Qualitative and quantitative phytochemical analysis and **in vitro** antioxidant activities of methanolic leaf extract of *Rhizophora apiculata* blume

M. Muthulingam¹*, K. Krishna Chaithanya²

**ABSTRACT**

**Introduction:** An oxidative stress results from the imbalance between the free radicals and the antioxidant system, and up regulated reactive oxygen and nitrogen species have been associated with oxidative stress diseases. *Rhizophora apiculata* Blume is an important medicinal plant long time used by many people in Asia and Africa continents for curing both the infectious and non-infectious diseases. **Objective:** The objective of the present study was to find the qualitative and quantitative phytochemical and evaluate the **in vitro** antioxidant activity of the methanolic leaf extract of *R. apiculata* Blume. **Materials and Methods:** The qualitative phytochemical analysis, quantitative estimations of total phenolic, alkaloid, flavonoid, tannin, and saponin content, and **in vitro** antioxidant activities (1,1-diphenyl-2-picrylhydrazyl [DPPH], hydrogen peroxide, superoxide, and hydroxy radical) of the methanolic leaf extract of *R. apiculata* Blume were performed using standard procedures. **Results:** The quantitative phytochemical analysis of the methanolic leaf extract of *R. apiculata* Blume revealed the presence of considerable amounts of tannins (95.14 µg/ml tannic acid equivalents/g), flavonoids (87.18 µg/ml quercetin equivalents/g), phenolic compounds (54.56 µg/ml gallic acid equivalents/g), saponins (4.17 µg/ml), and alkaloids (3.78 µg/ml), and further, the **in vitro** antioxidant activity of the ethanolic leaf extract of *R. apiculata* Blume showed significant scavenging activity on increasing order of H₂O₂ > DPPH > OH > O₂ compared to that of standard ascorbic acid. **Conclusion:** Based on the present results, the methanolic leaf extract of *R. apiculata* Blume contained high amount of flavonoid, tannin, and phenolic compounds, thereby existing significant **in vitro** antioxidant activity, and the ethanolic leaf extract of *R. apiculata* Blume can be used as a potential source of desired bioactive natural antioxidants for the development of therapeutic antioxidant drugs.

**KEY WORDS:** Antioxidants, Free radicals, Medicinal plant, Phytochemicals, *Rhizophora apiculata* Blume

**INTRODUCTION**

The high amounts of free radicals and other reactive species produced during aerobic respiration in the body can cause oxidative injury of essential macromolecules including amino acids, lipids, proteins, and DNA. It has been established that oxidative stress induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) is the major causative factors in the development of many chronic and degenerative diseases including atherosclerosis, heart disease, aging, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases, and others.¹² The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants are the substances capable of stabilizing or deactivating free radicals before they are damaging to the cell.³ Both exogenous and endogenous antioxidants act as an effective free radical scavenger by preventing and repairing damages caused by ROS and RNS and promoting their free radical decomposition and suppressing oxidative stress disorders.⁴ Several synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone are commercially available for the treatment of oxidative stress-related diseases, but the long-term use concerns due to their side effects such as kidney damage and mutagenesis.⁵ Therefore, there is a great tendency toward natural antioxidant sources such as plant derivatives. Several Indian medicinal plants were used for curing oxidative

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stress-related disease; therefore, there is a great tendency toward using natural antioxidant sources such as plant derivatives. The plant-derived secondary metabolites mainly the phenolic and flavonoids can minimize the generation of ROS and alleviate the chronic diseases caused by oxidative stress.

*Rhizophora apiculata* Blume which belongs to family Rhizophoraceae is an important medicinal plant used in traditional by many people in Asia and Africa. *R. apiculata* Blume has been used as traditional medicine for years with several biological activities such as antioxidant, antibacterial, antifungal, and antiviral. The objective of the present study was to evaluate the qualitative and quantitative phytochemical analysis and to find the *in vitro* antioxidant potential of the ethanolic leaf extract of *R. apiculata* Blume using standard *in vitro* antioxidant methods.

**MATERIALS AND METHODS**

**Collection of Plant Material**
The fresh leaves of *R. apiculata* Blume were collected from Pitchavaram, Chidambaram, Tamil Nadu, India, during March 2015. The plant was authenticated by Dr. Subramanian, Professor, Department of Botany, Annamalai University, Tamil Nadu, India.

**Preparation of Plant Extracts**
The fresh leaves of *R. apiculata* Blume were properly washed with distilled water, shade dried, and coarse powdered. Powder weighing 250 g was methanol by a hot percolation method using a Soxhlet apparatus. The successive methanolic extract was concentrated in a rotavapor according to the boiling temperatures of the solvents to dryness to obtain organic solvent crude extracts. The obtained methanolic (8.2 g) extracts were evaluated for phytochemical analysis and *in vitro* antioxidant activities.

**Preliminary Qualitative Phytochemical Screening**
The methanolic leaf extract of *R. apiculata* Blume was subjected to different phytochemical tests for the detection of different phytoconstituents using standard procedures.

**Test for Tannins**
1 ml of the methanolic leaf extract of *R. apiculata* Blume was added to test tube containing 0.02 M potassium ferricyanide then 1 ml of 0.02 M ferric chloride containing 0.1 M HCl was added and observed for blue–black coloration

**Test for Phlobatannins**
About 1 ml of the methanolic leaf extract of *R. apiculata* Blume was boiled with 2% aqueous HCl and the deposition of red precipitate was taken as evidence for the presence of phlobatannins.

**Test for Saponins**
Nearly 1 ml of the methanolic leaf extract of *R. apiculata* Blume was mixed with 5 ml of distilled water in a test tube, and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

**Test for Flavonoids**
About 5 ml of dilute ammonia solution was added to a portion of methanolic leaf extract of *R. apiculata* Blume followed by addition of concentrated H2SO4. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

**Test for Steroids**
Nearly 2 ml of acetic anhydride was added to 0.5 ml of the methanolic leaf extract of *R. apiculata* Blume with 2 ml of H2SO4. The color changed from violet to blue or green in sample indicate the presence of steroids.

**Test for Alkaloids**
Methanolic leaf extract of *R. apiculata* Blume was mixed with 2 ml of Wagner’s reagent. Reddish-brown-colored precipitate indicates the presence of alkaloids.

**Test for Quinones**
Dilute NaOH was added to the 1 ml of methanolic leaf extract of *R. apiculata* Blume. Blue-green or red coloration indicates the presence of quinones.

**Test for Coumarin**
About 10% of NaOH was added to the methanolic leaf extract of *R. apiculata* Blume and chloroform was added for the coloration of yellow color, which shows the presence of coumarin.

**Test for Terpenoids (Salkowski Test)**
5 ml of the methanolic leaf extract of *R. apiculata* Blume was mixed with 2 ml of chloroform, and added 3 ml of concentrated H2 SO4 carefully to form a layer. A reddish-brown coloration of the inner face was underplayed with 1 ml of concentrated H2SO4. Nearly 1 ml of the methanolic leaf extract of *R. apiculata* Blume was boiled with 5 ml of distilled water in a test tube, and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

**Test for Cardiac Glycosides (Keller-Kiliani Test)**
Five ml of the methanolic leaf extract of *R. apiculata* Blume was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H2SO4. A brown ring at the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid, a greenish ring may form just gradually throughout the thin layer.
Quantitative Determination of Phytochemical Constituents

Determination of total phenolic content (TPC)

TPC of the methanolic leaf extract of *R. apiculata* Blume was determined by the standard method[12] with little modifications, using tannic acid as a standard phenolic compound. The methanolic leaf extract was diluted with distilled water to a known concentration to obtain the readings within the standard curve range of 0.0–600 µg if tannic acid/ml. 250 µl of the diluted methanolic extract of tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin–Ciocalteu reagent. The absorbance of the resulting blue color solution was measured at 760 nm using spectrophotometer after including the samples for 90 min. All the experiments were conducted in three replicates.

Determination of saponins

Nearly 20 g of the methanolic leaf extract of *R. apiculata* Blume was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 48 h. After filtration, the extracts were concentrated on a water bath to 1/4th of the original drops to the extract until the precipitation was complete. The whole solution was collected, washed with dilute ammonium hydroxide, and then filtered. The residue obtained was dried and weighed.[13]

Determination of alkaloids

About 5.7 g of the methanolic leaf extract of *R. apiculata* Blume was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and then concentrated. The suspension was filtered and the residue reextracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel, and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in the water bath. After evaporation, the samples were dried in the oven to a constant weight and saponin content was calculated in percentage.[13]

Determination of flavonoids

About 10 g of methanolic leaf extract of *R. apiculata* Blume was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight.[14]

Determination of tannins

About 10 ml of standard solution was made up to 100 ml distilled water. 100–500 µl aliquots of the methanolic leaf extract of *R. apiculata* Blume were taken in clear test tubes. 0.5 µl of Folin–Denis reagent and 1 ml of sodium carbonate solution were added to each tube. Each tube was made up to 1000 µl with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 min and read at 760 nm against reagent blank.[14]

In Vitro Antioxidant Activity

1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH free radical scavenging assay is an established assay and is widely used to evaluate the radical scavenging activity of natural and synthetic antioxidant compounds. This assay is based on the reduction of DPPH in methanol in the presence of hydrogen-donating antioxidant constituents of plant extracts, due to the formation of the non-radical form of DPPH. The change in color is monitored at 517 nm. Briefly an aliquot of 1 ml, 0.3 mM DPPH ethanolic solution was added to 2.5 ml of various concentrations methanolic leaf extract of *R. apiculata* Blume (25, 50, 100, 200, and 500 µg/ml) and standard ascorbic acid (30 µg/ml) allowed to incubate at room temperature in the dark condition; after 30 min, the absorbance was measured at 517 nm. Ethanol was used as a blank. DPPH solution (1 ml, 0.3 mM) plus ethanol (2.5 ml) serves as a negative control. All the tests were performed in triplicates (*n* = 3), and average values are calculated. Half-maximal inhibitory concentration (IC$_{50}$) values were also calculated. Lower absorbance of the reaction mixtures indicates higher free radical scavenging activity.

\[
\% \text{ DPPH radical scavenging} = \left( \frac{A_{\text{control}517\text{nm}} - A_{\text{test}517\text{nm}}}{A_{\text{control}517\text{nm}}} \right) \times 100
\]

Superoxide scavenging activity

The superoxide free radical scavenging activity of the ethanolic leaf extract of *R. apiculata* Blume was measured by the method of Liu et al.[17] Briefly the assay mixture contained various concentrations of 25, 50, 100, 200, and 500 µg/ml of the methanolic leaf extract of *R. apiculata* Blume and ethylenediaminetetraacetic acid (EDTA) (6 mM containing 3 µg sodium cyanide), nitroblue tetrazolium (50 µm), riboflavin (2 µM), and phosphate buffer (58 µM, pH 7.8) in a total volume of 300 µl. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride and incubated for 20 min. The absorbance was measured by 560 nm using spectrophotometer.
M. Muthulingam and K. Krishna Chaithanya

% of Superoxideradical scavenging =
\[ \frac{A\text{control}_{560\text{nm}} - A\text{test}_{560\text{nm}}}{A\text{control}_{560\text{nm}}} \times 100 \]

**Hydrogen peroxide scavenging capacity**

The Hydrogen peroxide scavenging capacity was determined according to a method suggested by Zhang et al. Aliquot of 50 mM H\text{2}O\text{2} and various concentrations (25, 50, 100, 200 and 500 µg/ml) of the methanolic leaf extract of *R. apiculata* Blume were mixed (1:1 v/v) and incubated for 30 min at room temperature. After incubation, 90 µl of the H\text{2}O\text{2} sample solution was mixed with 10 µl high performance liquid chromatography (HPLC) grade methanol and 0.9 ml Fox reagent was added (previously prepared by mixing 9 volumes of 4.4 mM ascorbic acid in HPLC grade methanol with 1 volume of 1 mM xylene orange and 2.56 mM ammonium ferrous sulfate in 0.25 M H\text{2}SO\text{4}).

The reaction mixture was then vortexed and incubated at room temperature for 30 min. The absorbance of ferri-xylene orange complex was measured at 560 nm. All tests were carried out 3 times and sodium pyruvate was used in the reference compound.

% of Hydrogen peroxide scavenging =
\[ \frac{A\text{control}_{560\text{nm}} - A\text{test}_{560\text{nm}}}{A\text{control}_{560\text{nm}}} \times 100 \]

**Hydroxyl radical scavenging activity**

The scavenging activity for hydroxyl radical was measured according to the modified method of Halliwell et al. The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of FeCl\text{3}, 0.1 ml of H2 O\text{2}, 0.36 ml of deoxyribose, 1.0 ml of methanolic leaf extract of *R. apiculata* Blume of different concentrations (25, 50, 100, 200 and 500 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.4), and 0.1 ml of ascorbic acid in sequential manner. Then, this mixture was incubated at 37°C for 1 hour. About 1 ml portion of the incubated mixture was mixed with 1 ml of 10% tri chloroacetic acid (TCA) and 1 ml of 0.5% thiobarbituric acid to develop the pink chromogen, which was measured at 532 nm. The hydroxyl radical scavenging activity was calculated using the following equation.

% of Hydroxyl radical scavenging =
\[ \frac{A\text{control}_{532\text{nm}} - A\text{test}_{532\text{nm}}}{A\text{control}_{532\text{nm}}} \times 100 \]

**Statistical Analysis**

The experimental results were expressed as mean ± standard error of the mean of three replicates, where applicable, the data were subjected to one-way analysis of variance (ANOVA) and two-way ANOVA. All this analyses were performed by GraphPad Prism software program (Version 6.0). *P* < 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Qualitative Analysis of the Methanolic Leaf Extract of *R. apiculata* Blume**

The result of the preliminary phytochemical screening was carried out on the methanolic extract of *R. apiculata* and revealed the presence of a wide range of phytoconstituents (secondary metabolites) including flavonoids, tannins, alkaloids, and saponins supporting for its wide range of biological activities as shown in the Table 1. Surya and Hari reported that 70% methanolic leaf extract of *R. apiculata* Blume contained secondary metabolites such as flavonoids, saponins, and polyphenolic compounds. Then, the results of the present study are coexisting with the other reported results.

**Quantitative Analysis of the Methanolic Leaf Extract of *R. apiculata* Blume**

Medicinal plants constitute the group of plants mainly used for health care. The use of them as traditional medicine is known since time immemorial. Chemicals which present universally in all plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, and chlorophylls, while secondary phytochemicals as alkaloids to terpenoids and acetogenins to different phenols. These are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in human therapy, veterinary, agriculture, scientific research, and countless other areas.

**TPC**

The results of TPC are shown in Table 2. The standard calibration curve is shown in Figure. 1. The TPC of the methanolic leaf extract of *R. apiculata* Blume was found to be 27.36 and 54.56 µg at 50 and 100 µl, respectively, and graphically represented in Figure 2.

**Total Alkaloid Content**

The results of alkaloid content are shown in Table 3, and the alkaloid content was found to be 3.78% w/w in the methanolic extract of *R. apiculata* Blume.
was found to be 7.46 and 95.41 µg at 50 and 100 µl, respectively, and graphically represented in Figure 6.

**In Vitro Antioxidant Activity**

**DPPH radical scavenging assay**

The DPPH method is used for free radical scavenging activity or hydrogen-donating capacity of plant extract or antioxidant compounds. The antioxidant activity of methanolic leaf extract of *R. apiculata* Blume was found to be 7.46 and 95.41 µg at 50 and 100 µl, respectively, and graphically represented in Figure 6.

**Table 2: TPC of leaf methanolic extract of *R. apiculata* Blume**

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume (µl)</th>
<th>Absorbance</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (gallic acid)</td>
<td>100</td>
<td>0.151</td>
<td>89.60</td>
</tr>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>50</td>
<td>0.379</td>
<td>27.36</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.755</td>
<td>54.56</td>
</tr>
</tbody>
</table>

TPC: Total phenolic content, *R. apiculata*: Rhizophora apiculata

**Table 3: TAC of methanolic leaf extract of *R. apiculata* Blume**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample (g)</th>
<th>Weight of empty filter paper (g)</th>
<th>Weight of paper+precipitate (g)</th>
<th>TAC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>5.70</td>
<td>0.92</td>
<td>1.15</td>
<td>3.78</td>
</tr>
</tbody>
</table>

TAC: Total alkaloid content, *R. apiculata*: Rhizophora apiculata

**Table 4: TSC of the methanolic leaf extract of *R. apiculata* Blume**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample (g)</th>
<th>Weight of empty filter paper (g)</th>
<th>Weight of paper+precipitate (g)</th>
<th>TSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>20.0</td>
<td>98.50</td>
<td>99.33</td>
<td>4.17</td>
</tr>
</tbody>
</table>

TSC: Total saponin content, *R. apiculata*: Rhizophora apiculata

**Table 5: TFC of the methanolic leaf extract of *R. apiculata* Blume**

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume (µl)</th>
<th>Absorbance</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (quercetin)</td>
<td>100</td>
<td>0.111</td>
<td>89.90</td>
</tr>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>50</td>
<td>0.388</td>
<td>37.43</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.902</td>
<td>87.18</td>
</tr>
</tbody>
</table>

TFC: Total flavonoid content, *R. apiculata*: Rhizophora apiculata

**Figure 1:** Standard calibration curve for the determination of total phenolic content

**Figure 2:** Graphical representation of phenolic content in methanolic leaf extract of *Rhizophora apiculata* Blume for different volumes (50 and 100 µl)

**Total Saponin Content**

The results of saponin content are shown in Table 4. The saponin content was found to be 4.17% w/w in the methanolic extract of *R. apiculata* Blume.

**Total Flavonoid Content (TFC)**

The results of TFC are shown in Table 5. The standard calibration curve is shown in Figure 3. The TFC of the methanolic extract of *R. apiculata* Blume was found to be 37.43 and 87.18 µg at 50 and 100 µl, respectively, and graphically represented in Figure 4.

**Total Tannin Content (TTC)**

The results of TTC are shown in Table 6. The standard calibration curve is shown in Figure 5. The TTC of the methanolic leaf extract of *R. apiculata* Blume was found to be 7.46 and 95.41 µg at 50 and 100 µl, respectively, and graphically represented in Figure 6.
carried out with different doses, namely 25, 50, 100, 200, and 500 µg/ml. As shown in Table 7 and Figure 7, a dose-dependent DPPH free radical scavenging activity was found. The DPPH antioxidant activity of the methanolic leaf extract of *R. apiculata* Blume at 100 µg/ml was 90.0%, whereas the standard ascorbic acid exhibited 93.6% at 30 µg/ml, respectively. The IC<sub>50</sub> values of the methanolic leaf extract of *R. apiculata* Blume and ascorbic acid were 26.14 and 24.48 µg/ml, respectively.

### Table 6: TTC of the methanolic leaf extract of *R. apiculata* Blume

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume (µl)</th>
<th>Absorbance</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100</td>
<td>0.049</td>
<td>20.00</td>
</tr>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>50</td>
<td>0.018</td>
<td>7.46</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.225</td>
<td>95.41</td>
</tr>
</tbody>
</table>

TTC: Total tannin content, *R. apiculata*: Rhizophora apiculata

### Table 7: Determination of scavenging activity of the methanolic leaf extract of *R. apiculata* Blume by DPPH assay

<table>
<thead>
<tr>
<th>Description</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.250</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>30</td>
<td>0.016</td>
<td>93.6</td>
<td>24.48</td>
</tr>
<tr>
<td>Methanolic leaf extract of <em>R. apiculata</em> Blume</td>
<td>25</td>
<td>0.242</td>
<td>96.8</td>
<td>26.14</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.238</td>
<td>95.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.225</td>
<td>90.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.223</td>
<td>89.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.222</td>
<td>88.8</td>
<td></td>
</tr>
</tbody>
</table>

*R. apiculata*: Rhizophora apiculata, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, IC<sub>50</sub>: Inhibitory concentration

**Superoxide scavenging activity**

Superoxide anion radical scavenging activity of the methanolic leaf extract of *R. apiculata* Blume is shown in Figure 8, the decrease of absorbance [Table 8] indicates the consumption of superoxide anion in the reaction mixture by the methanolic leaf extract, and shown the maximum scavenging activity of 53.06% at 500 µg/ml with the IC50 value of the 116.12 µg/ml. Gerezgher *et al.* reported that ethanolic leaf extract...
Senna singueana shown 53.33% superoxide anion radical scavenging at 100 µg/ml with an IC\textsubscript{50} value of 191 µg/ml.

### Hydrogen Peroxide Scavenging Capacity

Hydrogen peroxide plays an important role in cell communication at low levels, but during pathological conditions, the high amount of H\textsubscript{2}O\textsubscript{2} was generated in biological system react with naturally occurring iron complexes in in vivo to generate extremely reactive hydroxyl radicals, and this may be the beginning of many of its toxic effects.\textsuperscript{[22]} As shown in Figure 9, the scavenging of hydrogen peroxide by the methanolic leaf extract of \textit{Rhizophora apiculata} Blume was increased in a dose-dependent manner, and the percentage inhibition of H\textsubscript{2}O\textsubscript{2} by the methanolic leaf extract was found to be 97.9% at 500 µg/ml with concentration-dependent manner with the IC\textsubscript{50} value of 24.32 µg/ml [Table 9].

### Hydroxyl Radical Scavenging Activity

Hydroxyl radical is reactive oxidizing radical that will react with most of the biomolecules and causing oxidative stress-related disease. A single hydroxyl radical can result in the development of many molecules of lipid hydroperoxides in the cell membrane, which may rigorously interrupt its function, and finally lead to cell death.\textsuperscript{[23]} As shown in Table 10 and Figure 10, the result represents the hydroxyl radical scavenging ability of \textit{Rhizophora apiculata} Blume.

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**Table 8: Determination of scavenging activity of the methanolic leaf extract of \textit{R. apiculata} Blume by superoxide dismutase assay**

<table>
<thead>
<tr>
<th>Description</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
<th>IC\textsubscript{50} values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.409</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanolic leaf extract of \textit{R. apiculata} Blume</td>
<td>25</td>
<td>0.296</td>
<td>27.63</td>
<td>116.12</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.278</td>
<td>32.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.236</td>
<td>42.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.207</td>
<td>49.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.192</td>
<td>53.06</td>
<td></td>
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</tbody>
</table>

\textit{R. apiculata: Rhizophora apiculata, IC\textsubscript{50}: Inhibitory concentration}
of the methanolic leaf extract of *R. apiculata* Blume. The various concentration of methanolic leaf extract of *R. apiculata* Blume exhibiting a concentration-dependent OH scavenging activity was found to be maximum at a concentration of 500 µg/ml with inhibition of 82.14% with IC$_{50}$ value of 60.13 µg/ml.

Phenolic compounds, flavonoids, and tannins are ubiquitous secondary metabolites in plants, and they are well known to be having antioxidant activity, which play a vital role in neutralizing the free radicals and quenching singlet and triplet oxygen.[29] In the present study, the TPC, TFC, and TTC were estimated, and it was found that the methanolic extract of the leaf extract of *R. apiculata* contained 54.56 µg/ml, 87.18 µg/ml, and 95.14 µg/ml, respectively.

**CONCLUSION**

The results obtained in the present study have shown that methanolic leaf extract of *R. apiculata* Blume has a high content of secondary metabolites such as flavonoids, polyphenolic compound, and tannins, without any toxicity and shown potential antioxidant activity. The methanolic leaf extract of *R. apiculata* was found to be an effective free radical scavenging activity against H$_2$O$_2$, > DPPH > OH > O$_2$ respectively, compared to that of ascorbic acid. This antioxidant activity of the methanolic leaf extract of *R. apiculata* Blume is due to their presence of high amount of flavonoid and tannin content. Hence, the methanolic leaf extract of *R. apiculata* Blume is a good source for the development of natural antioxidant medicines for oxidative stress-related diseases.

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