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In vitro Antioxidant, Free Radical Scavenging Activity and Chemometric Approach to Reveal Variability in Different Solvent Extracts From Selected Mangroves of Andaman and Nicobar Islands, India

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ABSTRACT

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Presence of various secondary metabolites is helpful for mangroves to tolerate biotic and abiotic stressors. In the recent years, the quest for more natural possibilities has led researchers to focus on secondary metabolites produced by plants such as alkaloids, phenolic compounds, terpenoids, flavonoids, glycosides, saponins, tannins, etc. which are reported to possess anti-oxidative, anti-microbial, anti-viral and anti-cancer properties. The present study was aimed to determine the antioxidant activity of mangrove leaf extracts obtained from two species, *Rhizophora apiculata* and *R. mucronata*. Four different solvent extracts, methanol, acetone, chloroform and aqueous of two different mangrove species, *R. apiculata* and *R. mucronata* were evaluated for their total phenol, flavonoid and antioxidant content such as total antioxidant activity (TAA), DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity, ABTS (2, 2-azinobiz-3-ethylbenthiazoline-6-sulfonic acid) radical cation scavenging activity and reducing power (RP). Results revealed that the methanolic extract of *R. apiculata* exhibited the maximum phenol (168.1±0.96 mg GAE/g) and flavonoid content (117.92±0.51 mg RE/g). Higher total antioxidant activity (103.22±4.17 mg ascorbic acid/g), DPPH (90.16±0.44%), ABTS (99.45±0.14%), radical scavenging activity and reducing power (36.51±0.33 mg/g) were observed in the methanolic extract of *R. apiculata*. Overall, the methanolic extract of *R. apiculata* showed better results among the two species, revealing higher antioxidant activity of *R. apiculate*, and also unravelling the strength of methanol in extracting the active compound.

ADDITIONAL INDEX WORDS: *Mangroves, antioxidant activity, total phenol, flavonoids, solvents.*

INTRODUCTION

Replacement of synthetic antioxidants with antioxidants of natural origin by the food industries (Hudson, 1990) has contributed significantly to the maintenance of good health (Halliwell and Gutteridge, 1989). Substantial evidence indicates that food containing antioxidants and in particular, the antioxidant nutrients may be of major importance in disease prevention by quenching free radicals generated in the body due to oxidative condition or other metabolic related disorders (Alam, Bristi, and Rafiquzzaman, 2013). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. These health effects have been attributed in part to the presence of phenolic compounds in plants, which may exert their effects as a result of their antioxidant properties. The antioxidant activities of phenolics are mainly due to the redox properties that allow them to act as a reducing agent, hydrogen donor and singlet oxygen quencher (Rice-Evans, Miller, and Paganga, 1996).

Though some are essential for regulating the cell growth, synthesising biologically important compounds and for phagocytosis, reactive oxygen species (ROS) has well established damaging effects on crucial biomolecules such as lipids, proteins, DNA, etc., which may cause cancer, atherosclerosis, hair loss, rheumatoid arthritis, haemorrhagic shocks, cardiovascular disorders, metabolic disorders, gastrointestinal disorders, as well as neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease and even Acquired immunodeficiency syndrome (AIDS) (Ahmed *et al*., 2006; Nishanthini, Ruba, and Mohan, 2012). Antioxidants are effective scavengers of free radicals such as peroxides, hydroperoxides and lipid peroxides. Various synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have become popular in the pharmaceutical industries, but uses of these synthetic antioxidants are generally accompanied by various side effects (Mohammad *et al.*, 2014). In recent years, search for more natural alternatives has led researchers to the secondary metabolites produced by plants such as alkaloids, terpenoids,

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flavonoids, glycosides, phenolic compounds, saponins, tannins, *etc*. which are reported to possess anti-oxidative, anti-microbial, anti-viral and anti-cancer, properties (Mohammad *et al.*, 2014).

The presence of various secondary metabolites in mangroves helps tolerate both biotic and abiotic stressors. Toleration to high salinity and moisture ranges, inundation of water and temperature variations by mangroves are mainly a result of these secondary metabolites which can mitigate biotic (oxidative potential) and abiotic stressors.

Recent studies have proven the activity of mangrove extracts against the various plant, animal and human pathogens, which are attributed to their biochemical complexities and bequeathed with a polyphenolic compound that protects them from the oxidative damage (Asha, Mathew, and Lakshmanan, 2012). Mangroves have been used as folklore medicine since time immemorial for treating diarrhoea, nose bleeds, dyspepsia, snake bite, etc. They also serve as a fish poison and feeding repellent as they contain considerable amounts of rotenoids, alkaloids, terpenoids which produces natural toxins (Bandaranayake, 2002). Around 55 species of mangroves belonging to 22 genera are found along the Indian ocean region (Kathiresan and Rajendran, 2005) of which about 25 species have been recorded from the Andaman sea (Zoological Survey of India, 2011). In recent years, there has been rise in the use of novel approaches for the study of free radicals and antioxidants in tropical mangroves for improving human health.

Rhizophoraceae is the most dominant family of mangroves found in the Andaman waters, of which *R. apiculata* and *R. mucronata* are the most commonly encountered species. Both these species have long reported history of use as folklore medicine in which, *R. mucronata* has been used as an astringent for the treatment of angina and haemorrhage (Perry, 1980). Premanathan *et al.* (1999) reported anti-HIV activity in the leaf extracts of *R. apiculate*, while its anti-bacterial activity against *Vibrio* sp. affecting lobster larvae was observed in the chloroform extract of *R. mucronata* leaves (Baskaran and Mohan, 2012). In this background, the present study was designed to determine the antioxidant activity of mangrove leaf extracts obtained from *R. apiculata* and *R. mucronata* using acetone, chloroform, aqueous and methanol.

METHODS

This section describes the methods adopted for crude extraction of mangrove leaves and screening of antioxidant and free radical activity.

Collection and Preparation of Sample

Leaf samples of *R. apiculata* and *R. mucronata* were collected from Chidiyatapu along the South Andaman coast (11°30'28.22"N; 92°41'58.89"E). Collected samples were cut into small pieces and shade dried for 3-4 days. The dried material was pulverized using a pestle and mortar and sieved to get particles of uniform size. One gram each of the ground samples was extracted using 50 ml of four different solvents, acetone, chloroform, methanol and double distilled water for 48 hours at room temperature. After 48 hours, the samples were centrifuged at 4500 g for 10 minutes and the supernatant was filtered in fresh tube using Whatman No. 1 filter paper and stored at 4ºC for further analysis.

Estimation of Total Phenol Content

The total phenol content of the sample was estimated by Folin-Ciocalteau (FC) assay (Singleton and Rossi, 1985) with minor modifications. 200 µL of extracted sample was mixed with 200 µL FC reagent (0.5 N) and 1.6 mL of 7.5% Na_2CO_3 and incubated in dark at room temperature for 2 hours. The absorbance was measured at 765 nm using UV-visible spectrophotometer. Gallic acid was used as standard and the phenolic contents were expressed as Gallic Acid Equivalents (mg GAE g^{-1}) of the leaf extract.

Estimation of Flavonoid Content

The spectrophotometric method of Zishen, Mengcheng, and Jianming (1999) with slight modification was used to determine the flavonoid content of the leaf extracts. After an interval of 5 minutes, 0.5 mL of sample was mixed with 0.3 mL 15% sodium nitrite, 0.6 mL 10% ammonium chloride hexahydrate and 3 mL of 1N sodium hydroxide. Absorbance was immediately measured at 510 nm. Flavonoid content was expressed as Rutin equivalents of the extract (mg RE g^{-1}).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The free radical scavenging effect of the leaf extracts was determined by the method of Yen and Chen (1995) with minor modifications. 200 µl aliquot of the extract was mixed with 100 µl of 0.16 mM DPPH methanolic solution. The mixture was incubated for 30 minutes in dark after which absorbance were recorded at 517 nm using a spectrophotometer. The half-maximal inhibitory concentration (IC_{50}) was calculated by the linear regression analysis and expressed as mean of determinations. Ascorbic acid was used as control.

$$\text{\% inhibition of DPPH radical (scavenging activity)} = (A_{control} - A_{sample} / A_{control}) \times 100 \quad (1)$$

ABTS (2, 2-azinobiz-3-ethylbenthiazoline-6-sulfonic acid) Radical Cation Scavenging Activity

ABTS radical cation scavenging activity of the extracts was determined following the method of Re *et al.* (1999) with minor modification. Trolox was used as standard. ABTS was prepared 24 hours prior to use, by mixing 20 mM ABTS solution with 70 mM potassium peroxodisulphate. 100 µL aliquot of the extracts was made up to 10 mL with distilled water and mixed with 3.9 mL of ABTS reagent and absorbance of these solutions was measured at 734 nm after 5 minutes.

$$\text{\% inhibition of ABTS radical} = (A_{control} - A_{sample} / A_{control}) \times 100 \quad (2)$$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the extract/standard.

Total Antioxidant Activity

Total antioxidant activity of the extracts was determined by the method described by Prieto *et al.* (1999) with slight modification. The 0.2 mg mL^{-1} of samples in different aliquots

was mixed with 1 mL each of the three reagent solutions (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated in water bath for 90 minutes at 95°C, after which the absorbance was measured at 695 nm. Total antioxidant activity was expressed as mg of ascorbic acid per gram of extract.

Reducing Power

The method described by Oyaizu (1986) with minor modification was used to determine the reducing power of the extracts. Different concentration of sample was mixed with 2.5 mL of phosphate buffer (0.2 M, 6.6 pH) and 2.5 mL of potassium ferric cyanide (1%) and incubated at 50°C for 30 minutes.

After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 minutes. Then 2.5 mL solution was taken from the upper layer and mixed with 2.5 mL of distilled water and 0.5 mL ferric chloride (0.1%) immediately after which the absorbance was measured at 700 nm. BHT methanolic solution was used as standard.

Statistical Analysis

Data were expressed as mean±standard error in three replicates. All the statistical analysis was carried out using SPSS 16.0. One-way analysis (ANOVA) and the Duncan's multiple range tests were applied to the result to determine any difference among the means and p values <0.05 regarded to be significant. The correlation and factor analysis was performed using SAS (Version 9.3, SAS Institute Inc., Carry, NC).

RESULTS

Oxidative damage is being induced as a result of the harsh climatic conditions of tropical Islands such as Andaman and Nicobar, where mangroves are able to sustain through secretion of various secondary metabolites, mainly phenols and flavonoids. The scavenging activity of rice is attributed to the presence of phenolic hydroxyls, particularly in the 3′OH and 4′OH of the three-carbon chain (Cho *et al.*, 2013). The present study revealed definite deviations in the flavonoid, total phenolic content, DPPH and ABTS radical scavenging activity, reducing power and total antioxidant activity among the two species and within the solvent system.

Total Phenol and Flavonoid Content

The total phenol and flavonoid contents recorded from the mangroves extracted using different solvents are given in Table 1. The phenolic content was found to be highest in the methanol extract (168.1±0.96 mg GAE/g) of *R. apiculata* followed by the aqueous extract (123.26±1.06 mg GAE/g) of the same species. It was observed that the acetone and chloroform extracts of both the species were significantly different (p <0.05) from the methanol and aqueous extracts of the species.

The maximum flavonoid content was observed in methanolic extract (117.92±0.51 mg RE/g) of *R. apiculata* followed by the methanol extract (94.58±0.79 mg RE/g) of *R. mucronata*. Comparatively, *R. apiculata* showed higher flavonoid content than *R. mucronata*, and significant difference (p < 0.05) among the various solvent extracts was also observed in *R. apiculata*.

DPPH and ABTS Radical Scavenging Activity

Both *R. apiculata* and *R. mucronata* revealed similar trends in the DPPH and ABTS radical scavenging activity (Table 2). Highest radical scavenging activity was observed in the methanolic extract of *R. apiculata* for both DPPH as 90.16±0.44 % (Figure 1a) and ABTS as 99.45±0.14 % (Figure 2a) with the increase in concentration from 2-20 mg/ml DPPH and ABTS radical scavenging activity of extracts of both the species was found in the order of methanol > water > acetone > chloroform. Chloroform extract of both the species showed the least activity in both the analysis, wherein *R. apiculata* displayed the maximum activity. The DPPH activity of chloroform extract of *R. apiculata* was 62.65±1.19% and the ABTS activity was 38.04±2.33%. The highest DPPH and ABTS scavenging activity in *R. mucronata* was observed in the methanolic extract (83.84±1.41) (Figure 1b) and 85.10±0.94% (Figure 2b) respectively, and a significant difference of p <0.05 was observed among all the solvent extracts of *R. mucronata* in both the analysis.

Total Antioxidant Activity

Extracts of *R. apiculata* showed higher antioxidant activity compared to *R. mucronata*, among which the methanolic extract of *R. apiculata* had the maximum activity of 103.22±4.17 mg ascorbic acid/g. A significant difference (p <0.05) was observed among the methanolic, aqueous, acetone and chloroform extracts of both the species. Acetone extract of *R. mucronata* showed the least total antioxidant activity of 38.75±1.12 mg ascorbic acid/g (Table 1).

Reducing Power

The reducing ability of different solvent extracts of *R. apiculata* and *R. mucronata* is shown in Table 2. Dose dependent increase in the absorbance was observed, wherein higher absorbance indicated higher reducing ability. Among the solvents tested, aqueous extract of *R. apiculata* (36.51±0.33 mg/g) and methanolic extract of *R. mucronata* (18.41±0.17 mg/g) revealed higher reducing values, in which *R. apiculata* demonstrated higher activity than *R. mucronata*.

DISCUSSION

Secondary metabolites are produced by the plants in order to protect them against the oxidative damage caused by the free radicals produced in response to environmental stress. Mangroves are plants growing in vicious environmental conditions facing harsh climatic conditions, yet they prove to be thriving without any damage which is attributed to the high levels of polyphenols produced by them.

Polyphenols are the non-enzymatic antioxidants that protect the plants from the oxidative damages caused by ROS (Sung-Suk *et al.*, 2014). These polyphenolic compounds include flavonoids, phenolic acids, tannins, *etc*. The antioxidant properties expressed by the polyphenolic compounds is accredited to their redox property which helps them to function as reducing agents, hydrogen donors and singlet oxygen quenchers (Nishanthini, Ruba, and Mohan, 2013). The antioxidant analysis of both the mangrove extracts tested in the present study revealed promising results.

Table 1. *Total phenol, flavonoid content and total antioxidant activity in different solvent extracts of R.* apiculata *and* R. mucronata.

Solvents	Parameters					
	Flavonoid		Phenol		TAA	
	R. apiculata	*R. mucronata*	*R. apiculata*	*R. mucronata*	*R. apiculata*	*R. mucronata*
Acetone Extract (AE)	45±0.66[b]	22.8±0.75[b]	22.61±0.15[c]	9.17±0.44[c]	58.82±3.18[b]	38.75±1.12[b]
Chloroform Extract (CE)	22.25±0.87[d]	6.92±1.3[d]	5.31±0.87[b]	3.18±0.28[b]	52.87±2.68[b]	40.16±4.88[b]
Methanol Extract (ME)	117.92±0.51[a]	94.58±0.79[a]	168.1±0.96[a]	113.22±0.73[a]	103.22±4.17[a]	53.2±1.61[a]
Water Extract (WE)	69.5±0.22[c]	41.4±0.44[c]	123.26±1.54[a]	74.17±1.02[a]	101.72±3.57[a]	52.33±4.61[a]

Table 2. *DPPH, ABTS radical scavenging activity and reducing power in different solvent extracts of* R. apiculata *and* R. mucronata.

Solvents	Parameters					
	DPPH (%)		ABTS (%)		RP	
	R. apiculata	*R. mucronata*	*R. apiculata*	*R. mucronata*	*R. apiculata*	*R. mucronata*
Acetone Extract (AE)	64.80±0.67[b]	63.39±0.64[c]	53.63±3.3[c]	40.01±0.59[c]	6.03±0.15[c]	2.32±0.066[c]
Chloroform Extract (CE)	62.65±1.19[b]	51.39±0.42[d]	38.04±2.33[d]	18.90±4.26[d]	3.41±0.058[d]	1.66±0.069[d]
Methanol Extract (ME)	90.16±0.44[a]	83.84±1.41[a]	99.45±0.14[a]	85.10±0.94[a]	36.51±1.23[b]	18.41±0.17[a]
Water Extract (WE)	90.03±2.21[a]	71.97±0.59[b]	70.02±0.52[b]	75.75±3.33[b]	23.41±0.33[a]	16.3±0.55[b]

***Each value is expressed as the mean± SE (n=3). Means with different letters are significantly different at p <0.05.*

Table 3. *IC_{50} values of different solvent extracts of* R. apiculata *and* R. mucronata.

Solvents	Parameter (IC_{50})			
	DPPH		ABTS	
	R. apiculata	*R. mucronata*	*R. apiculata*	*R. mucronata*
Acetone Extract (AE)	6.39±1.42[b]	9.86±0.65[c]	4.57±0.53[c]	11.96±0.14[c]
Chloroform Extract (CE)	9.08±0.40[a]	12.53±0.71[b]	7.98±1.36[c]	11.62±0.74[c]
Methanol Extract (ME)	11.02±0.08[a]	12.47±0.39[b]	17.31±4.31[b]	32.45±1[b]
Water Extract (WE)	10.74±0.58[a]	19.18±1.08[a]	29.63±2.6[a]	48.59±0.74[a]

***Values is expressed as the mean± SE (n=3), Means with different letters are significantly different at p <0.05*

In the present study, the level of total phenol was found in the range of 5.31±0.87 to 168.1±0.96 mg GAE/g for *R. apiculata* and 3.18±0.28 to 113.22±0.73 mg GAE/g for *R. mucronata*, which is very high compared to a previous study (Sung-Suk *et al.*, 2014) where the phenolic content in the leaf extracts of *Rhizophora stylosa*, was found to be 9.32±1.10 and 9.74±0.75 mg GAE/g in the aqueous and methanolic extracts. The phenol content observed in the present study was 168.1±0.96 and 113.22±0.73 mg GAE/g in the methanol extract, and 123.26±1.06 and 74.17±1.02 mg GAE/g in the aqueous extract of *R. apiculata* and *R. mucronata*. This difference in the concentration may be attributed to the difference in species and their distribution. Previous records on the total phenol content of *R. mucronata* species from the Sundarban, India (Banerjee *et al.*, 2008) revealed significantly lower content in the leaf and root extract, while the concentration in the stem bark was almost similar to the concentration in the methanol and aqueous leaf extract of *R. mucronata* observed in the present study. This difference in the total phenol content may be due to the efficiency of the solvent used for extraction, different parts of the plant used for analysis and possibly due to the differences in their geographic locations. Nishanthini, Ruba, and Mohan (2013) analysed two mangrove herbs (*Anthrocnemum fruticosum* and *Suaeda monoica*) and reported the flavonoid contents as 0.36 and 0.86 g/100g respectively, which is lower than the concentration observed in this present study for *R. apiculata* and *R. mucronata*. This difference in the concentration may be due to the fact that the former two species are mangrove herbs whereas the latter two are mangrove trees. Reductones associated with the reducing activity of any plant extract, displays antioxidant action when it donates hydrogen atoms to break the free radical chains (Nishanthini, Ruba, and Mohan, 2012). The antioxidant property of an extract can be correlated to the transformation or reduction of Fe^{3+} ion which serves as an indicator of electron donating activity; an important mechanism of phenolic antioxidant action. The result obtained in the present study indicated a dose-dependent increase in the absorption, which supports the reports of the previous studies (Nishanthini, Ruba, and Mohan, 2012, 2013; Reddy and Grace, 2016). All the four solvent extracts of *R. mucronata* showcased significantly higher reducing ability than the aqueous methanol extract of the same species reported from Sundarbans, India (Banerjee *et al.*, 2008).

Both DPPH and ABTS activity was highest in methanol extracts of both species, similar to the results obtained for DPPH activity by Sung-Suk *et al.* (2014) in *Rhizophora mangle* and *Sonneretia alba* where methanol extracts of the species showed higher activity than the aqueous extracts, but the scavenging activity observed in the species under analysis in the present study were higher than what was recorded in *R. mangle* and *S. alba*. The DPPH activity reported by Reddy and Grace (2016) in the methanolic leaf extract of *Aegiceras corniculatum*, *Excoecaria agallocha* and *Lumnitzera racemosa* were found to be lower than the activity observed in both the species analysed in this present study.

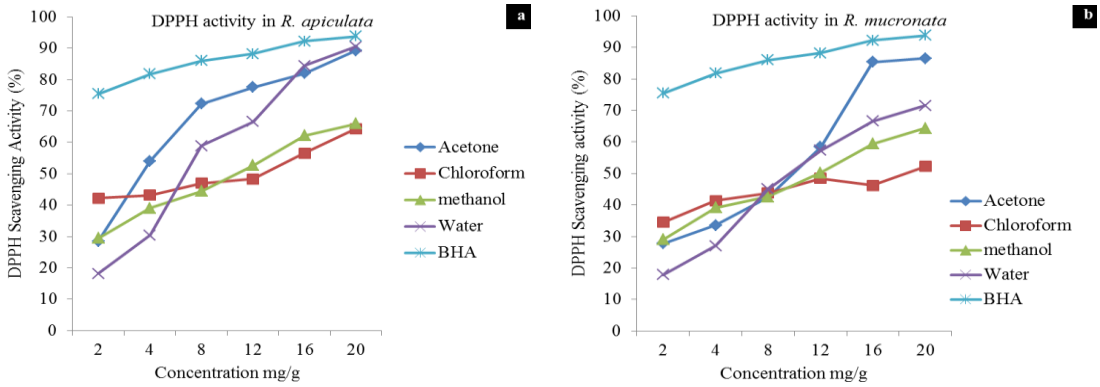

Figure 1. DPPH radical scavenging activity in different solvent extract of (a) *R. apiculata* and (b) *R. mucronata.*

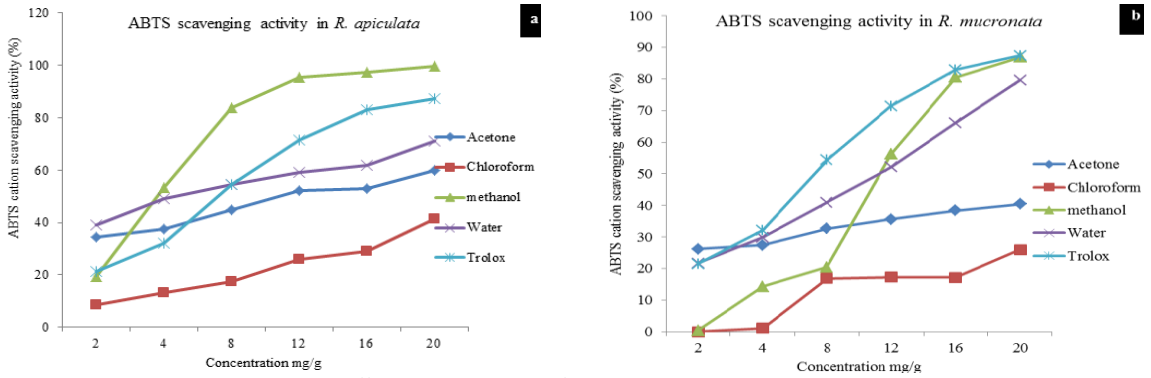

Figure 2. ABTS Cation Scavenging activity in different solvent extract of (a) *R. apiculata* and (b) *R. mucronata.*

The methanolic extract of Sesuvium protulacastrum leaf (Paulpriya et al., 2013), A. fruticosum stem and *S. monoica* leaf (Nishanthini, Ruba, and Mohan, 2013) revealed higher DPPH IC_{50} value than *R. apiculata* and *R. mucronata*, which revealed the higher DPPH radical scavenging activity of the studied species. The ABTS IC_{50} value for the methanol extract of *R. apiculata* and *R. mucronata* was recorded as 17.31±4.31 mg/ml and 32.45±1 respectively (Table 3). The values recorded by Paulpriya *et al.* (2013) in *S. protulacastrum* leaf (22.89) and Nishanthini, Ruba, and Mohan (2013) in *A. fruticosum* stem (28.63) and *S. monoica* leaf (22.62) was higher than *R. apiculata* but lower than *R. mucronata*, illustrating that *R. apiculata* has higher and *R. mucronata* lower ABTS radical cation scavenging activity.

Correlation and Factor Analysis

Phytochemical and radical scavenging data from two different mangrove species were submitted to factor and correlation analysis to identify association between variables and their ability to discriminate parameters among them. The correlation coefficient among phenolics, flavonoid, TAA, RP and radical scavenging capacity shown in Table 4 revealed that phenolic and flavonoids content had a strong positive correlation with the flavonoid content and antioxidant capacity (r^2>0.9; p <0.01-0.05), which was in agreement with most previous studies (Luo *et al.*, 2010 and Zakaria *et al.*, 2011) for marine algae. It is interesting to note that though flavonoids are the subclass of phenolic groups, it did not show significant correlation between TAA and DPPH, possibly due to the type of flavonoid compounds present in two mangroves which may not have free –OH groups for hydrogen transfer or electron delocalization (Rice-Evans, Miller, and Paganga, 1996). To understand how much of the variance in the variables has been accounted for by the variables is based on communalities values. From Table 5, it can be seen that over 99.7 and 98.9% (maximum) of variability is accounted for phenolic content value while minimum of 92.6% (DPPH) and 88.0% (flavonoids) variability were accounted for *R. apiculata* and *R. mucronata*. Of the six variables used, only one factor was selected, according to the criteria of an eigen value of >1.0. and two factors accounted for 95.97% of total variance (Table 4). After computation of a varimax rotation, it was observed that factor 1 and 2, with an eigen value of 5.72 (5.64) and 0.262 (0.247), represents 95.43 (94.03) % and 4.36 (4.49) % of overall variance in two species. From the screen plot (Figure 3a & 3b), the curve flattened after two components. Hence, up to two principal components were chosen to explain variability in six investigated phytochemicals.

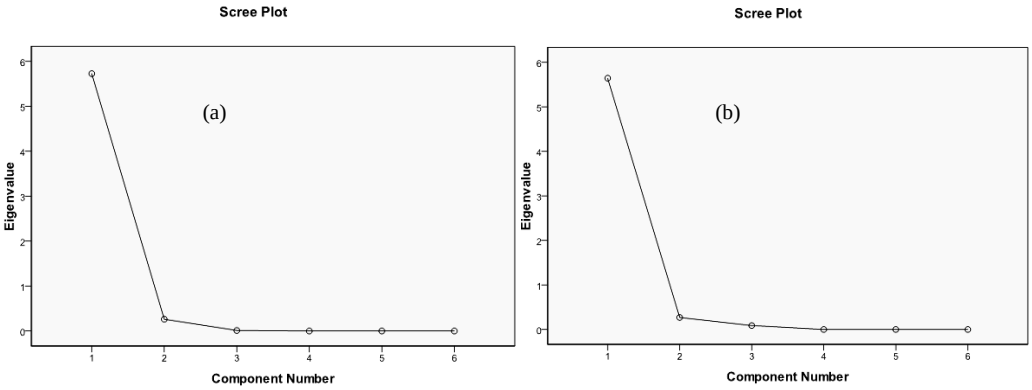

Figure 3. Scree plot (a) *R. apiculata* and (b) *R. mucronata.*

Table 4. *Pearson's correlation coefficient between phytochemicals of* R. apiculata *and* R. mucronata.

	Phenol	Flavonoids	TAA	RP	DPPH (%)	ABTS (%)
Phenol	1.00					
Flavonoids	0.95**	1.00				
TAA	0.97**	0.87	1.00			
RP	0.99***	0.97**	0.94**	1.00		
DPPH (%)	0.97**	0.85	0.99***	0.94**	1.00	
ABTS (%)	0.95**	0.99***	0.88	0.97**	0.86	1.00

***, ***significant at 5% and 1%, respectively.*

Table 5. *Communalities for* R. apiculata *and* R. mucronata

Variables	Initial	Extraction[a]	Extraction[b]
Phenol	1.000	0.997	0.989
Flavonoids	1.000	0.932	0.880
TAA	1.000	0.94	0.912
RP	1.000	0.989	0.962
DPPH (%)	1.000	0.926	0.935
ABTS (%)	1.000	0.941	0.964

Extraction method: Principal Component Analysis. a: R. apiculata; *b:* R. mucronata.

CONCLUSION

Reactive oxygen species (ROS), the radical derivatives of oxygen are the most important free radical in biological systems. The ROS are the harmful by-products generated during the normal cellular functions. Increasing intake of natural antioxidants may help to maintain a tolerable antioxidant status, perhaps the normal physiological functioning. The present study indicated that both *R. apiculata* and *R. mucronata* possess varying levels of antioxidant activity. The levels of total phenol and flavonoids were found to be high in both the species, which are generally the major contributors of antioxidant properties. The study also revealed promising DPPH and ABTS scavenging activities. The various antioxidants used in the study showed varying level of efficiency in extracting the active compounds among which methanol was found to be most promising. Further studies have to be carried out for the isolation of active compounds responsible for antioxidant properties.

Conflict of Interest

The authors declare that there is no conflict of interest.

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