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Research Article

Quantification of Free Radical Scavenging Potential and Antioxidant Activity of Methanolic Extract from *Rhizophora mucronata* Bark

Chitra Jairaman[1], Mohammed Yacoob Syed Ali[1*], AnuradhaVenkataraman[2] and Yogananth.Nagarajan[1]

[1]PG and Research Department of Biotechnology, Mohammed Sathak College of Arts & Science, Shollinganallur, Chennai-119, Tamil nadu, India
[2]PG and Research Department of Biochemistry, Mohammed Sathak College of Arts & Science, Shollinganallur, Chennai-119, Tamil nadu, India
*Corresponding Author: e-mail: syedmicro555@gmail.com Mob: +91 9842052929 & 7418406385

ABSTRACT

Plant based antioxidants are preferred due to the multiple mechanisms of actions and of the phytochemicals present in them. The present study was conducted to quantify the free radical scavenging activity and the antioxidant potential of the mangrove plant *Rhizophora mucronata (R. mucronata)*. The present study demonstrated the total phenolic content (TPC), total flavonoids content (TFC), Total Antioxidant Capacity (TAC) and free radical scavenging for 9 different parameters viz, DPPH, ABTS, lipid per oxidation, SO radical scavenging, Deoxyribose scavenging, SOD radical scavenging, β-carotene, Hydrogen peroxide, ferric reducing power assay. The results obtained for crude extract were DPPH: 59.56%, ABTS: 96.58%, Carotene: 72.87%, H_2O_2: 78.43%, SO: 99.45%, SOD: 95.82%, ferric reducing power assay: 59.56%, lipid per oxidation: 96.28%, deoxy ribose: 97.71%, total phenolic content (TPC): 56.51± 0.056 µg/gm Gallic acid equivalent, total flavonoids content (TFC): 79.36±0.047 µg/gm Querectin equivalent and Total antioxidant: 66.3± 0.063 µg/gm ascorbic acid equivalent The GC-MS Analysis for the secondary metabolite isolation and characterization showed many bioactive compounds with pharmacological value. GC-MS analysis revealed compounds viz, reducing sugars, fatty acids and sterols.

KEYWORDS: Methanolic Bark Extract, DPPH Assay, Antioxidant Properties, Scavenging Assay, GC-MS Analysis

1. INTRODUCTION

Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years. According to WHO the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine. Traditional medicine has been used almost by 70% of India's population [35]. There are about 45,000 plant species found in the Indian subcontinent. Of these, about 3500 species of both higher and lower plant groups are of medicinal value. Forest land consists of approximately 500 medicinal plant species used by Ayurveda which has been obtained from wild areas which constitutes about

80% of the medicinal value [11]. A rich source of different types of medicines has been obtained by plants which possess a wide variety of bioactive compounds. Because of its easy availability, efficacy and low cost, in recent years herbal medicine gained a huge importance [37]. Since plant based drugs contains nutraceuticals effects it is essential to evaluate the anti oxidant activity of the plants.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Free radicals are produced as the oxidation which damages the cell as it forms a series of chain reactions. According to many experts, this damage is a factor in the development of blood vessel disease (atherosclerosis), cancer, and other conditions. The antioxidant activity of Polyphenols due to their redox properties, play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides which is the indication of the antioxidant property. This exerts beneficial pharmacological effects on neurological disorders on the basis of in vitro observations [24]. In order to survive and counteract the reactive oxygen species (ROS) plant and its parts produces various anti oxidative compounds [14]. The various forms of activated oxygen (ROS), include free radicals such as superoxide anion radicals (O_2-), hydroxyl radicals (OH.) and non free-radical species such as H_2O_2 and singled oxygen. These molecules are considered as exacerbating factors in cellular injury and aging process [40]. ROS can cause lipid per-oxidation in foods, which leads to the deterioration of the food [19]. Thus leading to rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods, due to the formation of secondary, potentially toxic compounds. Thus, in order to increase the shelf-life of foods, various anti-oxidants were added. [17].

The important source of drugs are often found in medicinal plants in the form of secondary metabolites viz, alkaloids, glycosides, steroids and flavonoids [54]. Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. In addition, they have a metal chelation potential [16]. The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [23]. The importance of natural phenolic compounds from plants materials is also raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects [8].

Rhizophora mucronata, plant of Mangrove family, found on the coastal region of India has been utilized in treatment of various disorders and illness. In traditional medicine its bark and leaf extracts has been used as astringent, anti-septic and haemostatic with antibacterial, anti-ulcerogenic and anti inflammatory activities [31], [49] and [48]. In order to get relief from fish stings the poultice of the leaves has been applied [57]. A polysaccharide extracted from the leaves of *R.mucronata* showed positive activity against human immunodeficiency viruses [26]. The alkaloid rhizophorine is a major constituent of the leaves of *R.mucronata* [36]. Previous phytochemical investigation of *R.mucronata* indicated the presence of alkaloids, anthocyanidins, carbohydrates, carotenoids, tannins, gibberellins, flavonoids, inositols, lipids, minerals, polysaccharides, polyphenols, procyanidins, proteins, saponins, steroids, and triterpenes [36].

The literature revealed that the plant possesses hepatoprotective, antioxidant [56], anti-HIV [26] anti diabetic [56], antiplasmodial activity. In the present study, methanolic extracts of *R. mucronata* bark were subjected for the antioxidant screening using different in vitro methods.

2. MATERIALS AND METHODS

2.1 COLLECTION OF PLANTS AND ITS EXTRACTION

The barks of *Rhizophora mucronata* were collected from Pitchavaram mangrove forest. Collected plant materials were shade dried, powdered and used for extraction. The dried

powder material of the bark was extracted with methanol. The solvent was removed under pressure to obtain total extracts. The crude extract is stored at 4ºC for further use.

2.2 COLUMN CHROMATOGRAPHIC SEPARATION

The known weight of the crude extract was taken and subjected to Column Chromatography to obtain different fractions viz (Fraction 1 to Fraction-5). All the six (Crude extract and 5 fractions) were subjected to Scavenging assay (9 assays) and Total Phenolic Content, Total Flavonoid Content, total antioxidant capacity and GC-MS analysis for the characterization of the bioactive compounds.

2.3 QUANTITATIVE DETERMINATION OF PHYTOCONSTITUENTS

2.3.1 DETERMINATION OF TOTAL PHENOLIC CONTENT

Phenolic contents of crude extracts and the fractions were estimated by the method of [5]. 2 ml of 2% Na_2CO_3 was mixed with 100 µl of aliquot sample let it stand for 2 min at room temperature. 100 µl of 50% Folin Ciocalteau's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Phenolic content is expressed as Gallic acid equivalent per gram). All the experiment was conducted in three replicates.

2.3.2 TOTAL FLAVONOID CONTENT

The total flavonoid content was determined according to the method of [28]. Briefly, in 0.5 ml of the stock sample along with 150 µl of 10% AlCl3.H2O solution, 250 µl of 5% $NaNO_2$ solution was added. After 5 min, 0.5 ml of 1M NaOH solution was added and then the total volume was made up of 2.5 ml with distilled water and the absorbance was read at 510 nm.

2.4 TOTAL ANTIOXIDANT ACTIVITY

Total antioxidant activity was measured by the method of [25]. In order to measure the Total Antioxidant Capacity (TAC) reagent, 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml distilled water. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

2.5 FREE RADICAL SCAVENGING ASSAYS

2.5.1 TOTAL DPPH ASSAY

The scavenging effects of samples for DPPH radical were determined by the method [15]. Briefly, 2.0 ml of 0.16 mM DPPH methanolic solution was added to 2.0 ml of aliquot of test samples. The mixture was then vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The scavenging effect (%) was calculated by using the formulae given by Duan et al., 2006.

Scavenging effect (%) = [1-(A sample -A sample blank)/A control] × 100

2.5.2 ABTS RADICAL SCAVENGING

The ABTS scavenging capacity of the extract was compared with that of BHT and/or ascorbic acid and percentage inhibition calculated. For ABTS assay, the procedure followed with some modifications. The antioxidant effect of the leaf extracts was studied using ABTS (2, 2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of [46]. ABTS radical cations ($ABTS^+$) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium per sulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the different extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer (Genesys 10-S, USA) and the per cent inhibition was calculated using the formula

% ABTS radical scavenging activity = control - test/ control x 100

2.5.3 HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY

The ability of the crude extract to scavenge hydrogen peroxide was determined by the standard procedure of [42]. Hydrogen peroxide 10 mM solution was prepared in the phosphate buffer saline of 0.1 M, pH 7.4, 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide) after 10 min of incubation at 37ºC.

2.5.4 SCAVENGING OF SUPEROXIDE RADICAL

Scavenging of superoxide radical was studied using the method elaborated by [3]. Assay tubes contained 0.2 ml of the extract (corresponding to 20 mg extract) with 0.2 ml EDTA, 0.1 ml Nitro blue tetrazolium, 0.05 ml riboflavin and 2.64 ml phosphate buffer. The control tubes were set up with DMSO (Dimethyl sulfoxide) solution instead of the extracts. The initial optical densities of the solutions were recorded at 560 nm and the tubes were illuminated uniformly with the fluorescent lamp for 30 mins. A 560 was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition by the sample was calculated by comparing with O.D of the control tubes.

2.5.5 SUPEROXIDE DISMUTASE RADICAL SCAVENGING ACTIVITY

The Superoxide Dismutase Radical Scavenging Activity was measured by [2] method. Superoxide anions were generated using PMS / NADH system. The superoxide anions are subsequently made to reduce nitro blue tetrazolium (NBT) which yields a chromogenic product, which is measured at 560 nm. Test solution (20-100 mg/ml) in 0.1M phosphate buffer pH 7.4, 625 µl of 468 µM NADH solution, 625 µl of 150 µM NBT solution and 625µl of 60 µM PMS solution were added to a test tube and incubated at room temperature for 5 min. The absorbance was read at 560 nm. Linear graph of concentration Vs percentage inhibition was prepared and IC50 values were calculated.

% Superoxide dismutase radical scavenging activity = control- test/ control X 100

2.5.6 FERRIC REDUCING ANTIOXIDANT POWER ASSAY

Reducing power of different crude extract was determined by the method of [6]. Briefly, 1.0 ml of different solvent extract containing different concentration of samples were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50ºC for 20 min. After incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 min. From the upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml of FeCl3 (0.1%). Absorbance of all the solution was measured at 700 nm. Ferric reducing antioxidant Power is expressed as the number of equivalents of ascorbic acid.

2.5.7 DEOXYRIBOSE RADICAL SCAVENGING ASSAY

It was used to determine the hydroxyl radical scavenging activity in an aqueous medium [5]. The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without sample at various concentrations (10-250 µg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37ºC. The mixture was heated at 95 0C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. All readings were corrected for any interference from brown colour of the extract or antioxidant by including appropriate controls. The negative control without any antioxidant or sample was considered 100% Deoxyribose oxidation. The % hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Ascorbic acid was taken as the positive control.

2.5.8 LIPID PEROXIDATION BY EGG YOLK METHOD

Inhibitions of lipid peroxidation in the egg of hen were determined using a modified [4] thiobarbituric acidreactive species (TBARS) assay as previously described [32]. Egg homogenate (0.5 ml, 10% in dis- tilled water, v/v) and 0.1 ml of each fraction were mixed separately in a test tube and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml FeSO4 (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxi- dation. Thereafter, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA (w/v) in 1.1% sodium dodecyl sulfate (SDS) and 0.05 ml 20% TCA was added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Also, 10% of liver and brain homogenates obtained from rat were also used in place of egg homogenate for the evaluation of lipid peroxidation.

2.5.9 β- CAROTENE BLEACHING ASSAY

The antioxidant capacity of each of the sample extracts was estimated by the β-carotene bleaching method following the procedure described by [23] with modifications. One milliliter of β-carotene (0.2mg/ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were added to 0.2ml of sample extracts, standard (α-tocopherol) and control (80% methanol). Thereafter, chloroform was evaporated to dryness under vacuum using rotary evaporator. After evaporation, 100 ml of deionized water was added into the mixture and shaken vigorously until emulsion was obtained. Two milliliters of aliquots of the emulsions were pipetted into the test tubes and immediately placed in water bath at 45°C for 2 hours. The absorbance was read at 20 min interval at 470 nm, using a UV-visible spectrophotometer (Secomam, Anthelie Advanced 5) at initial time (t=0). Degradation rate (dr) of the sample was calculated according to the first order kinetics as described by Al-Saikhan et al. (1995):

$$\textbf{dr of sample} = (\ln [A_0 / A_t]) / t$$

where: ln = natural log; A_o = initial absorbance at time 0; A_t = absorbance at 20 min of incubation; t = 120 min and dr = degradation rate.

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control by using the equation:

$$\textbf{AA\%} = ([\textbf{dr control} - \textbf{dr sample}] / \textbf{dr control}) \times 100$$

2.6 GC-MS ANALYSIS

GC-MS analysis of active methanolic crude extract of bark of *Rhizophora mucronata* was done using Agilent GC-MS 5975 Inert XL MSD (United States) gas chromatography equipped with J&W 122-5532G DB-5mm 30 × 0.25 mm × 0.25 μm and mass detector (EM with replaceable horn) was operated in EMV mode. Helium was used as carrier gas with the flow rate of 1 ml/min. The column oven temperature was kept at 80°C for 2 min then programmed at 10–250°C/min, which was held for zero min, and 5–280°C/min which was held at 9 min. Electron impact spectra in positive ionization mode were acquired between m/z 40 and 450.

3.0 RESULTS

3.1 TOTAL PHENOLIC CONTENT

The total phenolic content of the crude and the fractions were determined and the results are presented in Fig.1a.The highest phenolic content was observed in the crude extract 56.51± 0.056 μg/gm Gallic acid equivalent, followed by Fraction-5 with 47.81 ± 0.047 μg/gm Gallic acid equivalent, then F-2 and F-3 have equal values with 46.03%± 0.04 μg/gm Gallic acid equivalent and finally with F-1 and F-4 with the values 44.68± 0.044 μg/gm Gallic acid equivalent and 44.53± 0.04 μg/gm Gallic acid equivalent respectively.

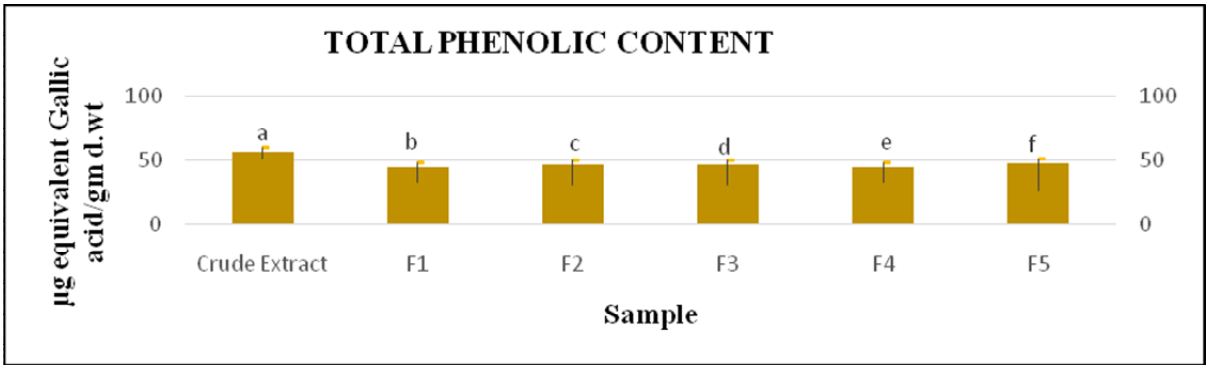


FIGURE: 1a. Total phenolic content found on the crude extract and different fractions (F1 to F5) expressed as µg/gm Gallic acid

3.2 TOTAL FLAVONOID CONTENT

The total flavonoid content of the crude and the fractions were determined and the results are presented in Fig.1b. The maximum flavonoid content was seen in F-5 with 79.36%± 1.984 and the least in the crude extract with 65.98%± 1.649.

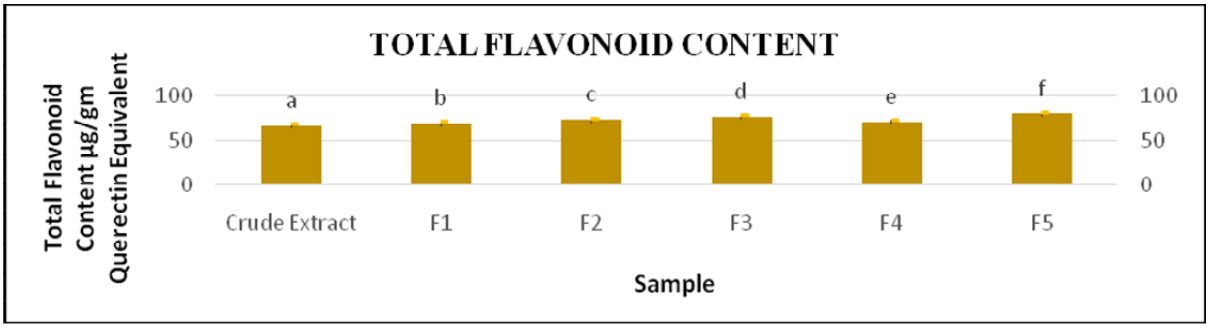


FIGURE: 1b. Total flavonoid content found on the crude extract and different fractions (F1 to F5) expressed as µg/gm Querectin Equivalent

3.3 TOTAL ANTIOXIDANT CAPACITY

The total antioxidant activity of the crude and the fractions were determined and the results are presented in Fig.1c. The antioxidant capacity has been expressed in µg/mg ascorbic acid equivalent. The highest antioxidant capacity was observed in F-3 with 74.48 ± 0.074µg/mg ascorbic acid equivalent. The crude extract had observed with 66.3± 0.063 µg/mg ascorbic acid equivalents and the least was observed in F-1 with 64.1± 0.063 µg/mg ascorbic acid equivalent.

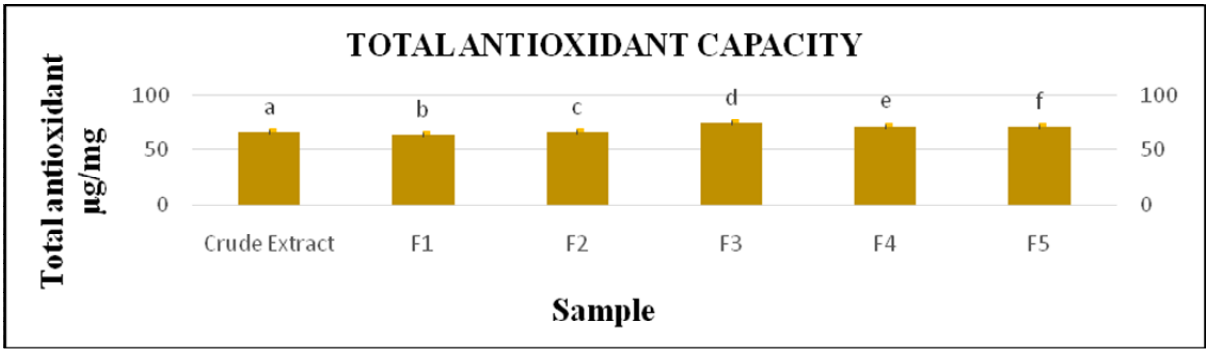


FIGURE: 1c. Total antioxidant capacity found on the crude extract and different fractions (F1 to F5) expressed as µg/gm Ascorbic acid Equivalent

3.4 DPPH ASSAY

The DPPH radical scavenging activities (%) of the extracts and fractions are presented in Fig.1d. The maximum DPPH radical scavenging was observed in the crude extract with 59.56 %± 0.057 and the least is observed in F-3 with 32.66%± 0.048.

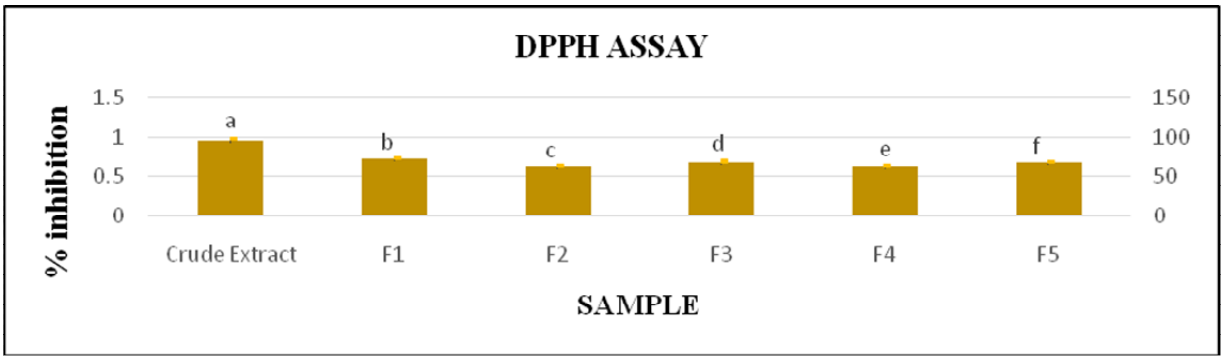


FIGURE: 1d. DPPH activity shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.5 ABTS RADICAL SCAVENGING ASSAY

The ABTS radical scavenging of the crude and the fractions were determined and the results are presented in Fig-1e. The maximum activity of 97.91%± 0.023 was observed in the fraction F3, followed by the crude extract with 96.58% ± 0.0236. The least in the fraction F-1 with 92.16%± 0.021 ABTS scavenging effect.

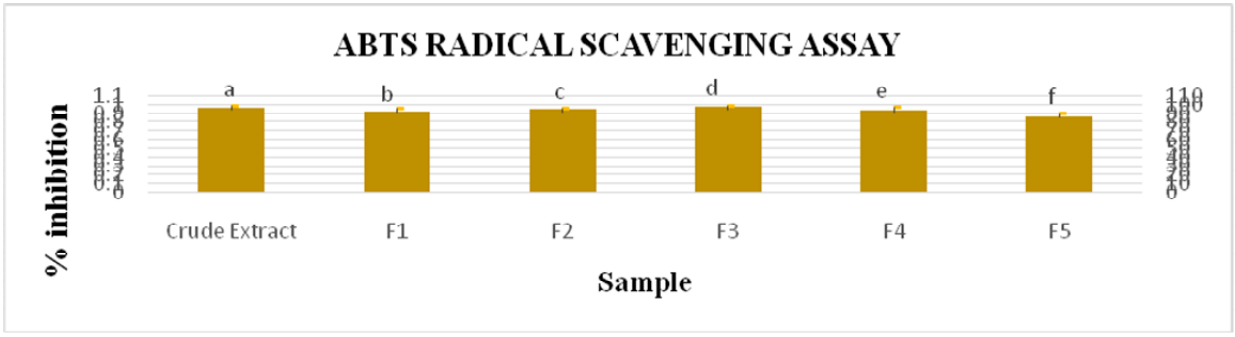


FIGURE: 1e. ABTS activity shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.6 H_2O_2 RADICAL SCAVENGING ASSAY

The hydrogen peroxide radical scavenging activities (%) of the extracts and fractions are presented in Fig.1f. The maximum hydrogen peroxide scavenging assay was observed in the crude extract 78.43%± 0.0997 and the least was seen in F-2 with 58.53%± 0.0978.

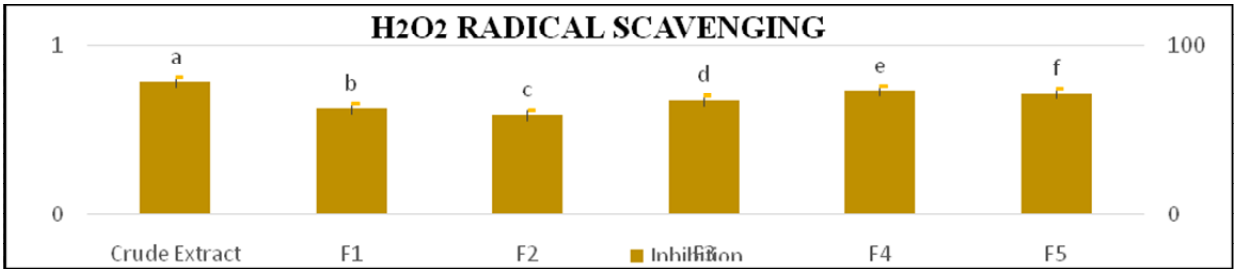


FIGURE: 1f. H_2O_2 radical scavenging shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.7 SUPEROXIDE RADICAL SCAVENGING ASSAY

The super oxide radical scavenging activities (%) of the extracts and fractions are presented in Fig.1g. The maximum superoxide scavenging assay was observed in the crude extract with the value of 99.45%± 0.082 and the least was observed in F-3 with 72.55%± 0.082.

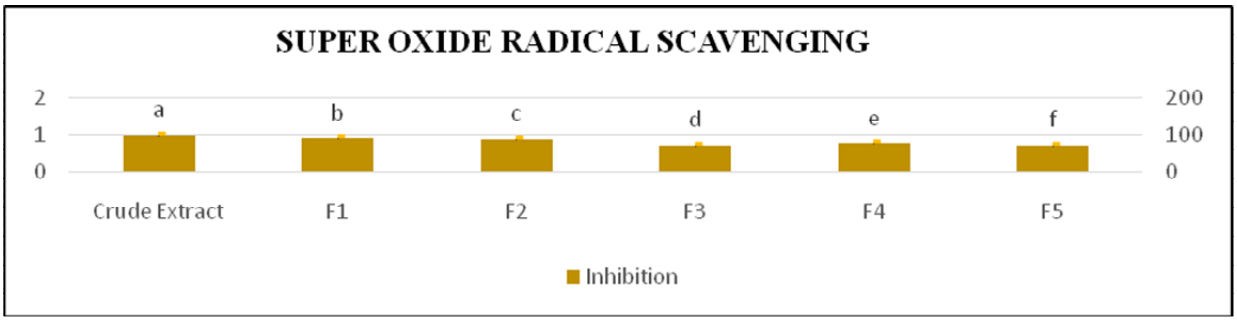


FIGURE: 1g. Super oxide radical scavenging shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.8 SUPER OXIDE DISMUTASE RADICAL SCAVENGING ASSAY

The superoxide dismutase radical scavenging of the crude and the fractions were determined and the results are presented in Fig-1h. The maximum SOD scavenging activity was observed in the crude extract with 95.82%± 0.094 and the least was seen at F-5 with 40.71%± 0.094.

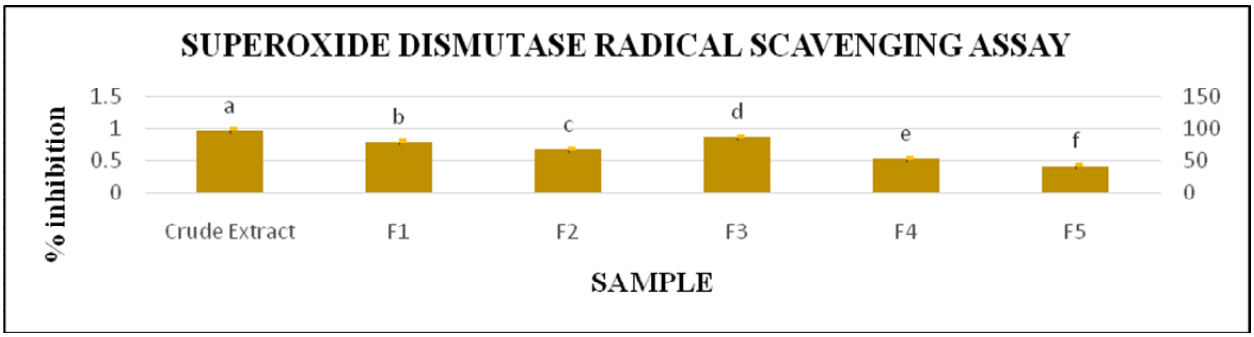


FIGURE: 1h. Superoxide Dismutase radical scavenging shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.9 FERRIC REDUCING ANTIOXIDANT POWER

The ferric reducing antioxidant capacity of the crude and the fractions were determined and the results are presented in Fig-1i. The maximum was observed at the crude extract with 41.24%± 0.289 and least was observed in F-4 with 6.11%± 0.29.

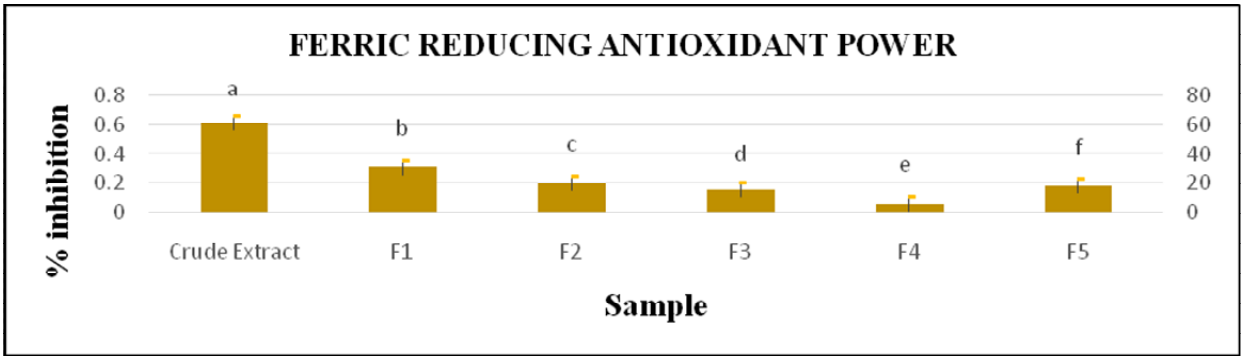


FIGURE: 1i. Ferric reducing antioxidant power shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.10 DEOXY RIBOSE RADICAL SCAVENGING

The scavenging of deoxyribose radical of the crude and the fractions were determined and the results are presented in Fig-1j. The maximum value was observed in the crude extract with 97.71% ± 0.002 inhibition. The least was observed in F-5 with 53.55%± 0.001.

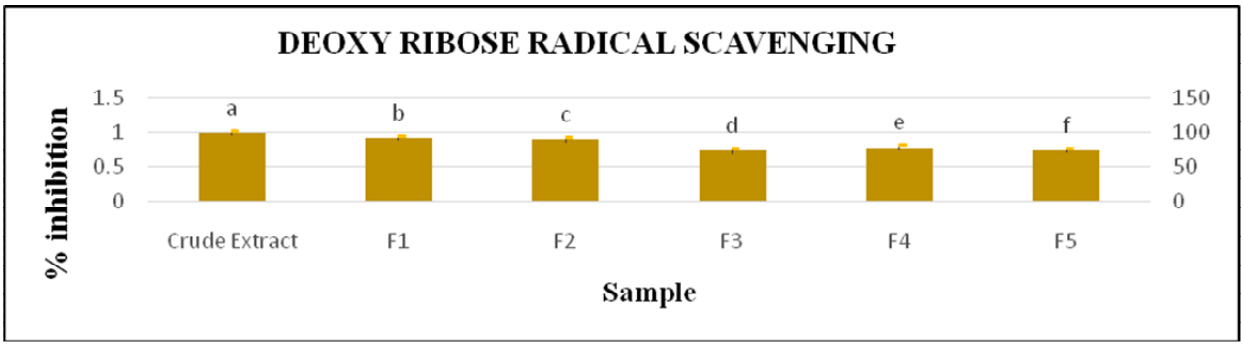


FIGURE: 1j. Deoxy ribose radical scavenging shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.11 LIPID PEROXIDATION BY EGG YOLK METHOD

The anti-lipid per oxidation of the crude and the fractions were determined and the results are presented in Fig-1k. The maximum % inhibition observed in the crude extract with 96.28%± 0.033 and the least in F-5 with 87.17%± 0.0033 inhibitions.

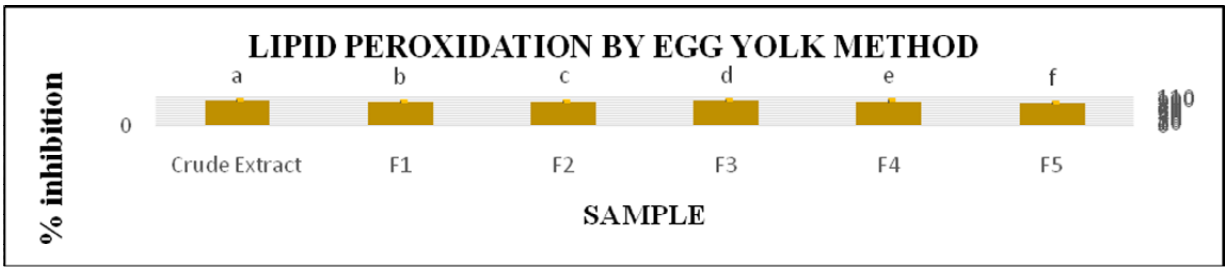


FIGURE: 1k. Lipid per oxidation shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.12 β- CAROTENE BLEACHING ASSAY

The β- carotene bleaching of the crude and the fractions were determined and the results are presented in Fig-1l. The maximum value was observed in the crude extract with 72.87%± 0.061 and least seen in F-4 with 35.85%± 0.063.

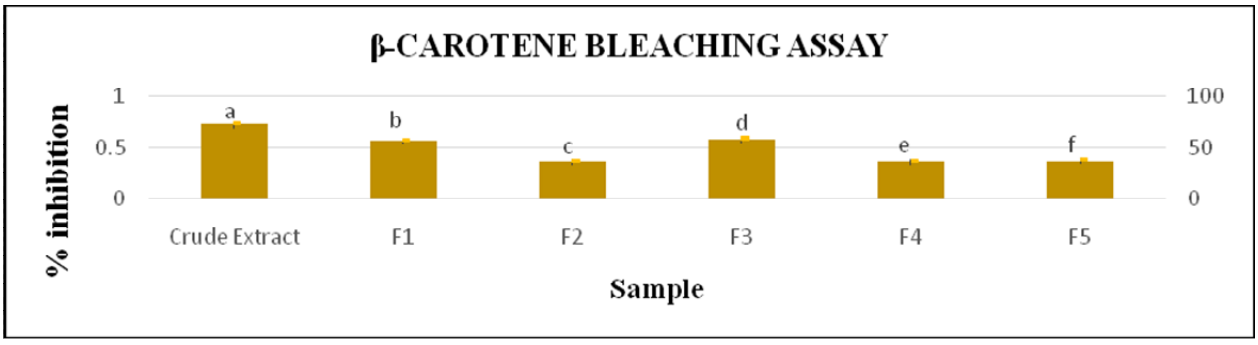


FIGURE: 1l. β- carotene bleaching assay shown by the crude extract and different fractions (F1 to F5) expressed as MEAN ± Standard Deviation

3.13 GC-MS ANALYSIS

The characterization of bioactive compounds analyzed by GC-MS analysis is illustrated in the below Table: 1 and Figure-1m. The table clearly indicates the presence of secondary compounds from fatty acids, reducing sugars, sterols, anti-inflammatory compounds and flavonoids. There are 10 major

metabolites identified, viz, Ethyl iso-allocholate, lactose, 9-Octadecenoic acid methyl ester, Metacetamol, 3, O-methyl-D-glucose, Cyclopropanebutanoic acid, Dasycarpidan-1-methanol acetate (ester), 1-Monolinoleoylglycerol trimethylsilyl ether, Octasiloxane and Heptasiloxane. The table also shared the retention time, area percentage, the structure and the bioactivity reported of the respective compounds**.**

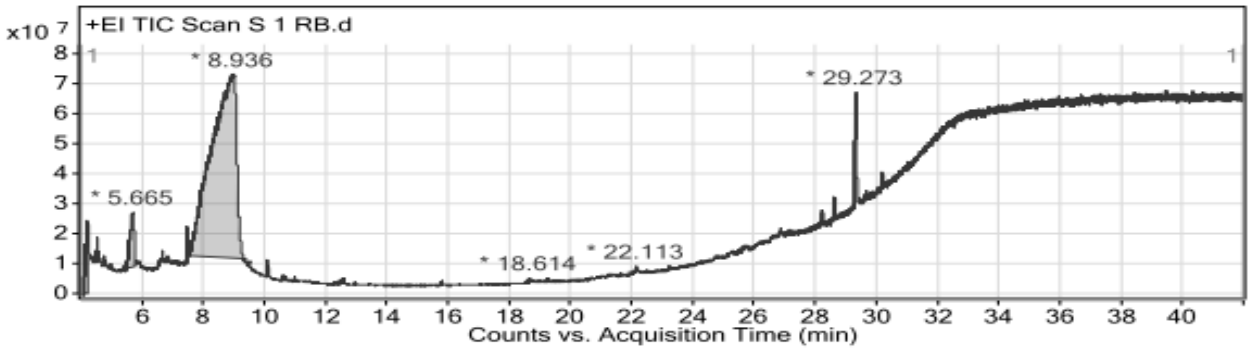
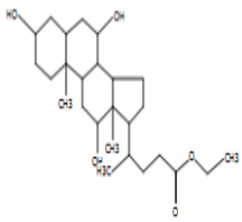
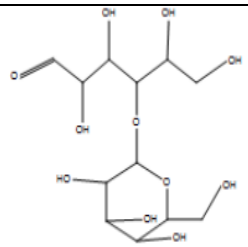
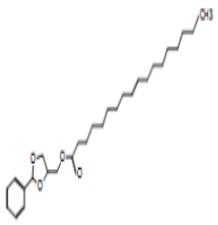
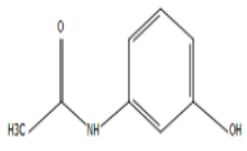
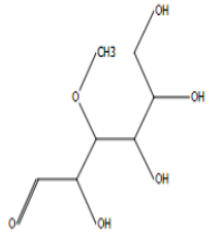
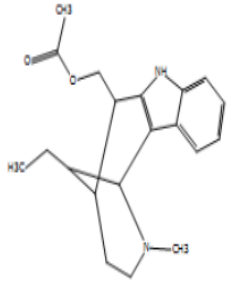
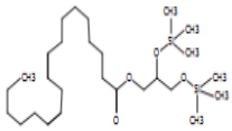
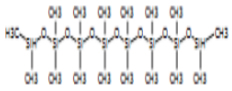
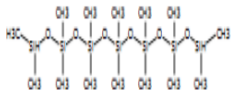


Figure: 1m. GC-MS analysis of the methanolic crude extract of Bark of *Rhizophora mucronata*

S.NO	RETENTION TIME	COMPONENTS	AREA %	STRUCTURE	FORMULAE	COMPOUND NATURE	BIOACTIVITY REPORTED
1.	4.196	Ethyl iso-allocholate	4.21		$C_{26}H_{44}O_5$	Steroid	Anti-inflammatory, anticancer antimicrobial, anti asthma, diuretic
2.	5.665	Lactose	5.51		$C_{12}H_{22}O_{11}$	Sugar Compound	Anti hypertensive and Anti microbial
3.	6.638	9, Octadecenoic Acid methyl ester	0.49		$C_{19}H_{36}O_2$	Linoleic Acid	Nematicide, Anti arthritic, Hepatoprotective, Anti androgenic, Hypocholesterolemic 5-Alpha reductase inhibitor, Antihistaminic, Anti coronary, Insectifuge, Antieczemic, Anti acne
4.	7.451	Metacetamol	1.5		$C_8H_9NO_2$	3-Acetamido phenol	Anti-bacterial and Anti-helminthic

5.	8.936	3,O-methyl-D-glucose	100		$C_7H_{14}O_6$	Sugar Moiety	anti-cancer, anti inflammatory
6.	10.075	Cyclopropanebutanoic acid	0.31		$C_{25}H_{42}O_2$	Ester	Anti microbial Anti- inflammatory
7.	18.614	Dasycarpidan-1-methanol acetate (ester)	0.19		$C_{20}H_{26}N_2O_2$	Ester	antimicrobial, antioxidant and Anti-inflammatory
8.	22.113	1-Monolinoleoylglycerol trimethylsilyl ether	0.36		$C_{27}H_{54}O_4Si_2$	Steroid	Antimicrobial Antioxidant Anti-inflammatory, Ant arthritic Anti asthma, Diuretic
9.	28.177	Octasiloxane	0.49		$C_{16}H_{50}O_7Si_8$	Volatile organic compounds	Anti microbial
10.	28.576	Heptasiloxane	0.6		$C_{14}H_{44}O_6Si_7$	Volatile organic compounds	Anti microbial

Source: Dr. Duke's phytochemical and Ethno botanical database.

TABLE: 1 Show the result for GC-MS Analysis, with bioactive compounds, their retention time, structure, their area %, formulae, nature of the compound and activity of the isolated bioactives.

4.0 DISCUSSION

The anti-oxidant activity found in the natural phenolics exerts their beneficial health effects [39]. These compounds acts by preventing 1st chain initiation by scavenging initial radicals such as hydroxyl radicals, decomposing primary products of oxidation, binding metal ion catalysts, to non radical species and breaking chains to prevent continued hydrogen abstraction from substances and are capable of decreasing oxygen concentration, intercepting singlet oxygen [41]. The overall antioxidant activities of the plant foods can be attributed to the phenolic compounds present in them. The phenolic content in the crude extract was found to be maximum with 56.51± 0.056µg/gm Gallic acid equivalent.

In order to determine the total flavonoid contents of the crude extract and different fractions obtained through Column Chromatography, aluminium chloride colorimetric method was used. Total flavonoid

contents were calculated using the standard curve of querectin and was expressed as querectin equivalents (QE) per gram of the plant extract. Flavonoid contents of the extracts were found to decrease in the following order: F5 > F3 > F2 > F4 > F1> Crude extract. Flavonoids play an important role in antioxidant system in plants. The anti oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [19]. Depending on their structure, flavonoids are able to scavenge practically all known reactive oxygen species (ROS).

Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Total antioxidant capacity of the crude extract and fractions of *Rhizophora mucronata* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The antioxidant potential of the bark extract of *Rhizophora mucornata* was investigated in the search for new bioactive compounds from natural resources. The highest antioxidant capacity was observed in F-3 with 74.48 ± 0.074µg/mg ascorbic acid equivalent. The crude extract had observed with 66.3± 0.063 µg/mg ascorbic acid equivalents.

DPPH radicals are widely used in the model system to test the scavenging activity of several natural phytoconstituents. The result of DPPH scavenging activity in this study indicates that the plant was potentially active. The crude methanolic extract showed % inhibition of 59.56 as compared to the fractions in which F-3 showed the least % inhibition with 32.66 .The DPPH contains an odd electron, which is responsible for purple colour, and absorbance wavelength of 517nm [45]. The crude and fractionated methanolic extracts of *Rhizophora mucronata* bark were estimated using potassium ferric cyanide reduction method. In this assay, the yellow colour formed in the reaction is significant indicator of antioxidant activity.

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline-6sulphonic acid) assay is based on the scavenging of light by ABTS radicals [33]. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in absorption which can be followed spectrophotometrically. The relatively stable ABTS radical has a green colour and is quantified spectrophotometrically at 734nm. The crude extracts produced maximum ABTS scavenging capacity followed by different fractions.

The extracts were assessed for their radical scavenging potential using deoxyribose degradation assay. The antioxidant compounds are responsible for the reduction of ferric (Fe^{3+}) form to ferrous (Fe^{2+}) form. The addition of $FeCl_3$ to the ferrous form led to the formation of blue colored complex. So the reduction ability can be determined by measuring the colored complex at700nm [38]. The results obtained clearly indicate that the bark extract has the capacity to scavenge the hydrogen peroxide radicals.

Reducing power of the fractions was assessed using ferric to ferrous reducing activity as determined spectrophotometrically from the formation of Perl's Prussian blue colour complex [35]. Reducing power of different extracts of *R.mucornata* was compared with ascorbic acid and BHT. Among that, the crude extract exhibited the most reducing power. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power.

Lipid per oxidation plays an important role in aging process and some chronic diseases including diabetes, nervous disorder, cardiovascular diseases and cancer [11], [14]. [28]. The result obtained clearly showed that the crude extract has the maximum capacity for anti-lipid per oxidation followed by different extracts.

Carotene, the compound commonly found in the green vegetable is also active [10]. In the β-

carotene bleaching assay, the oxidation of linoleic acid produces free radicals due to the removing of hydrogen atom from diallylic methylene groups of linoleic acid [21]. The highly unsaturated β- carotene then will be oxidized by the generated free radical. Degradation of the orange coloured chromophore of β- carotene could be monitored spectrophotometrically. However, the presence of antioxidant constituents could prevent the bleaching of β-carotene because of their ability to neutralize the free radicals [44], [47]. The antioxidant activity was expressed as percent inhibition relative to the control. The result obtained in this study clearly indicates that the crude extract showed the highest capacity for the bleaching activity.

Among the detected compounds via GC-MS analysis few reported to be of highly bioactive compound. For instance, 9, octadecadienoic acid methyl ester is effective antihistaminic, anti-coronary, Insectifuge and antieczemic [1] and [31].These findings are also supported by the study done by [60] that revealed the insecticidal activity of Bersama abyssinica extracts.

On the other hand ethyl iso-allocholate was reported to exhibit anti-inflammatory, anticancer antimicrobial [54], [56], whereas hexadecanoic is effective antioxidant, hypocholesterolemic, nematicide and pesticide properties [53]. The other compounds viz, Heptasiloxane and Octasiloxane showed good anti microbial activity.

Different plant compounds can exhibit similar activity and this could be due to the presence of similar functional group. Moreover, antimicrobial, antioxidant and anti-inflammatory activities were displayed by most compounds in this study clearly indicated that the plant is biologically very active.

5.0 CONCLUSION

This study affirms the in vitro antioxidant potential of methanolic extracts of bark of *Rhizophora mucronata*. Results are compared with values of different standards such as Gallic acid, BHT, querectin and ascorbic acid. GC-MS analysis also identified the potential bioactive compounds with the valuable therapeutic activity. Based on the above results, the Bark extract of *Rhizophora mucronata* showed the potential as a source of antioxidant and a high capacity to scavenge a wide range of reactive oxygen species and further studies may lead to drug development. Gas chromatogram and mass spectrometry (GC-MS) analysis of bark extract of *Rhizophora mucronata* can be used as a promising multipurpose medicinal source whereas further clinical trial is required to prove its efficacy. This indicated that *Rhizophora mucronata* contained potential antioxidant bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential anti proliferative properties.

FINANCIAL DISCLOSURE

This study has been undertaken by author's own interest, No funds have been received by the department to pursue this research.

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