical and sub-tropical coasts [8]. Mangroves are biochemically unique, producing a wide array of novel natural products to treat human diseases [9]. *Rhizophora mucronata* (Rhizophoraceae) commonly known as an Asiatic mangrove, widely distributed along the tropical and sub-tropical coastal regions. This mangrove plant has been reported to posses several medicinal properties. In countries like Burma, India and China bark of *Rhizophora mucronata* has been used as traditional medicine in the treatment of diarrhoea, dysentery, blood in urine, fever, angina, diabetes, hematuria, and hemorrhage [10,11). Indo-chinese used the roots for angina and hemorrhage. Malayans used old leaves and or roots for child birth. Burmese used the bark for bloody urine, Chinese and Japanese use it for diarrhoea; Indo-chinese used for angina [12]. Many studies have been reported that the mangrove plant derived extract may be a rich source of novel compounds along with enormous biological activities [13,6]. In this point of view, the present investigation is to be undertaken to determine the qualitative and quantitative analysis of phytochemicals from the extract of mangrove plant, *R. mucronata.*

MATERIALS AND METHODS

Chemicals

Chemical reagents such as Nitro blue tetrazolium (NBT), was purchased from Sigma, USA, Gallic acid (Standard solution) (Loba Chemie, Mumbai), Sodium carbonate (Hi Media, Mumbai) , Sodium Nitroprusside (10 mM) solution and Trichloro Acetic Acid (TCA) (S.D. Fine Chemicals, Mumbai). All other reagents used were of analytical grade.

Collection of plant material

Leaves of the mangrove plant, *R. mucronata* was collected from the Pichavaram mangrove forest (Lat.11^{o} 27' N; Long.79^{o} 47' E), Southeast coast of Tamil Nadu, India. After that the dried specimen was identified and its halotype (No. R-90) has been deposited in herbarium at C.A.S. in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai. The fresh leaves washed in distilled water and air dried at room temperature. The dried leaves were made into powder form by using electrical grinder.

Preparation of extracts: One kg of powdered material of *R. mucronata* was soaked in 4 L of solvents for 24 hrs. and extracted with three different solvents such as methanol, ethanol and chloroform at 25^{o}C. The extraction was repeated thrice to obtain a sizable quantity of extract, after that the extracts were pooled, filtered using Whatmann No. 1 paper and concentrated by using rotary evaporator (Buchi Rotavapor R-124). Finally, the resultant residues of crude extracts were kept at 4^{o}C for further investigation.

Qualitative phytochemical analysis

The methanolic, ethanolic and chloroform extracts of *R. mucronata* were screened for the presence of phytochemicals such as proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, total phenolic content, total flavonoid and total antioxidant by the following standard methods [14].

Test for Proteins

Millon's test: The methanolic, ethanolic and chloroform extracts of *R. mucronata* mixed with 2 mL of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test: The methanolic, ethanolic and chloroform extracts of *R. mucronata* were boiled with 2 mL of 0.2% solution of Ninhydrin. Whereas, the pink colour appeared confirmed the presence of amino acids and proteins.

Test for Carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together with 2 mL of crude extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: The methanolic, ethanolic and chloroform extracts of *R. mucronata* were mixed with 2 mL of Benedict's reagent and boiled. Finally, a reddish brown precipitate was formed, which indicated the presence of the carbohydrates.

Molisch's test: The methanolic, ethanolic and chloroform extracts of *R. mucronata* were mixed with 2 mL of Molisch's reagent and the mixture was shaken properly. After that, 2 mL of concentrated H_2SO_4 was poured carefully along the side of test tube. Appearance of a violet ring at the interphase that is concluded and it is confirmed the presence of the carbohydrate.

Iodine test: The methanolic, ethanolic and chloroform extracts of *R. mucronata* was mixed with 2 mL of Iodine solution. Finally, a dark blue or purple colouration is appeared and it indicated the presence of carbohydrate.

Test for Phenols and Tannins

The crude extracts of *R. mucronata* were mixed with 2 mL of 2 % solution of $FeCl_3$. A blue-green or black colouration is appeared and that indicated the presence of phenols and tannins.

Test for Flavonoids

Shinoda test: The crude extracts of *R. mucronata* were mixed with few fragments of magnesium ribbon and added concentrated HCl in drop wise. Pink scarlet colour is appeared after few minutes which concluded and indicated the presence of flavonoids.

Alkaline reagent test: The crude extracts of *R. mucronata* mixed with 2 mL of 2 % solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated and confirmed the presence of flavonoids.

Test for Saponins: The crude extracts of *R. mucronata* were mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of Saponins.

Test for Glycosides

Liebermann's test: The crude extracts of *R. mucronata* were mixed with each of 2 mL of chloroform and 2 mL of acetic acid. The mixture was cooled in ice and added carefully concentrated H_2SO_4. Finally, a colour change from violet to blue to green indicated the presence of steroidal nucleus, (*i.e.*), glycone portion of glycoside.

Salkowski's test: The crude extracts were mixed with 2 mL of chloroform. Then added a 2 mL of conc. H_2SO_4 and shaken gently. Whereas, a reddish brown colour appeared indicated and confirmed the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-Kilani test: The crude extracts of *R. mucronata* were mixed with 2 mL of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 mL of concentrated H_2SO_4. A brown ring presence at the interphase indicated and confirmed the presence of cardiac glycosides.

Test for Steroids: The crude extracts were mixed with 2 mL of chloroform and added concentrated H_2SO_4 in sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extracts with 2 mL of chloroform. Then 2 mL of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish colouration indicated and confirmed the presence of steroids.

Test for Terpenoids

The crude extracts of *R. mucronata* were dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added and heated for about 2 min. A grayish colour indicated the presence of terpenoids.

Test for Alkaloids

The crude extracts were mixed with 2 mL of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the

mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Determination of Total Phenol

The amount of protein in the three different extracts were determined by Folin-Ciocalteu reagent, according to the method using gallic acid as a standard phenolic compound [15] 1.0 mL of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 mL of distilled water in methanol. 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. After three minutes 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue colour that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract [16]. All determinations were performed in triplicate. The concentration of total content of phenolic compounds in plant extracts was determined ug of gallic acid equivalent (GAE) was calculated by the following formula

C ¼ c _ V=m;

Where: C– total content of phenolic compounds, mg/g plant extract in GAE, c – The concentration of gallic acid established from the calibration curve, mg/mL, V – The volume of extract, mL, m – The weight of pure plant extract

Total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine the flavonoid content. 1 mL of plant extracts were mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6mL of distilled water and remains at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/mL). All the tests were performed in triplicates. Flavonoid content were determined from the standard curve and expressed as quercetin equivalent (mg/g of extracted compound) [17].

Total Terpenoids Content

The quantitative analysis of total terpenoids was estimated with the modified procedure of [18]. The compounds were isolated from plant constituents by methanolic, ethanolic and chloroform extracts. The extracts were treated for total terpenoids. The absorbance was measured at 538 nm. Ursolic acid was used as standard (1mg/mL). All the tests were performed in triplicates. Terpenoids content was determined from the standard curve and expressed as ursolic equivalent (mg/g of extracted compound)

Total Antioxidant

Total antioxidant activities of methanolic, ethanolic and chloroform extracts of *R. mucronata* were determined according to the method of Prieto, [19]. Briefly, 0.03 mL crude extract were mixed with 3.0 mL reagents solution 0.6 M Sulphuric acid, 28mM sodium phosphate and 4mM ammonium in molybdate. Reaction mixture was incubated at 95ºC for few minutes in water bath. Absorbance of all the extracts mixture was measured at 695 nm. Total antioxidant activities are expressed as the number of equivalent of ascorbic acid in milligram per gram extract.

RESULTS

Phytochemical analysis plays a major resource for information on analytical and instrumental methodology in plant sciences. A preliminary study was done to identify the active constituents from methanolic, ethanolic and chloroform extracts of *R. mucronata*. The phytochemical characteristics of three different solvent extracts of *R. mucronata* tested and summarized table 1. From the **Table 1,** it could be observed that protein, phenols, flavonoids, saponins, Glycosides, terpenoids and tannins were presence in all the three solvent extracts. Whereas, the Steroids were deficient in chloroform and methanol extract. Alkaloids were also absent in only ethanol extract. **Fig 1** showed the total phenolic content of methanolic, ethanolic and chloroform crude extracts of *R. mucronata* at the concentration of 0.5mg/g. The methanol extract showed 264 mg/g and it was expressed in gallic acid equivalents. Whereas, the ethanol

extract was observed 259 mg/g. Finally, the chloroform extract showed 240 mg/g. The results were also revealed that the higher level of phenolic compound were present in methanol extract compared to ethanol and chloroform extracts **Fig. 2** showed the total flavonoids content of methanol, ethanol and chloroform crude extracts of *R. mucronata* were investigated. Results were found that, the methanol extract recorded 242 mg/g. Ethanol extract showed 233 mg/g. Whereas, the chloroform extract determined 228 mg/g respectively. The results revealed that the higher level of total flavonoids presented in methanol extract compare to ethanol and chloroform extracts. Among three different solvent extracts of mangrove plant *R. mucronata,* the methanol extract observed a higher level of total flavonoids. **Fig 3** showed the total terpenoids content of methanol, ethanol and chloroform crude extracts of *R. mucronata*. Results were found that the methanol extract was recorded 64 mg/g. Ethanol extract was showed of 54 mg/g. whereas, the chloroform extract was determined 43 mg/g respectively. The results revealed that the higher level of total terpenoids present in methanol extract compare to ethanol and chloroform extracts. Among three different solvent extracts of mangrove plant *R. mucronata,* the methanol extract has high content of total terpenoids. **Fig. 4** and **Table 2** showed methanolic, ethanolic and chloroform crude extracts of *R. mucronata* were tested against total antioxidant activity. The results showed that among three different solvent extracts, the methanol extract observed highest anti-oxidant activity effects compare to ethanol and chloroform crude extracts.

DISCUSSION

Properties of a good solvent in the plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate [20]. In the present study, three different solvents are used for the mangrove plant extraction based on literature and biological activities. The results exhibited most of the phytochemicals are present in methanolic and ethanolic extracts of mangrove plant *R. mucronata* than the chloroform extract. Terpenoids and tannins are present in all three extracts but higher level of terpenoids present in chloroform extracts showed the same result which were observed in the previous study [21].

The medicinal values of the plant leaves may be related to their constituent phytochemicals. According to Varadarajan [22] phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [14]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. The present investigation revealed that the phytochemical constituents from *R. mucronata* extracts such as protein, phenols, flavonoids, saponins, glycosides, terpenoids and tannins. Previously, Cowan [21] had observed the presence of steroids in chloroform and methanol extracts and presence of alkaloids in ethanol extract respectively. The phenolic compounds are one of the largest and the most ubiquitous groups of plant metabolites [23]. They possess biological properties such as anti- apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities [24]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [25,26]. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [27]. Similarly, terpenoids and vitamins are act as regulators of metabolism and play a protective role as antioxidants [28]. The present study, also determined that the phenolic compound is present in all three extracts, but high level phenolic compound observed in methanol extract (264 mg/g) followed by ethanol extract (259 mg/g) and chloroform extract (240 mg/g).

Table 1: Phytochemical constituents of three different solvent extract of mangrove species *R. mucronata*

Phytochemicals	Methanol	Ethanol	Chloroform
Protein	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Steroids	-	+	-
Terpenoids	+	+	+
Alkaloids	+	-	+
Tannins	+	+	+

(+ indicates present, - indicates absent)

Table 2: Total antioxidant content in mangrove species of *R. mucronata* extracts (mg/g)

Methanol (mg/g)	Ethanol (mg/g)	Chloroform (mg/g)
499.33	456.34	388.23

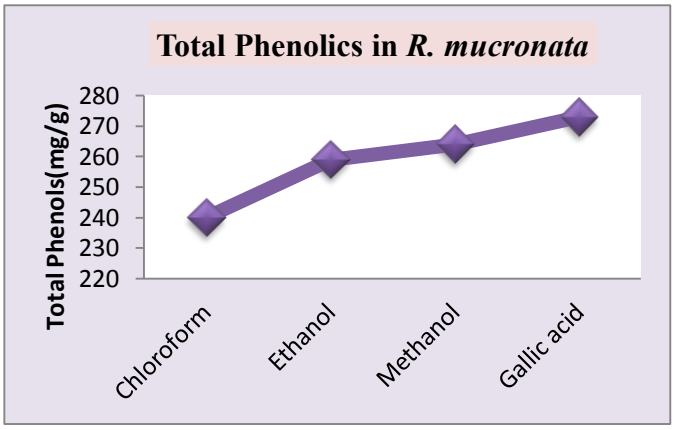

Fig.1- Total phenolics in *R.mucronata* extracts

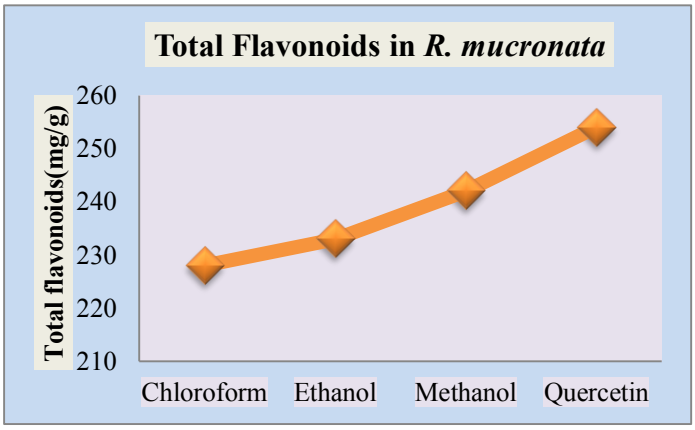

Fig 2. Total flavonoids present in *R.mucronata* extracts

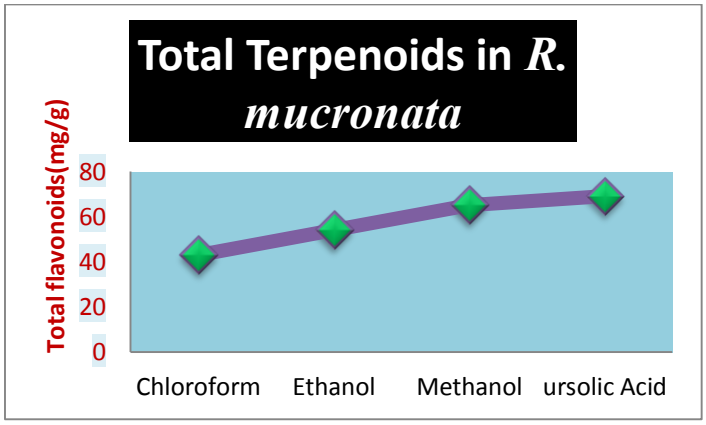

Fig.3-Total Terpenoinds present in *R.mucronata* extracts

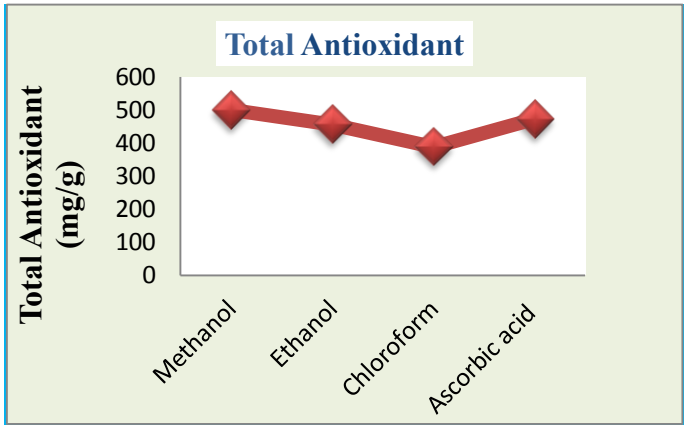

Fig.4-Total antioxidant levels in *R.mucronata*

Earlier, Cheung [29] reported that methanol extract of *Lentinus edodes, Volvariella volvacea* having the highest amount of phenolic compounds than petroleum ether, ethyl acetate and water extracts. Flavonoid is a hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and found to be antimicrobial substances against wide array of microorganisms *in vitro*. The current study also revealed that the flavonoid was high in methanol extract compare to ethanol and chloroform extracts. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell for anticancer activities [30,31]. The results revealed the presence of medicinally important constituents in the plants studied. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

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