ORIGINAL RESEARCH ARTICLE
Phytochemical Characterization and Antimicrobial Efficiency of Mangrove Plants
Avicennia marina and Avicennia officinalis

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ABSTRACT
Mangrove plants have been used in folklore medicines. In the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in Avicennia officinalis when compared to the Avicennia marina. In the DPPH scavenging assay, both the mangrove extracts showed high antioxidant activity. The Avicennia marina samples have more effective antioxidant activity when compared to the Avicennia officinalis. And the percentage of scavenging was found to be about 89.85% for Avicennia marina and 68.67% for Avicennia officinalis sample. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hRf = >12, 25 and 93 of both the mangrove extracts and hRf = 53 in Avicennia marina alone were proved to be having antioxidant activity. The results of antimicrobial activity by the well diffusion assay also clearly expressed that Avicennia marina has high concentration of active principles when compared to the Avicennia officinalis.

Key words: Avicennia marina, Avicennia officinalis, Mangrove plants, alkaloids, anti-atherosclerotic activities, anti-inflammatory.

INTRODUCTION
Mangroves are woody trees and shrubs that grow in the intertidal zones of tropical and sub-tropical regions [1]. Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven activity against human, animal and plant pathogens. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance [2] [3]. Had also reported the bioactive compounds from mangrove plants.

MATERIALS AND METHODS
The leaves of Avicennia marina and Avicennia officinalis were collected from the southeast coast of Parangipettai, Tamilnadu, India. The mangrove leaves sample was dried in the shadow; it was...
powdered and stored at room temperature. About 5g of the leaves were extracted with 100 ml of Methanol for 1 week at room temperature. These samples were filtered using Whatmann filter paper and the filtrate was evaporated to dryness under vacuum at 40°C. Each concentrated extracts were made into different concentrations (200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml) using ethanol.

### Preliminary Phytochemical Analysis

The preliminary phytochemicals from the mangrove sample extracts were determined. In the preliminary phytochemical analysis of crude extracts of *Avicennia marina* and *Avicennia officinalis* for screened the presence of glycosides, tannin, terpenoids, phenolics, saponins, amino acid, Sterol, alkaloid, phenolics and flavanoids were carried out by [14] method.

### Antioxidant Assay

The antioxidant properties of the mangrove extracts were studied by their ability to scavenge free radicals using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reducing power.

#### Preparation of Test Extracts

5ml of hydroponics test sample was dissolved in 5ml of pure methanol for analysis.

#### Preparation of DPPH (2, 2-Diphenyl-1-picryl hydrazyl)

0.0025g of 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) was dissolved in 25 ml of methanol (0.25mM concentration). The content should be made and kept in dark condition, because DPPH is light sensitive.

#### DPPH free Radical Scavenging Assay

The free radical scavenging activity of mangrove extracts was measured by the DPPH method proposed [15]. Percentage inhibition or DPPH scavenging activity was calculated by following expressions.

$_{\text{Percentage of scavenging}} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$

Where $A_0$ is the absorbance of control and $A_1$ is the absorbance of test sample.

#### Rapid screening with TLC method

Thin Layer Chromatography was used to detect antioxidant activity of two mangrove samples based on spraying the plates with oxidizing reagents. The separated compounds on TLC plates were spraying with 0.004% DPPH stable radical in methanol [16] to located and detect antioxidant active compounds. The protecting against the scavenging DPPH radical gave pale yellow coloured spots were taken as positive results.

### Antimicrobial Activity

Five pathogens were chosen for the present investigation and obtained from the Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India. Thus, the antimicrobial activity of the crude extract of *Avicennia marina* and *Avicennia officinalis* were determined by measuring the zone of inhibition in the Agar well diffusion method. The results were compared with a standard antibiotic, tetracycline (20µg/ml).

### Results

#### Phytochemical analysis

In the preliminary phytochemical analysis of crude extracts of *Avicennia marina* and *Avicennia officinalis* contains Alkaloid, Flavanoid, terpenoids and phenolics. In *Avicennia marina* extract obtained Saponins and Amino acid but absence in *Avicennia officinalis*. Tannins, Sterols and Glycosides were absent in *Avicennia marina* but present in *Avicennia officinalis* (Table 1).

### Table 1: Preliminary phytochemical screening of crude extracts of *Avicennia marina* and *Avicennia officinalis*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the test</th>
<th>Avicennia marina</th>
<th>Avicennia officinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoid</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Phenolics</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Sterols</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>Amino acid</td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

#### Antioxidant activity

#### DPPH scavenging assay

In the present study the mangrove extracts has high DPPH scavenging capacity, which increased with increasing concentration (Fig 1). The DPPH assay was carried out at different concentrations of mangrove samples, namely 200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml. DPPH assay did not show any significant difference at 200µg/ml and 400µg/ml concentrations in *Avicennia marina* sample; however, it was significant for 600µg/ml and 800µg/ml for the extracts.

DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses colour stoichiometrically depending on the number of electrons taken up. Hence this assay provided information on reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.
Fig 1: Effect of crude extract of mangrove samples on scavenging of DPPH

Rapid screening with TLC method
Rapid TLC-screening assay based on decolorization of ethanolic DPPH· radical that sprayed into TLC plates, as a rapid test to evaluate the antioxidant activity of natural compounds. The development of pale yellow spots on the separated TLC plate confirms the antioxidant activity of the two samples. Among all separated bands, bands at hRf = >10, 25, 53 and 93 of Avicennia marina and Avicennia officinalis extracts showed an excellent antioxidant activity (Table 2).

Table 2: TLC profile of Avicennia marina and Avicennia officinalis

<table>
<thead>
<tr>
<th>No. of spots obtained</th>
<th>Avicennia marina</th>
<th>Avicennia officinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rf value</td>
<td>hRf value</td>
</tr>
<tr>
<td>1</td>
<td>0.22</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>0.53</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>0.74</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>93</td>
</tr>
</tbody>
</table>

Antimicrobial activity
The antimicrobial activity of crude extracts of Avicennia marina and Avicennia officinalis against nine human pathogenic bacterial strains were done and their zone of inhibition compared with standard antibiotic, tetracycline. The mangrove extracts were shown more active antimicrobial proficiency against Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli when compared to the standard antibiotic, but little antibacterial activity against Enterobacter aeruginosa, Proteus sp, Salmonella paratyphi, Citrobacter sp. is a highly resistant against both test samples as well as standard antibiotics. (Table 3).

Table 3: Antimicrobial activity of the crude extract of Avicennia marina and Avicennia officinalis against human pathogenic bacteria

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogenic bacteria</th>
<th>Avicennia marina</th>
<th>Avicennia officinalis</th>
<th>Standard antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>18</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella pneumonia</td>
<td>24</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas aeruginosa</td>
<td>26</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus subtilis</td>
<td>16</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>Escherichia coli</td>
<td>27</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Enterobacter aeruginosa</td>
<td>8</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>Proteus sp</td>
<td>12</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Salmonella paratyphi</td>
<td>5</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Citrobacter sp</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

From the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in test extracts, they are beneficial one for its biological activity. In the DPPH scavenging assay, the mangrove extracts showed high antioxidant activity. The test extract samples have more effective antioxidant activity, the percentage of scavenging was found to be about 88.93% for Avicennia marina and 67.67 % in Avicennia officinalis. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hRf = >10, 25 and 93 of both the mangrove extracts and hRf = 53 in Avicennia marina alone were proved to be having antioxidant activity.

DISCUSSION
The mangroves are a promising source of natural products. Mangroves have been a source on several bioactive compounds. Those bands that have developed into yellow spots were suspected as carotenoids and other phenolic compounds with respect to references (Hanaa et al., 2008). Phenolic compounds from plants are known to be good natural anti-oxidants. However, the activity of synthetic anti-oxidants was often observed to be higher than that of natural anti-oxidants [18]. The results of antimicrobial activity by the well diffusion assay also clearly expressed that test extracts have high concentration of active principles.

CONCLUSION
This work is a successful attempt of phytochemical characterization and antimicrobial efficiency of mangrove plants Avicennia marina and Avicennia officinalis. In recent years,
screening of mangrove plants for a variety of biological activities, further attention should be paid to develop the novel drugs from natural product.

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REFERENCES