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CHROMATOGRAPHIC ANALYSIS OF *RHIZOPHORA MUCRONATA* BARK- BASED ON ANTIOXIDANT AND FREE RADICAL SCAVENGING POTENTIAL

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ABSTRACT
The present study aims at performing the total anti oxidant capacity, total phenolics, total flavonoids, free radical scavenging which includes DPPH Assay, H_2O_2 scavenging assay, SO radical scavenging of the different bark extract of *Rhizophora mucronata* and characterization of the components present in it by HPTLC fingerprinting. The various solvent extracts of *Rhizophora mucronata* bark were subjected to anti oxidant assay and free radical scavenging assay. Total Phenolic content ranged from 22.17 to 85.4 mg/gm of gallic acid equivalent, Total Flavonoid from 21.27 to 84.72 mg/gm Quercetin equivalent and Total antioxidant capacity was recorded between 29 to 78.5 mg/gm Ascorbic acid equivalent. The DPPH assay has been found to be maximum for ethanol: water (7:3) showed 94.56% inhibition and least recorded for diethyl ether with 41.45% inhibition. Similar results were obtained for other scavenging assay viz SO and H_2O_2 radical scavenging assay. The preliminary phytochemical analysis of these extracts revealed the presence of alkaloids, terpenoids, phenols, phyto sterols, carbohydrates, flavonoids, saponins, tannins. Based on the phytochemical analysis, ethanol: water (7:3) was selected as the most suitable extract for further analysis as it has eluded 8 major components. Thus, ethanol: water (7:3) was then subjected to column chromatography to yield different fractions. The HPTLC results obtained clearly indicated the flavonoid: Quercetin is found both in crude and fractions BE-3 and BE-4 as well. Thus, it can be concluded that the bark of *Rhizophora* contains powerful bioactive compounds with huge therapeutic activity.

KEYWORDS: Rhizophora mucronata, Phytoconstituents, Anti oxidant capacity, DPPH Assay, HPTLC Fingerprinting.

1. INTRODUCTION
The plant, *Rhizophora mucronata*, belonging to Mangrove family and commonly known as the loop root mangrove, red mangrove and Asiatic mangrove.[1] In Asia, *R. mucron*ata found in Cambodia, India, Indonesia, Malaysia Pakistan, Sri Lanka, Thailand and Vietnam. Rhizophora mucronata species also found in South Pacific Solomon Islands, Vanuatu and Australia northern territory.[1]

The natural habitat of Rhizophora mucronata is estuaries, tidal creeks and flat coastal areas subject to daily tidal flooding. It seems to be more tolerant of inundation than other mangrove species and often forms an evergreen fringe to mangrove areas.

R.mucronata is used in the treatment of various diseases, found on the coastal region of India.[2] It has folkloric curative properties against diabetes, diarrhoea, nausea, haematuria, haemorrhages and angina. The pertinent data from literature corroborated that R. mucronata posses antiviral[3], antibacterial[4], cytotoxic, analgesic, anti diuretic activity[5], nematocidal[6], antidiarrhoeal[7], hepatoprotective and antioxidant properties.[8] In ethno medicine, the *R.mucronata* bark is also mentioned for its anti diarrheal properties.[9] It is also been scientifically proved to have antiviral activities.[10] The diverse biological activities manifested by the *R. mucronata* are due to the presence of various types of steroids, diterpenoids, alkaloids, flavonoids, phenols and triterpenoids.[11]

In the last few years, various methods have been validated for procuring the active molecules from the

plants. In order to get a series of bioactive compounds from plants, the extraction procedure is very vital. The extraction procedure should be selected in such a way that mostly all the phytochemicals can be eluted and explored for a huge number of disorders. So, the present study was aimed to investigate the possible chemical components present in different bark extract of *R.mucronata* viz Diethyl ether, Acetone, Iso-propanol, Ethyl acetate, Methanol, Ethanol: water (7:3). The different extracts were then subjected to anti-oxidant activity and free radical scavenging assay.

Antioxidants are those substances which has a unique property of breaking the chain reaction of free radicals. It has been found that free radicals which produce oxidative stress results in the induction of chronic and degenerative diseases viz, cancer, heart related diseases, ageing process, immune suppression, diabetes, nervous disorders, artherssclerosis and many other metabolic disorders.[12] A large number of herbal and non-herbal plants possess various chemical compounds which exhibits anti oxidant properties. Various parts viz, stems, leaf, bark, root, fruts, flowers and twigs of the plants contains a wide range of anti oxidant compounds, many of which have commercial applications in the pharmaceutical, medical, cosmetic, food industries and agriculture. Thus, the current study aimed to investigate the antioxidant capacity, total phenolics and flavonoids and the free radical scavenging assay DPPH, H_2O_2 assay and Super oxide radical scavenging of the different extracts of *R. mucronata* bark.

2.0 MATERIALS AND METHODS

2.1 Plant material collection and its extraction

The barks of *R. mucronata* were collected from Pitchavaram mangrove forest. Collected plant materials were shade dried, powdered and used for extraction. The dried barks were then grinded in coarse powder using high capacity grinding machine. The powdered plant material (20 gm) was successively extracted in a Soxhlet extractor at elevated temperature using 200 ml of distilled petroleum ether (40-60°C) which was followed by Diethyl ether, Acetone, Iso-propanol, Ethyl acetate, Methanol, Ethanol: water (7:3). All the extracts were filtered individually through filter paper and poured on petri dishes to evaporate the liquid solvents from the extract to get dry extracts. After drying, crude extracts were weighed and stored in stock vials and kept in refrigerator (0- 4°C) for further use.

2.2 Quantification of Secondary Metabolites

2.2.1 Total Phenolic content

Total phenols were recorded by Folin Ciocalteu reagent[13] with slight modifications. 5 ml of Folin Ciocalteu reagent (1:10 diluted with distilled water) and 4 ml of aqueous Na_2CO3 (1M) was mixed with 0.5 ml of dilute extract (1:10 g/ml) or gallic acid used as standard. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for colour development and the absorbance was measured by UV-Spectroscopy at 765 nm against blank.

2.2.2 Total Flavonoid Content

The total flavonoid content was determined according to the method of[14] Briefly, a 250 µl of 5% $NaNO_2$ solution was added to 0.5 ml of the stock sample along with 150 µl of 10% $AlCl_3.H_2O$ solution. 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 ionized distilled water and the absorbance was read 510 nm against a methanol blank.

2.2.3 Total Anti oxidant activity

Total antioxidant activities of various solvent extracts obtained from the bark of *R.mucronata* were determined.[15] Briefly, 0.3 ml of sample was mixed with 63.0ml reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695nm. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

2.3 Free Radical Scavenging Assay

2.3.1 DPPH Radical Scavenging Assay

The scavenging effects of the various solvent extracts were determined[16] briefly, 2.0 ml of 0.16mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in the dark. The absorbance of all the sample solutions was measured at 517nm.[9] The scavenging effect (%) was calculated by using the formulae:

DPPH radical scavenging activity[%] =[1- (Asample–Asample blank / Acontrol) × 100]

Where, A sample is the Absorbance of DPPH solution & test sample, A sample blank is the absorbance of the sample only without DPPH solution). Synthetic antioxidant Ascorbic acid was used as positive controls.

2.3.2 H_2O_2 Radical Scavenging Assay

The ability of the marine plants to scavenge H_2O_2 was determined with the slight modification[17] briefly, 40mM H_2O_2 was prepared in phosphate buffer (pH-7.4) and the H_2O_2 concentration was determined spectrophotometrically by measuring the absorption with the extinction coefficient for H_2O_2 of 81M-1cm-1. Extracts (100 µg/ml) in distilled water and ascorbic acid (20 – 100 µg/ml, positive control) were added to 0.6 ml of 40mM H_2O_2 solution and the absorbance of H_2O_2 was determined at 230 nm after 10 minutes incubation against a blank solution containing phosphate buffer without H_2O_2.

The percentage of scavenging of H_2O_2 was calculated as the percentage of scavenging of hydrogen peroxide was calculated using the following formula:

H2O2 radical scavenging activity [%]
= [(A0 – A1) / A0] × 100

(Where A0 – Absorbance of control; A1 – Absorbance of sample)

2.3.3 Super oxide Radical Scavenging Assay
Scavenging of superoxide radical was studied using the method elaborated by[18] 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 algal 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. A560 was measured again and the difference in O.D was taken as the quantum of superoxide production. The percentage of inhibition by the algal sample was calculated by comparing with O.D of the control tubes.

2.4 Chromatographic Analysis: In order to isolate the bioactive compound from the crude extracts they were further fractionated using column chromatography silica gel (40 g, 60-120 #, LOBA Chemie Pvt. Ltd.) as stationary phase. The known weight of the crude extract was taken and subjected to Column Chromatography to obtain different fractions viz (Fraction 1 to Fraction-5). The fractions are collected at regular intervals in a screw capped bottle which is stored at 4ºC for further use. Later, the crude extract and the fractions (F1 to F5) were subjected to HPTLC fingerprinting.

2.5 HPTLC Fingerprinting: All the extracts (crude and 5 fractions) were were inoculated on the pre-coated Silica gel 60 F254 aluminium sheets.Chromatogram was developed on 10 × 20cm aluminium Thin Layer Chromatography (TLC) plate pre-coated with 0.2mm layer of silica gel 60 F254 (E. Merck, Germany) stored in a desiccator. The application was done by Camag Linomat syringe (100µL sec-1), mounted on a Linomat V applicator. Application of bands of each extract with different concentration were carried out at a distance of 8mm with the help of Linomat V applicator attached to Camag HPTLC system, which was programmed through win CATS software (Version 1.3.0) at λ max 254 and 366nm using deuterium light source, the slit dimensions were 8 × 0.4mm and at λ max 410nm using tungsten light source. The chromatograms were recorded [Sethi, 1996, Wagner and Baldt, 2001].

The samples were applied in the form of bands at a distance of 8mm (distance to the lower edge was 10mm) was performed at 25°C with hexane: ethyl acetate: methanol: formic acid [0.5:2:7:0.5] as mobile system. After development, the plate was dried at 60°C in an oven for 5mins. Scanning was then performed with a Camag TLC Scanner III equipped with the win CATS Software. The chromatograms were scanned by the densitometer at 254 and 366nm. The Rf values and fingerprint data were recorded and the plate was kept in photo documentation chamber and captured the images.

3.0 RESULTS

3.1 Quantification of Secondary Metabolites

3.1.1 Total Phenolic content

Total phenolic content of the different bark extracts of *R.mucronata* was determined by using the Folin-Ciocalteau's reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the Gallic acid was used for constructing the standard curve (0 to 200 µg/ml; Y= 0.013X and R2= 0.9906) Figure-1. Ethanol: water (7:3) extract of *R.mucronata* bark was found to contain the highest amount of phenols (Phenol contents of the extracts were found to decrease in the following order: Ethanol: water (7:3) extract > Methanol extract > Ethyl acetate extract > Iso-propanol extract > Acetone> Diethyl ether (Figure-2) (Table 1). Thus, from the results it can be concluded that the tested plant extract contains the important phenolics bioactives in them.

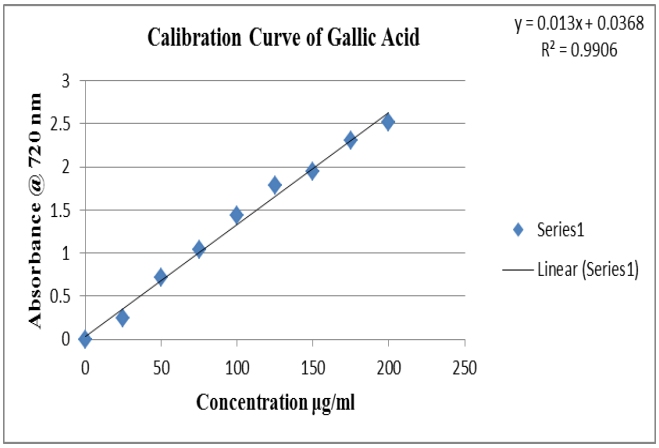


Figure. 1. Calibration curve of standard Gallic acid.

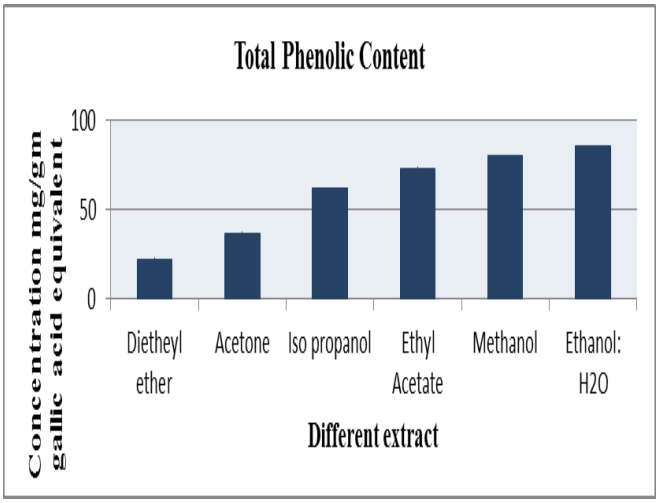


Figure. 2. Total phenolic contents of the different extracts of bark of R. mucronata.

Table. 1: Total phenol contents of the different extracts of R. mucronata bark. Values are the mean of duplicate experiments and represented as mean ± SD.

S. No	Extract	Total Phenol Contents (mg/gm, Gallic Acid Equivalent)
1	Diethyl ether	22.17 ± 0.099
2	Acetone	36.63± 0.139
3	Iso-propanol	61.96 ± 0.345
4	Ethyl acetate	73.09 ± 0.356
5	Methanol	79.86 ± 0.265
6	Ethanol: water (7:3)	85.4 ± 0.095

Table. 2: Total flavonoid content of the different extracts of R. mucronata bark. Values are the mean of duplicate experiments and represented as mean ± SD.

S. No	Extract	Total Flavonoid Content (mg/gm, Quercetin Equivalent)
1	Diethyl ether	21.27 ± 0.5499
2	Acetone	44.15 ± 1.1388
3	Iso-propanol	55.3 ± 1.2773
4	Ethyl acetate	63.5 ± 1.1388
5	Methanol	76.16 ± 1.4116
6	Ethanol: water (7:3)	84.72 ± 0.2684

3.1.2 Total Flavonoid Content

Total flavonoid contents was calculated using the standard curve of Quercetin (0 to 200 µg/ml; Y= 0.0139X and R^2= 0.9932) (Figure-3) and the total flavonoid compounds concentration in the bark extract was expressed as milligrams of Quercetin equivalent per gram of dry weight (mg Quercetin/g) of extract. Ethanol: water (7:3) extract of *R.mucronata* bark was found to contain the highest amount of flavonoids (Total flavonoids of the extracts were found to decrease in the following order: Ethanol: water (7:3) extract > Methanol extract > Ethyl acetate extract > Iso-propanol extract > Acetone> Diethyl ether (Figure-4) (Table 2).

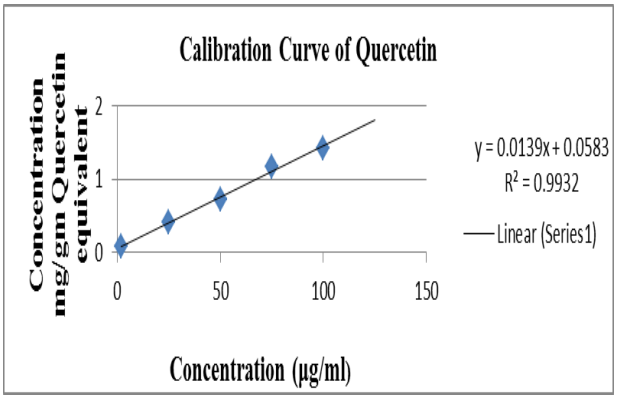


Figure. 3: Calibration curve of standard Quercetin.

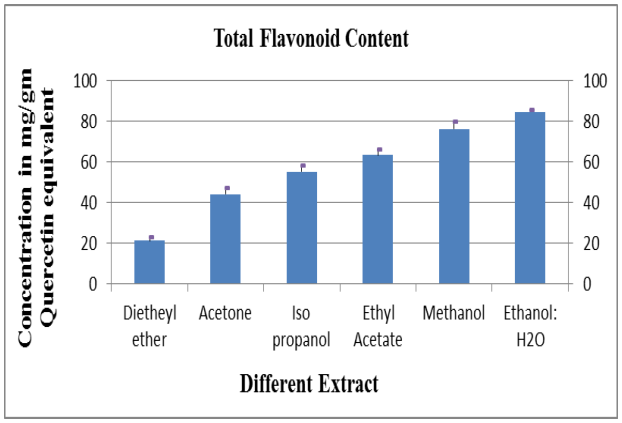


Figure. 4: Total flavonoid content of the different extracts of bark of R. mucronata.

3.1.3 Total Anti oxidant Capacity

Total antioxidant capacity of the different extracts of *R.mucronata* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (0-200µg/ml; Y= 0.002X and R2= 0.016) (Figure-5) and the total anti oxidant capacity in the bark extract was expressed as milligrams of Ascorbic acid equivalent per gram of dry weight (mg Ascorbic acid /g) of extract. Ethanol: water (7:3) extract of *R.mucronata* bark was found to contain the highest amount of antioxidant capacity (Total anti oxidant capacity of the extracts were found to decrease in the following order: Ethanol: water (7:3) extract > Methanol extract > Ethyl acetate extract > Iso-propanol extract > Acetone> Diethyl ether (Figure-6) (Table 3).

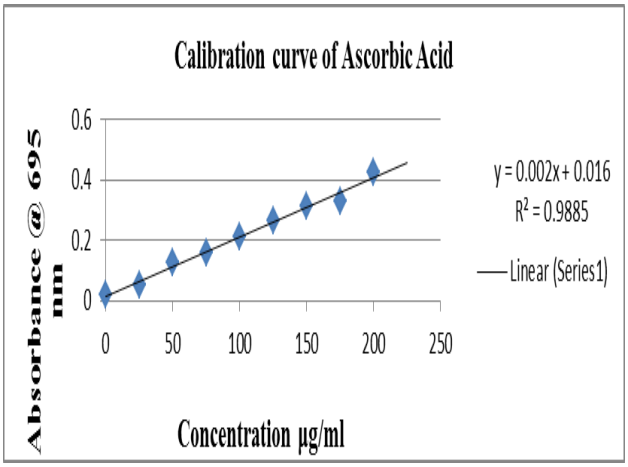


Figure. 5: Calibration curve of standard Ascorbic Acid.

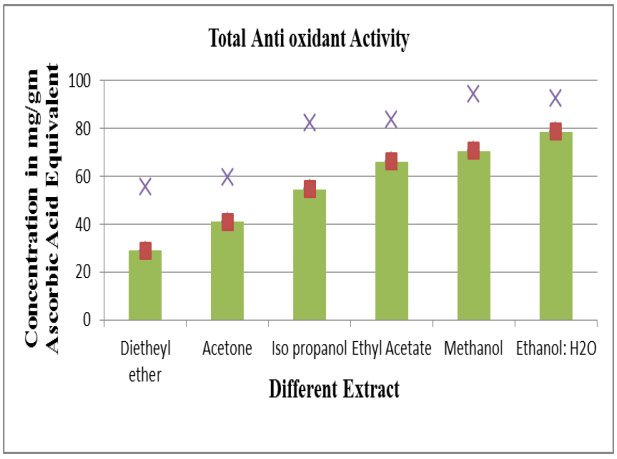


Figure. 5: Total anti oxidant capacity of the different extracts of bark of R. mucronata.

Table 3: Total anti oxidant capacity of the different extracts of R. mucronata bark. Values are the mean of duplicate experiments and represented as mean ± SD.

S. No	Extract	Total Anti oxidant capacity(mg/gm, Ascorbic acid Equivalent)
1	Diethyl ether	29.0 ± 11.539
2	Acetone	41.0 ± 7.997
3	Iso-propanol	54.5 ± 12.04
4	Ethyl acetate	66.05 ± 7.543
5	Methanol	70.5 ± 10.292
6	Ethanol: water (7:3)	78.5 ± 6.075

3.2 FREE RADICAL SCAVENGING ASSAY

3.2.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of different extracts of *R.mucronata* bark was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. Ethanol: water (7: 3), Methanol, Ethyl acetate and Iso- propanol showed maximum inhibition 95.00%, 86.17%, 81.36% and 72.45% respectively whereas the Diethyl ether and Acetone showed less 43.87% and 55.29% respectively. (Figure- 6).

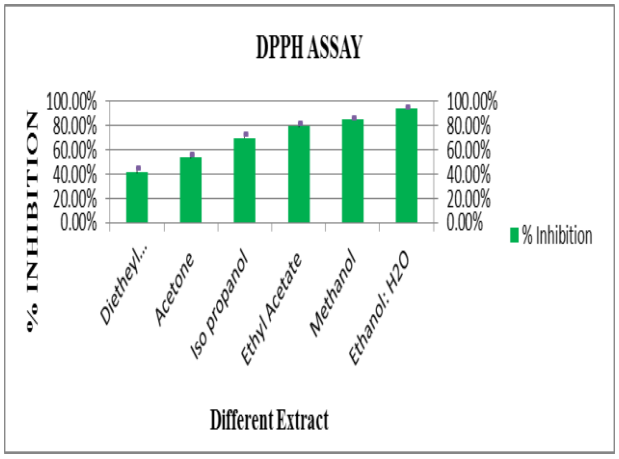


Figure. 6: DPPH Radical Scavenging Assay of the different extract of bark of R. mucronata. Values are the mean of duplicate experiments and represented as mean ± SD.

3.2.2 H_2O_2 Radical Scavenging Assay

Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects.[26] Scavenging of hydrogen peroxide of different extracts of *R.mucronata* bark extract is presented in Figure 7. Among the 6 extracts, Ethanol: water (7: 3), Methanol, Ethyl acetate and Iso-propanol showed good activity taking Ethanol: water (7: 3) in the top position in depleting H2O2 radical. The percentage of H2O2 scavenging activity of Ethanol: water (7: 3) extract was found to be 79.13% which is highest among six extract followed by Methanol extract > Ethyl acetate extract > Iso-propanol extract > Acetone> Diethyl ether with 75.74%, 66.05%, 55.17%, 46.25% and 34.25% respectively.

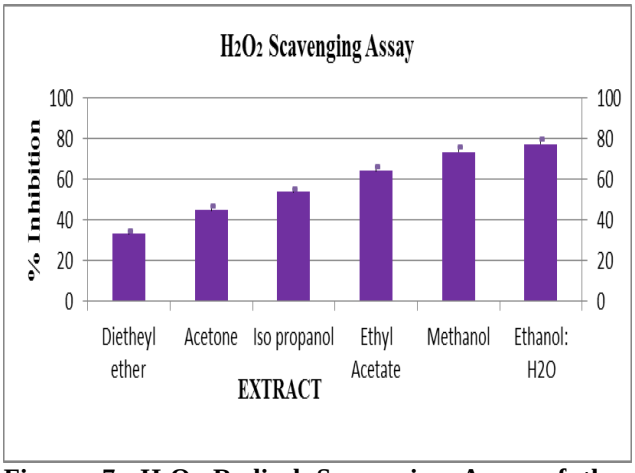
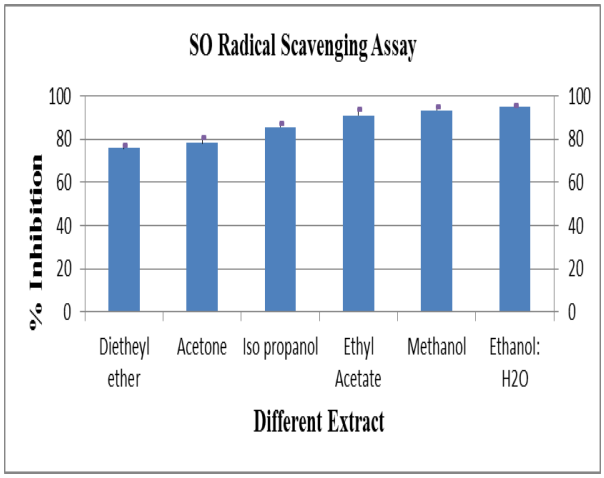


Figure. 7: H_2O_2 Radical Scavenging Assay of the different extract of bark of *R. mucronata*. Values are the mean of duplicate experiments and represented as mean ± SD.

3.2.3 Super oxide Radical Scavenging Assay

The Scavenging of Super oxide radical of the different extracts of bark of *R. mucronata* were determined and the results are presented in Figure- 8. The maximum

value was observed for the Ethanol: water (7:3), Methanol, Ethyl acetate and Iso- propanol showed good activity. The percentage of Super oxide scavenging activity of Ethanol: water (7: 3) extract was found to be 95.03% which is highest among six extract followed by Methanol extract > Ethyl acetate extract > Iso-propanol extract > Acetone> Diethyl ether with 93.33%, 91.04%, 85.56%, 78.21% and 75.75% respectively.



3.3 HPTLC RESULTS

The plates were developed and observed under UV at both 254 nm and 366nm. The Rf values were calculated for different bands. The standard Quercetin peak value, Rf at 366 nm is mentioned in (Table-4, Figure-9). The HPTLC fingerprint of the crude extract revealed 9 peaks with Rf value ranging from -0.04, 0.02, 0.15, 0.21, 0.34, 0.48, 0.54, 0.66 and 0.81 at 633 nm (Table-5 Figure-10) for the crude methanolic extract. Similarly for fractions, BE-1 obtained 5 Rf values (Table-6. Figure-11), BE-2- 4 Rf values (Table-7, Figure-12), BE-3- ranged 6 Rf values (Table-8, Figure-13), BE-4 got 5 Rf values (Table-9, Figure-14) and BE-5 with 6 Rf values (Table-10, Figure-15) respectively.

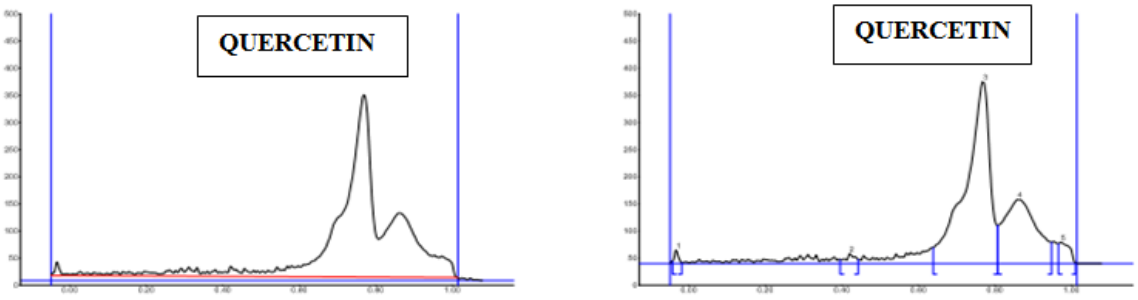


Figure. 9: Graph showing the Standard Quercetin peak value.

Table. 4. Illustartes the Rf value, Area, area % and assigned compound for the standard Querectin.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.04	2.8	-0.03	23.8	4.95	-0.02	4.1	185.7	0.94	Unknown
2	0.63	19.3	0.77	433.4	90.03	0.81	21.9	18957.8	96.38	Quercetin
3	0.85	23.1	0.86	24.2	5.02	0.90	6.7	526.8	2.68	Unknown

Track: 1 Crude Extract

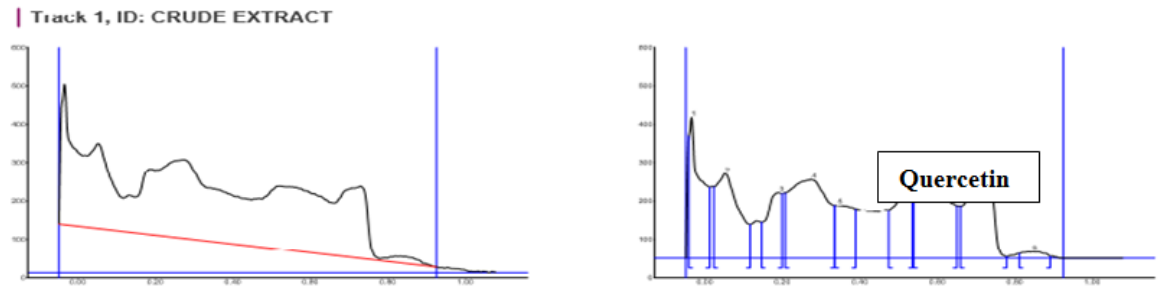


Figure. 10: Graph showing different peaks obtained for the crude bark extract of Rhizophora mucronata.

Table. 5. Illustrates the Rf value, Area, area % and assigned compound for the crude bark extract of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.04	317.5	-0.03	367.8	22.43	0.01	185.2	8017.3	11.66	unknown
2	0.02	186.3	0.05	221.0	13.48	0.12	87.8	9490.4	13.80	unknown
3	0.15	93.8	0.19	171.0	10.43	0.20	169.2	4757.3	6.92	unknown
4	0.21	170.6	0.28	205.2	12.52	0.34	138.3	14523.4	21.12	unknown
5	0.34	138.5	0.34	139.2	8.49	0.39	129.0	4618.4	6.72	unknown
6	0.48	126.0	0.53	164.3	10.02	0.54	164.2	5883.4	8.56	unknown
7	0.54	164.2	0.55	165.9	10.12	0.65	137.4	10949.0	15.92	unknown
8	0.66	136.9	0.73	187.6	11.44	0.78	4.5	9784.3	14.23	Expected Quercetin
9	0.81	12.3	0.88	17.6	1.08	0.89	5.0	733.0	1.07	unknown

TRACK-2 BE-1

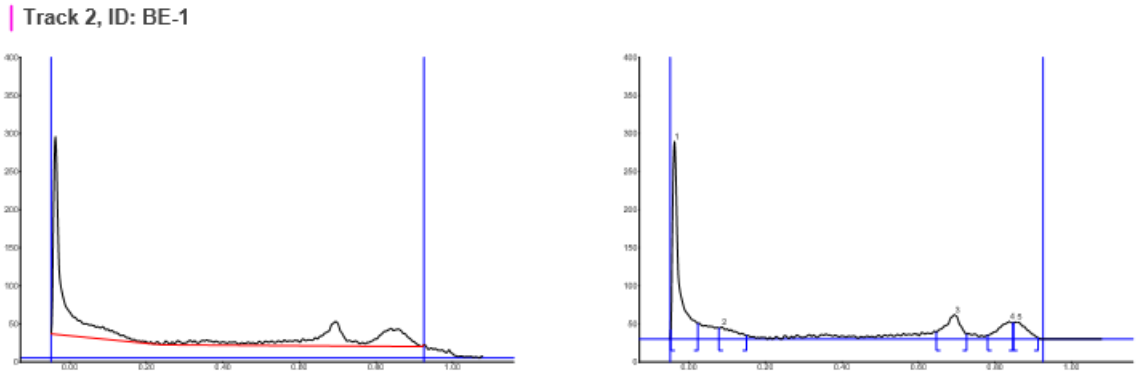


Figure. 11: Graph showing different peaks obtained for the fraction (BE-1) of Rhizophora mucronata.

Table: 6 Illustrates the Rf value, Area, area % and assigned compound for the fraction (BE-1) of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.05	12.4	-0.04	259.7	73.28	0.02	20.7	3224.2	55.26	unknown
2	0.08	14.5	0.09	16.1	4.55	0.15	4.6	482.4	8.27	unknown
3	0.65	9.1	0.70	32.0	9.02	0.73	6.5	943.3	16.17	unknown
4	0.78	4.9	0.84	23.6	6.66	0.05	20.9	627.5	10.75	unknown
5	0.85	21.4	0.86	23.0	6.49	0.91	1.8	557.7	9.56	unknown

Track-3 BE-2

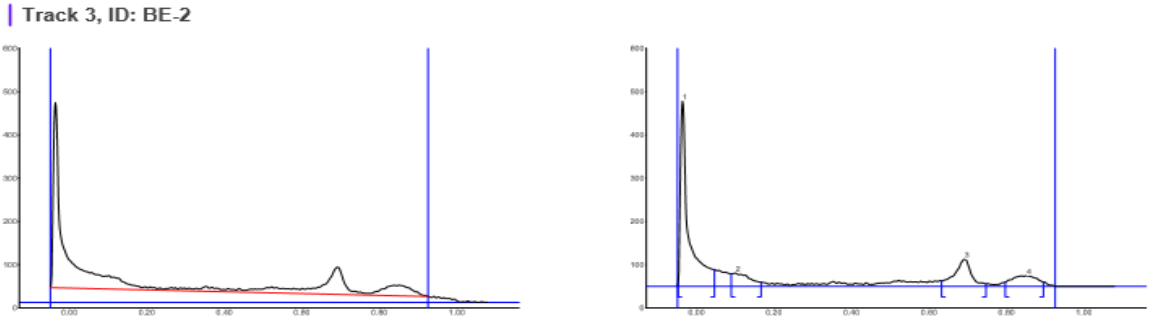


Figure- 12 Graph showing different peaks obtained for the fraction (BE-2) of Rhizophora mucronata.

Table. 7. Illustrates the Rf value, Area, area % and assigned compound for the fraction (BE-2) of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.05	8.0	-0.04	428.3	78.30	0.05	38.81	6514.8	61.00	unknown
2	0.09	29.2	0.10	31.0	5.67	0.17	9.7	1047.1	9.80	unknown
3	0.63	12.7	0.69	63.0	11.51	0.75	5.9	1941.3	18.18	unknown
4	0.80	9.4	0.85	24.7	4.52	0.90	4.9	1176.2	11.01	unknown

TRACK 4: BE-3

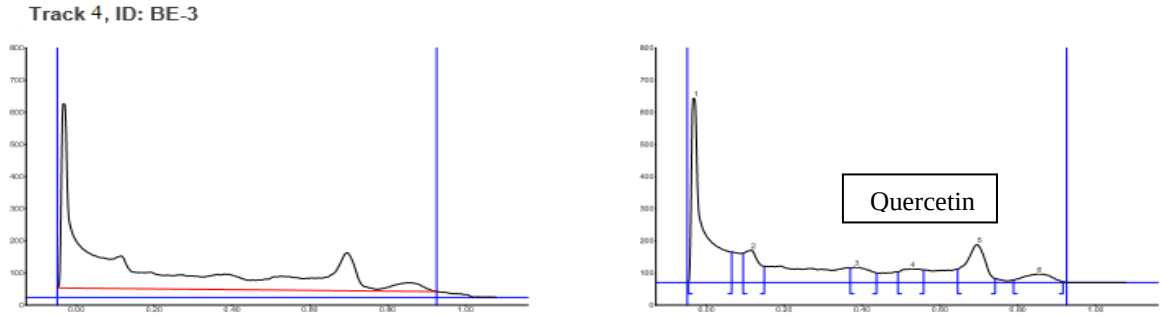


Figure. 13. Graph showing different peaks obtained for the fraction (BE-3) of Rhizophora mucronata.

Table: 8 Illustrates the Rf value, Area, area % and assigned compound for the fraction (BE-3) of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.05	6.1	0.03	573.7	62.99	0.07	94.9	12599.8	51.54	unknown
2	0.10	91.4	0.12	100.9	11.08	0.15	50.9	2815.6	11.52	unknown
3	0.37	45.2	0.38	48.0	5.27	0.44	29.5	1785.1	7.30	unknown
4	0.49	33.4	0.53	43.1	4.73	0.56	39.3	1702.2	6.96	unknown
5	0.65	41.7	0.70	118.4	13.00	0.74	12.4	4117.5	16.84	Expected Quercetin
6	0.79	8.2	0.85	26.7	2.93	0.92	1.9	1425.4	5.83	unknown

TRACK-5: BE-4

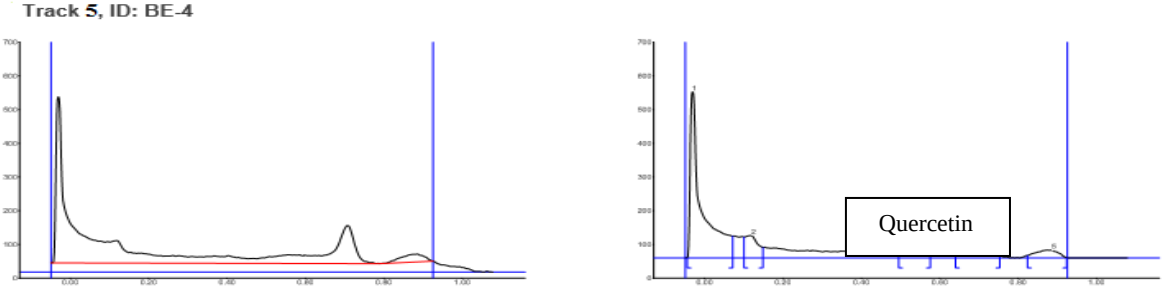


Figure. 14. Graph showing different peaks obtained for the fraction (BE-4) of Rhizophora mucronata.

Table. 9: Illustrates the Rf value, Area, area % and assigned compound for the fraction (BE-4) of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.04	0.4	-0.03	482.9	68.38	0.07	64.5	10158.4	58.03	unknown
2	0.10	61.9	0.12	66.5	9.22	0.15	30.9	1641.1	9.38	unknown
3	0.50	16.3	0.56	25.6	3.55	0.58	24.7	1134.0	6.48	unknown
4	0.64	22.4	0.71	112.8	15.65	0.75	6.1	3587.8	20.50	Expected Quercetin
5	0.83	7.2	0.89	23.1	3.20	0.93	0.5	982.8	5.61	unknown

TRACK: 6 BE-5

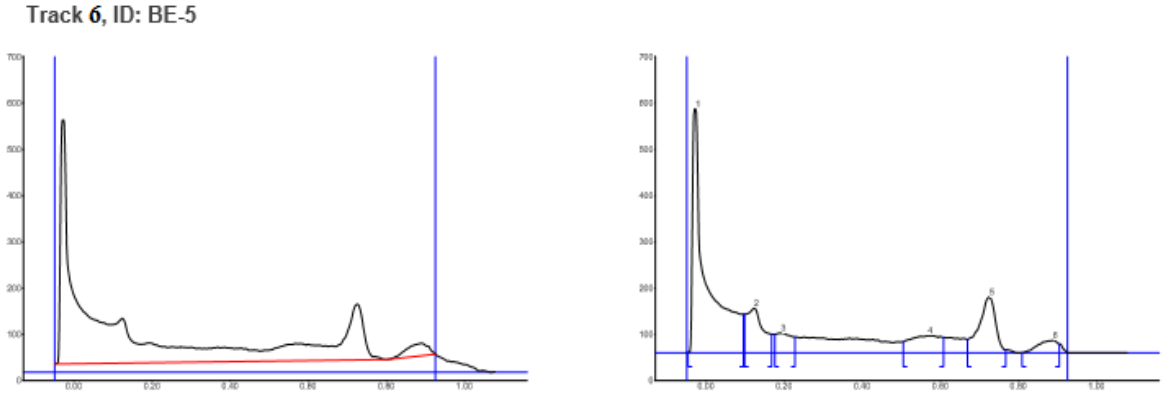


Figure. 15: Graph showing different peaks obtained for the fraction (BE-5) of Rhizophora mucronata.

Table. 10: Illustrates the Rf value, Area, area % and assigned compound for the fraction (BE-5) of Rhizophora mucronata.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Ht	Area	Area%	Assigned Compound
1	-0.05	1.3	-0.03	527.4	61.93	0.10	83.5	13220.5	54.19	unknown
2	0.10	83.6	0.12	96.6	11.34	0.17	40.5	3033.6	13.43	unknown
3	0.18	40.4	0.19	42.8	5.03	0.23	34.2	1299.5	5.33	unknown
4	0.51	24.1	0.57	37.2	4.37	0.61	33.2	2168.4	8.89	unknown
5	0.67	29.5	0.73	120.6	14.16	0.77	6.4	3691.0	15.13	unknown
6	0.89	0.5	0.89	27.0	3.17	0.91	19.4	985.4	4.04	unknown

4.0 DISCUSSION

Plants contain many bioactive compounds which are commonly known as phytochemicals or phytochemicals responsible for exhibiting various diverse ethno pharmacological activities hence they are also known as bioactive compounds.[19] There are many secondary metabolites found in plants which acts as a natural source of anti-oxidants and hence preferred over synthetic ones because of its low cost, easy availability and with no side effects on the usage.[20] The bioactive secondary metabolites have been shown to reduce the risk and progression of diseases such as cancer, cardiovascular, neurodegenerative diseases, etc.[21]

The initial phytochemical screening of plant extracts qualitatively is an important tool for the pharmacological and pathological discovery of novel drugs, thus proving the essential information with regards to the chemical compounds present in the plant. The phytochemicals present in the bark extract viz, alkaloids, flavonoids, terpenoids, carbohydrates, etc showed that the plant contained potent bioactive compounds of medicinal value. This obtained result confirms to the findings of.[22] All metabolites have been shown to be responsible for therapeutic activity of plants.

In recent times, there has been a great interest in exploring different plant parts to obtain anti oxidants to reduce the oxidative stress induced tissue injury. Amongst the numerous anti oxidants that occur naturally, phenolic compounds, alkaloids, flavonoids and terpenoids are found to be most effective in neutralizing the ill effects of free radicals generated within our body.

There are various techniques involved to study the mechanism of free radical formation and the scavenging of the same. DPPH is the most commonly used assay for the anti oxidant evaluation of many plant extracts because of its stability and simplicity.[24] In the present study, ethanol: water (7:3) showed % inhibition of 95 as maximum value indicating the plant is highly active and potentially potent with different bioactive compounds.

The other extracts viz, Methanol, Ethyl acetate and Iso-propanol showed 86.17%, 81.36% and 72.45% respectively and Diethyl ether and Acetone showed less 43.87% and 55.29% respectively.

H_2O_2 although not a radical species to play a role to contribute for the oxidative stress. But the generation of H_2O_2 in lower concentrations, inside our body is an important parameter to be considered, as lower amounts it reacts with the naturally occurring iron complexes to generate highly reactive Hydroxyl radicals which leads to the manifestations of various toxic diseases. Thus, it needs to be expelled out, or quenched away from our body for proper functioning. In the present study the H2O2 activity was found least in the Diethyl ether extract 33.03% and maximum in the ethanol: water (7:3) with 77.44%.

This study is further extended to HPTLC profiling where in the targeted phytochemical was flavonoids. Flavonoids are the polyphenolic bioactive compounds with variant phenolic structures; they are mostly composed of flavanonols, flavanols, flavonone and flavones that makes up a huge group of bioactive compounds in plants with the huge therapeutic value. They are usually found in barks, stems, roots, fruits, vegetables, flowers and twigs.[23] They have significant biological activities viz, anti-inflammatory[24], anti-cancer[25], anti-oxidant[26] and anti microbial activity.[27] Thus in the present study flavonoids preferably Querectin was targeted.

According to HPTLC fingerprinting, the crude bark extract of R.mucronata showed the presence of Flavonoid, Querectin at Rf value as 0.66 and the area as 14.23% with respect to the standard Rf at 0.63 with the area % as 96.38. Apart from the crude, the fractions BE-3 and BE-4 also consisted Querectin with Rf value as 0.65 and 0.64 respectively. Thus, the HPTLC fingerprinting clearly indicated the presence of potent bioactive in the crude extract as well as in the fractions BE-3 and BE-4 respectively. Similar study has been undertaken in order to check the anti-diarrhoeal and anti-inflammatory activities of various compounds isolated from R.mucronata bark[28, 29] wherein they identified Querectin as one of the bioactive compound which co relates with the present study.

CONCLUSION

The phytochemical analysis revealed the presence of wide range of bioactives, ranging from, steroids, fatty acids, carbohydrates, flavonoids, tannins, alkaloids which is the clear indication of diverse components in the bark extract of *R.mucronata* with high therapeutic and pharmacological value. The anti oxidant activity of the extracts and free radical scavenging assay provides a clarity that the bark of R.mucronata possess potent bioactive compounds with huge therapeutic activity. On addition, the HPTLC fingerprinting of the crude and the fractions also supports the above findings clearly signifies the presence of flavonoid, Querectin and other bioactives. Thus we can conclude that the extract is the potent source for various phytochemicals with a wide range of bioactive compounds. The other phytochemicals obtained in the extract can also be explored for the respective therapeutic activity in the future.

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