Comparative Evaluation of some Selected Bioactive Constituents in the Leaves and Bark of *Avicennia marina* (Forsk.) Veirh from the Sudanese Red Sea Coast

Nahid A. Osman\(^{1}\)* and Faiza A. Abkar\(^{1}\)

\(^{1}\)Faculty of Marine Science and Fisheries, Red Sea University, Port Sudan, Sudan. P. O. box 24. 

* Corresponding author (nahidcoast@yahoo.com).

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Abstract-The presence of selected phytoconstituents in the leaves and bark of *Avicennia marina* (Forsk.) Veirh collected from a stand along the Sudanese Red Sea coast is assessed with regular phytochemical methods. The qualitative screening revealed that both plant parts contained alkaloids, flavonoids, sterols, and tannins. Quantitatively, the bark contained more alkaloids (12.75%), flavonoids (4.5%), and tannins (34.6%) than the leaves which contained 11.63%, 3.6% and 15.90% respectively. The findings were correlated with the prevailing environmental conditions in the study area. The potentiality of this tree as a possible source of phytochemicals with pharmaceutical prospective was also discussed.

Index terms: Phytochemicals, *Avicennia marina*, leaves, bark, Sudan

I. INTRODUCTION

Mangroves thrive under stressful and extreme tropical environmental conditions [1] such as high solar radiation, temperature, salinity, and anaerobic conditions that may have unfavorable effects on the photosynthesis of these plants. Hence, mangroves have evolved special adaptation to survive these conditions. Adaptations of mangrove plants to their harsh habitat occur at morphological, physiological, and biochemical levels spanning canopy architecture, salt regulation, and oxygen conversion [2]. Although the main function of these adaptations is to counteract the unfavourable effects of the aforementioned abiotic stresses, it has resulted in unique attributes which distinguish mangroves among other plants.

One of these attributes are the secondary metabolites produced by the mangroves which have been used traditionally by medicinal local practitioners due to their proved medicinal values [3,4]. Contemporary research has verified the traditional knowledge on mangrove and revealed that mangrove plants contain a wide array of novel bioactive metabolites with pharmaceutical potential [5], however, of varying efficiency on inhibition of pathogens and tumors.

Along the Red Sea coasts mangroves survive in an extreme tropical arid environment with significant solar radiation, insufficient and temporal freshwater influx, and highly saline seawater (≥ 40‰). The true mangrove plant *Avicennia marina* (Forsk.) Veirh is the keystone species of the mangrove stands along the Red Sea coast of Sudan. The plant that was recently placed in the family Acanthaceae is commonly known as the grey or white mangrove. It is a small tree or shrub (4 to 7m in height) grows in the intertidal areas receiving freshwater discharges along the tropical and subtropical sea coasts.

Although *A. marina* has been reported as a medicinal plant in both conventional medicine [6,7] and contemporary pharmacognosy research on mangrove plants little research was done to investigate phytoconstituents of the Sudanese species. Recently, Mouafi et al. [8] reported the presence of seven phytochemicals from *A. marina* leaves collected from the Sudanese Red Sea coast and the ability of its extract to inhibit the growth of some antibiotic resistant bacteria and one fungi species.

Recognizing the paucity of information on quantitative values of phytochemicals of Sudanese *A. Marina* the present paper aims to compare the availability of selected bioactive constituents in leaves and bark of this true mangrove tree and to determine their relative concentrations in these parts. Besides, the paper aimed to quantify these bioconstituents for comparison with relevant values of equivalent plants from the wet tropical region to help...
understand the influence of environmental conditions on the synthesis of these compounds.

II. MATERIALS AND METHODS

The experimental work was based on the recommendations given by Harbone [9]. The qualitative screening tested the presence of 4 phytochemical groups of compounds namely: alkaloids, flavonoids, sterols, and tannins. The quantitative measurements determined the content of 3 of the 4 tested phytochemicals namely: alkaloids, flavonoids, and tannins. Regular phytochemical procedures were followed for the qualitative and quantitative analysis. Experiments were performed in replicates and/or triplicates to ensure reliability of the results.

Study area

The leaves and bark of *Avicennia marina* were collected from a mangrove stand located 8 kilometers south of Port Sudan harbor between latitudes E 19° 35' 40" and E 19° 36' 40" and longitudes N 37° 14' 54" and N 37° 14' 55".

Plant materials

Mature healthy leaves and the stem bark of healthy trees were collected from the stand and washed with tap and distilled water respectively. The samples were then dried in the shade at room temperature, milled to a homogenous powder and kept in tight container.

Extraction

The serial exhausted extraction method [10] was followed to extract plant materials. Ten grams of plant tissues were first macerated in 100 ml of ether for 3 days at room temperature with frequent shaking. The mixture was centrifuged (10000g/10m) and filtered with Whatman No. 1 filter paper into a clean glass vial and kept at 4°C until further analysis. Then the residues were successively extracted with chloroform, methanol, and distilled water respectively.

Qualitative screening of bioactive constituents

The extracts were tested for the presence of alkaloids, flavonoids, sterols, and tannins phytochemical groups of compounds. This was based on development of colouration and precipitation upon addition of certain chemical reagents to the plant parts extracts.

Test of alkaloids

The presence of alkaloids in the plant extract was tested with Wagner’s reagent following the procedure described by Sabri, et al. (2012) [11]. Ten ml of the extract were evaporated to dryness. Two ml of 2% HCl acid solution were added to the dry residues. Few drops of the reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

Test of flavonoids

This test was done as described in Pamar et al. (2012) [12]. Few drops of NaOH were added to 2 ml of extract. The formation of intense yellow colouration that changed to colourless when few drops of dilute HCl were added indicates the presence of flavonoids.

Test of sterols

Two ml of concentrated sulphuric acid were added to 2 ml extract [13]. Formation of red precipitate indicated the presence of sterols.

Test of tannin

Tannins were detected according to the methods described in Ugochuhwu et al. (2013) [14]. To 1 ml of extract solution, 1 ml of 3% ferric chloride solution was added. Development of brownish green or a blue black coloration indicated the presence of tannins.

Quantitative determination of bioactive constituents

The crude concentration of three of the four tested phytochemical groups of compounds was gravimetrically determined in the leaves and bark of *A. marina* on dry weigh basis. The concentration of alkaloids was determined according to Harbone (1973) as described by Agoreyo et al. (2012) [15]. The method of Kocip-Abyazan (1994) described by Eleazu et al. (2012) [16] was performed to determine the concentration of flavonoids. Tannins were quantified with the methods illustrated by Vetter and Barbosa (1995) [17]. Below are the descriptions of the procedures performed.
**Moisture content**
Two grams of the plant tissue were incubated in an oven at 105°C until the sample reached a constant weight. Moisture content (MC) was determined gravimetrically with the following formula.

\[
MC (\%) = \frac{\text{weight of sample after oven}}{\text{weight of sample}} \times 100
\]

**Alkaloids**
Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol were added. The mixture was covered and allowed to stand for four hours. Then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide solution was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was filtered, washed with dilute ammonium hydroxide solution, dried and weighed. The content of alkaloids was determine with the following formula

\[
\text{Alkaloids (\%)} = \frac{\text{weight of precipitate}}{\text{weight of sample}-MC} \times 100
\]

**Flavonoids**
Ten grams of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated over a water bath to dryness and weighed when constant weight was achieved. The total content of flavonoids was determined as follows.

\[
\text{Flavonoids (\%)} = \frac{\text{weight of precipitate}}{\text{weight of sample}-MC} \times 100
\]

**Tannins**
Two grams of plant tissue were extracted with 100 ml distilled water for one hour at 90°C. The mixture was decanted through a filter paper and the residue was extracted again. The 2 filtrates were combined, allowed to cool down, and made up to 500 ml with distilled water. One hundred ml of this solution were transferred to a beaker and 10 ml of 40% formaldehyde and 5 ml of concentrated sulphuric acids were added. The mixture was refluxed for 30 minutes, allowed to cool down and filtered. The precipitate was dried and weighed. Tannin content was obtained with the following formula.

\[
\text{Tannins (\%)} = \frac{\text{weight of precipitate}}{\text{weight of sample}-MC} \times 100
\]

**III. RESULT AND DISCUSSION**

Alkaloids, flavonoids, sterols, and tannin were detected and measured in both leaves and bark of Sudanese *A. marina*.

**Presence of the bioactive constituents in the leaves and bark of *A. marina***

The presence of phytochemical compounds of Sudanese *A. marina* (Forssk.) Veirh varied in the different extracts of leaves and bark (Table 1). In the leaves extracts, alkaloids were present in both methanolic and distilled water extracts. Flavonoids were detected only in the distilled water extract. Sterols were absent from the ether extract of the leaves and were detectable in the chloroform, methanol, and distilled water extracts. Presence of tannins in the leaves was observed in both methanolic and distilled water extracts. Among the bark extracts, alkaloids were only soluble in the methanolic one. Flavonoids were detectable in both methanolic and distilled water extracts. The sterols and tannins of the *A. marina* bark were soluble in all the solvents employed for the extraction. The variation in the presence of the phytochemical compounds in the different extracts may largely be attributed to the solubility of these compounds in the solvents used for extraction. The solubility of any phytochemical in a given solvent is influenced by many factors. Some of these factors are related to the physiochemical properties of the phytochemical compound and the other may be related to the solvent characteristics. Examples of the factors related to the phytochemical compound properties are polarity, the functional group of the phytochemical, and its molecular weight. During extraction the solvent diffuses into the solid plant materials and dissolve compounds with similar polarity [10]. Applying this to the currently obtained solubility pattern of phytoconstituents in the extracts of leaves and bark of *A. marina* may suggest that the leaves and bark would contain the same phytochemical groups of compounds; however their chemical structures and type may differ. For example, and within the context of this study, the leaves may contain 2 types of alkaloids one soluble in methanol and the other is soluble in distilled water. Bark seems to contain one group of alkaloids soluble in methanol. This may also be applicable for flavonoids of both leaves and bark. Likewise, the detection of sterol in 3 leaves
extracts and 4 bark extracts could also indicate that Sudanese *A. marina* contain an array of sterols that remains to be investigated. Similarly, the solubility of tannins in 2 different extracts of the leaves and in all the 4 different extracts of the bark may preliminary indicate the diversity of tannins compounds produced by this tree and signify its potentiality as a medicinal plants and highlight the need for further research to characterize these bioactive compounds. **The bioactive contents in the leaves and bark of *A. marina***

In general the bark of the Sudanese *A. marina* (Forssk.) Veirh contained greater concentrations of the tested phytochemical groups of compounds compared to the leaves (Table 2).

### Table 1

Presence of the bioactive constituents in the leaves and bark of *A. marina* from Sudan.

<table>
<thead>
<tr>
<th>Phytochemical groups of compounds</th>
<th>Presence of phytochemicals in leaves and bark of <em>A. marina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td></td>
<td>E</td>
</tr>
<tr>
<td>Alkaloids.</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids.</td>
<td>-</td>
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<tr>
<td>Sterols.</td>
<td>-</td>
</tr>
<tr>
<td>Tannins.</td>
<td>-</td>
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<tr>
<td>Percentage yield (%)</td>
<td>2.43</td>
</tr>
</tbody>
</table>


### Table 2

Content of the alkaloids, flavonoids, and tannins in the leaves and bark of *A. marina* from Sudan.

<table>
<thead>
<tr>
<th><em>A. marina</em> part</th>
<th>Alkaloids (%)</th>
<th>Flavonoids (%)</th>
<th>Tannins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>11.5±0.15</td>
<td>3.55±0.05</td>
<td>15.85±0.05</td>
</tr>
<tr>
<td>Bark</td>
<td>12.73±0.025</td>
<td>4.50±0.0</td>
<td>34.65±0.07</td>
</tr>
</tbody>
</table>

Comparatively the bark of *A. marina* contained more alkaloids (12.75%), more flavonoids (4.5%) and higher tannins percent (34.60%) than the leaves which contained 11.63%, 3.6% and 15.90% respectively. Presence of alkaloids has been reported from species of the genus Avicennia including *A. marina* [8,18,19]. Species of this genus from Australia were considered as a poor source of alkaloids [2]. The present values of the concentrations of alkaloids of the leaves and bark of the Sudanese *A. marina* indicate that this tree could represent a potential source of alkaloids, particularly when compared with the values of alkaloids obtained for some medicinal plants. For example the value of 1.04% alkaloids in the medicinal plant *Sida acuta* (Malvaceae) was the maximum value reported by Edeoga et al (2005) [20] among the 10 medicinal plants investigated. This is also similar to the value of 1.13% reported by Khan et al (2011) [21] for *Adhatoda vasica*. Possible explanations for the comparatively high level of alkaloids in the tissues of Sudanese *A. marina* may almost certainly be related to biotic and abiotic stress conditions. The latter is manifested in the harsh environmental condition of aridity prevailing in the plant habitat. Recent studies and reviews reported the protective role of higher plant secondary metabolites against abiotic stresses including drought, high salinity, UV light exposure, high and low temperatures [22;23;24,25;26]. Under these stressing conditions, reactive oxygen species (ROS) are excessively released [26]. ROS cause disturbance in plant cell homeostasis and eventually leads to cell death. Therefore, plants suffer from these stresses generate high oversupply of bioactive compounds to prevent the damaging effect of ROS. Elevated levels of alkaloids, flavonoids, and phenolics were reported from plants subjected to drought and salt stress conditions.
In some instance, an increase of 187% in total alkaloids content in Catharanthus roseus plant subjected to drought stress was reported [30]. The extreme tropical arid climate prevailing in the Sudanese Red Sea coast may has produced a significant bearing on the composition and content of A. marina bioactive constituents including alkaloids. It seems that the Sudanese species may have accumulated alkaloids to survive these conditions. This is in conformity with Selmar (2008) [24] who discussed the analogous quality differences observed between equivalent medicinal plants grown in moderate Atlantic climate and those grown in semi-arid region with regard to secondary products contents. The content of these products is less in the former group of plant compared to the second one.

Under biotic stress conditions the plants use alkaloids as a chemical defense against herbivory and pathogens [31]. Alkaloids tend to be accumulated in the plant part to be protected at sufficient concentration to be an adequate defense. For example quinolizidine and pyrrolizidine alkaloids are concentrated in the peripheral layers of the stems of Lupinus and Senecio and are 10 to 20% higher than the mean value of the whole stem [32]. In the present communication alkaloids were extracted from the most vulnerable parts of the Sudanese A. marina, leave and bark that are subjected to camel browsing and extreme solar radiations. Some alkaloids exert pharmacological activities in human and lower animals. In human they influence the nervous system particularly the action of the chemical transmitters [32].

Alkaloids have many other pharmacological benefits including antihypertension, anticancer, antimalarial, antirhythmic, antibiotic and antiseptic activities. The values of flavonoids obtained for both leaves and bark of A. marina in this study are remarkably higher than those reported for other mangroves from wet tropical region [33] where the concentration of flavonoids in the bark of Aegceras corniculatum was 0.44%, Bruguiera gymnorrhiza was 0.26%, Cynometra iripa was 0.49% and that of Lumnitzera racemosa was 0.89%. The higher levels of flavonoids in the Sudanese mangrove species may largely be an adaptation to the extreme solar radiation in the Red Sea region that is known to experience some of the hottest and most arid conditions which occur in any marine area on Earth [34]. This is also in conformity with Bandaranayake (1994) [2] who reported that tropical mangroves have high concentration of flavonoids in the leaf epidermis and that these compounds perform a protective role against the damaging effects of ultraviolet-B radiation. It has been suggested that a flavonoids-DNA complex provide mutual protection against oxidative damage of harmful solar radiation [35] through acting as a screen in the epidermal cell layer thus adjusting the antioxidant system at both cell and whole organism levels. Accordingly, flavonoids are of great pharmacological benefits to human health particularly in preventing and curing of ailments resulting from the free radicals and inflammatory agents.

Plant sterols know as phytosterols have several bioactive properties with possible benefits for human health [36]. They contribute to lowering serum cholesterol level and were reported to produce anti-inflammatory, anti-bacterial, anti-artherosclerotic, anti-oxidant, anti-ulcerative, and antitumor influences. Besides, phytosterols serve as precursor for the production of hormone pharmaceuticals.

Similarly, the concentration of tannins in Sudanese species is noticeably higher that it’s concentration in Aegceras corniculatum (5.08%), Bruguiera gymnorrhiza (3.70%), Cynometra iripa (5.22%), and that of Lumnitzera racemosa (10.27%). The members of the genus Avicennia were reported to exhibit an exceptionally strong positive reaction for tannins tests in qualitative phytochemical screening [2]. Additionally, drought stressed plants were reported to yield a 10% higher amount of phenolic compounds [24]. The biological role of tannin in plant is related to protection against infection, insect and animal herbivory. In traditional healing tannin-containing plant extracts were used as astringent, diuretics, antiinflamatory, antiseptics, and haemostatic pharmaceuticals [37]. Recently compounds representative of tannins were found to have antiviral, antibacterial, and anticancer activities in biological tests.

IV. CONCLUSION

The search for new lead compounds for the development of new pharmaceuticals has become increasingly important especially with the drug resistance exhibited by disease causing agents. Surviving in extremely harsh environmental conditions as well as being...
subjected to anthropogenic stresses, the Sudanese
A. marina may contain novel pharmacological
therapeutics. Further investigations on the
isolation, characterization and biological assays
of the bioactive constituents of this tree may help
to provide a template for new potent drugs.

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