Proximate analysis and Phytochemical screening of Grey Mangrove (Avicenna marina) species

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Abstract
Mangroves along sheltered tropics and sub-tropics coasts fulfill important functions in terms of conservation of biological diversity, coasts protection, and consolidation of habitats, nutrients and feed for a variety of organisms. In Sudan mangroves occupied scattered stands along the Red Sea Coasts. In many parts of the world mangroves are expected to extend land side due to sea-level rise. Growing human population urges the immense need to exploit the existing livestock resources; hence ruminants are expected to be more dependent on fresh and preserved forage to avoid competition upon edible grains. In the present study proximate feed analysis and phytochemical screening is carried for Avicenna marina different age groups. Crude extracts for fresh and dead leaves, stems flowers and seeds were tested. Tissue water content reported values between (6.13% - 8.00%), while values ranges were (17.7 % - 13.0 %), (10.57% - 5.98%), (32.0% - 15.33 %), (48.57 % –38.72 %) and (35.17 % - 13. 13%). screening results presence of Tannins, Coumarins, Triterpenes and Saponins, Sterols, Flavonoids and Anthraquinones. In addition the plant productivity expressed in LA (Leaf Area) estimated. Questionnaires carried for animal herds results revealed that animals feeding on mangroves had increased milk and meat yield and health wise.

Key words: Mangroves; Avicenna marina; proximate analysis I; Phytochemical screening

Introduction:
Mangroves the characteristic intertidal plant formations in the coasts of sheltered tropical and subtropical shorelines, usually referred to as coastal woodlands, mangals, tidal forests or mangrove forests. The true mangrove plants are restricted to the typical intertidal zone where as semi mangrove plants grow on the landward fringe mangrove habitat or in terrestrial marginal zones (Marilyn,1988). Mangroves are largely confined to the region between 30 north and south of the equator (Chapman,1975). Mangroves are among the most productive carbon sink, habitats which proliferate luxuriantly in the coastal areas, river estuaries and back water areas where the litter and the dead plant portions composed a rich provenance for nutrients in the muddy swamps creating important nurseries for commercial fishes and many micro and macro associated fauna and flora (Gong and Ong, 1991; Supaporn and Nathsuda 2012).

Avicenna marina (Forssk.) Vierh. (Family: Avicenniaceae), commonly known as grey mangrove or white mangrove, is the most widespread throughout the world. It’s found in the arid regions forming an important unique ecosystem on both sides of the Red Sea in Sudan, Egypt, Eritrea, Yemen and Saudi Arabia (Mohamed1984).
seems that some mangroves locations depart from their global decline areas, where a recent study about South Saudi Arabia mangroves shows no decline in mangrove stands, where the area covered by mangroves increased by about 12% over the 41 years between 1972 to 2013 (Almahasheer, 2016). Given the correct hydrological conditions, mangroves can grow and thrive in a variety of coastal environments, and within two decades approach the biomass, stand structure and productivity of natural forests, considering tidal fluctuations and flushing which are important factors for restoration and endurance of mangrove subsequent flourishment (Ellison, 2000). *Avicenna marina* grows successfully in many parts of the world either in their natural habitats or by modifying the hosting environment by adding low saline unpolluted water (Stubbs and Saenger, 2002; Sugga, 2015). Mangrove planting is carried out in many parts of the world for a variety of reasons, including timber production, shore protection, fisheries and wildlife enhancement. Although there is a peak for seed production, but seeds were available around the whole year in considerable quantities in mangrove forests (Stubbs and Saenger, 2002).

Several mangrove species have been used in traditional medicine as a source of phytochemical with some novel biochemically unique active metabolites such as alkaloids, phenol, steroids, Terpenoids and tannins that have bioactivity as antioxidant, antimicrobial, anticancer and antiviral and proved to enhance the ability of animals to beat disease, with benefit to their health and welfare. They also have applications as insecticide and pesticide (Sharaf, et al., 2000; Mahera, et al., 2011; 2000). Mouafi, et al. (2013) reported that *Avicenna marina* could be used for silage production with high nutritive value.
Although mangroves provide valuable ecosystem services that contribute to human wellbeing, they are generally undervalued in both private and public decisions in relation to their use, conservation and restoration, due to lack of understanding, and demerit of information on their values. Without information on the economic value of mangrove ecosystem, the importance of them tends to be ignored. Most of mangrove studies provide some attention of their ecological values, but do not highlight the values of mangrove ecosystems to markets (Ellison, 2000, Khufu’s; et al., 2016).

Use of mangroves as fresh pasture for grazing livestock such as goats, camels, and cattle has been reported in many parts of the world such as Pakistan, Qatar, Saudi Arabia, China, and Malaysia. These studies identify *Avicenna marina* as a key fodder species but in all cases the examples of animal grazing are regarded as either unsustainable and/or poorly managed, representing a threat to mangrove wise use. Importance of *Avicenna marina* foliage as a salt enrichment nutrient for animals proves that it is an exciting fodder candidate (Wilkie 1995; Mouafi, 2013).

In Sudan 37% of the total animal feed is estimated as (92.9) million tons in (2012) derived from rangelands, which is equivalent to 70% of the available total animal feed, agricultural by-products contribute 28.3% and irrigated forage contributes 1% of the feed, 0.5% from cereals, cakes and concentrates (HCENR, Reports 2014). The drought period 1980 – 1983 had adverse effects on rangelands, vegetation resources, livestock and agricultural production systems in Sudan. The Red Sea State (RSS) in the coastal area had a distinct climate with characteristic vegetation endemic to the vicinity, was adversely affected and mangroves had been a source for the benefit of man and his domestic livestock during the period of Sahelian drought, that inflicted disturbance of natural vegetation along the Red Sea Coast (Sabir and Mohamed, 1990). *Avicenna marina* seedlings were observed to be eaten in high rates in mangrove stands (Bashir, 1998).

**2. Material and Methods:**

**2.1 Samples Collection:**

Plant Material for the species *Avicenna marina* was collected from Red Sea region in Sudan from "Am –wage" mangrove stand which is located at the Red Sea coast about 8km north Port Sudan town, during May 2015. The specimens were identified at the Department of Biological Oceanography, Faculty of Marine Sciences and Fisheries, Red Sea University, Sudan.

**2.2 Sample preparation:**

*Avicenna marina* fresh samples were cleaned with tap water and shade dried for 72 hrs under appropriate condition. The samples were pulverized using mechanical grinder. 25g of fine sample was added to a conical flask along with solvent (methanol) for extraction of phytochemicals. In the present study methanol is used for preparation of extract from *Avicenna marina*.

**2.3 Proximate analysis:**

To evaluate nutrients in *Avicenna marina* samples including organic and inorganic compounds, analysis was carried according to Hambleton and Rodney (1975).

**Chemical Analysis:**

Chemical analysis experiments were achieved in Animal Production Department University of Khartoum and the National Centre of Research, Sudan. Residual ash was assayed at 600°C for 4h. Protein and fats were determined according to Kjeldahl method.
Moisture content was determined by drying 50 g of sample at 105°C to constant weight. Difference in weight indicates the moisture content:

\[
\text{Moisture content} \% = \left( \frac{\text{Wet weigh} - \text{Dry weight}}{\text{Wet weight}} \right) \times 100.
\]

**Cold Percolation Extraction Method:**

After drying the collected samples, the samples were pulverized into small pieces using sterilized pestle and mortar. An amount (30 g) of crushed material was taken separately into 500 ml of methanol and sterilized water, kept on a rotary shaker at 120 rpm for 24 h. After shaking, it was filtered through eight layers of muslin cloth, centrifuged at 5000 x g for 15 min. Resultant extracts were evaporated and concentrated to dryness using the rotary evaporator at 45% C. The samples were then dissolved in the same solvents used for extraction and stored at 4% C.

**2.4. Phytochemical screening:**

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, sterols, Triterpenes, Coumarins and Anthraquinones was carried out using standard procedures described by Edeoga et al. (2005), as follows:

**Test for Alkaloids:** One ml of the filtrate with 2 ml of Dragendorff’s reagent shows turbid orange color. Test for Terpenoids: One ml of the filtrate with 2 ml CHCl3 is carefully added to few drops of concentrated H2 SO4. An interface with a reddish brown coloration is formed. Test for Steroids: To one ml of the filtrate, 10 ml chloroform and 10 ml of H2 SO 4 is slowly added by the sides of the test tube. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

**Test for Tannins:** One ml of filtrate with 2 ml of Ferric chloride gives dark green color. Test for Flavonoids: One ml of filtrate with 2 ml of dilute NaOH shows development of golden yellow color.

**Test for Saponins:** One ml of filtrate with 2 ml distilled water, is shaken vigorously and allowed to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins.

**Test for Anthraquinones:** To one ml of the filtrate, 10 ml benzene was added, followed by filtering and addition of 5 ml of 10% (v/v) ammonia to the filtrate and shake well. Development of pinkish colored solution indicates the presence of anthraquinones.

**Test for Anthocyanides:** Add one ml of filtrate with 5 ml of dilute HCl, the appearance of pale pink color indicates the presence of the above compound.

**Estimation of Leaf Area Specific Indicator (LASI):**

Leaf area was estimated (Abu-Shagra, 1984), and the dry leaf weight was determined using a digital balance to the second decimal. (LASI) was calculated by dividing leaf weight (g) / leaf area (cm2). There is a reverse relationship between (LA) and (LASI). Leaves with low (LASI) are highly productive.

Additional information was collected by questionnaire and direct interview.

**3. Results and Discussion:**

The present study was conducted to investigate the nutritive value, the phytochemical constituents and the productivity expressed in specific leaf area indicator (SLAI), for the mangrove species *Avicenna marina*, as potential source of fodder. As shown in Table (1) the Sudanese mangrove contains crude fats of (17.70-13.00%) compared to (12.08%) in *Avicenna sp* from Pakistan. For crude fiber and crude protein, the values were lower (15.33-32.00%) compared to (46.3%) and (5.98-10.57) compared to (31.33%) (Kurian and Patiola, 1980)
Table (1): Proximate Analysis of *Avicenna marina*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture%</th>
<th>Fats%</th>
<th>Protein%</th>
<th>Fibers%</th>
<th>Carbo-hy%</th>
<th>Ash%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Leaf(1)</td>
<td>6.40</td>
<td>15.80</td>
<td>10.57</td>
<td>20.73</td>
<td>46.50</td>
<td>20.40</td>
</tr>
<tr>
<td>Green Leaf(2)</td>
<td>7.90</td>
<td>17.70</td>
<td>10.50</td>
<td>15.33</td>
<td>48.57</td>
<td>13.00</td>
</tr>
<tr>
<td>Dead Leaf</td>
<td>7.30</td>
<td>15.33</td>
<td>9.00</td>
<td>29.65</td>
<td>38.72</td>
<td>35.17</td>
</tr>
<tr>
<td>Mature Seam</td>
<td>6.50</td>
<td>13.10</td>
<td>8.63</td>
<td>31.00</td>
<td>40.77</td>
<td>21.20</td>
</tr>
<tr>
<td>Young Steam</td>
<td>8.00</td>
<td>12.90</td>
<td>6.93</td>
<td>24.17</td>
<td>48.00</td>
<td>13.13</td>
</tr>
<tr>
<td>Mature Seeds</td>
<td>6.50</td>
<td>16.17</td>
<td>5.98</td>
<td>26.05</td>
<td>45.30</td>
<td>4.05</td>
</tr>
<tr>
<td>Ripen Seeds</td>
<td>6.80</td>
<td>15.84</td>
<td>8.95</td>
<td>23.41</td>
<td>45.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Flowers</td>
<td>6.13</td>
<td>13.00</td>
<td>9.17</td>
<td>32.00</td>
<td>39.70</td>
<td>16.40</td>
</tr>
</tbody>
</table>

Mangroves are used in different parts of the world as a substitute to traditional feed for livestock (MacNAE, 1968; Wilkie, 1995). Leaves of *Avicenna marina* have desirable protein, and carbohydrates, where the propagules were reported to be used as food at famine times and/or war, for their high potentialities as food for human and fodder for domesticated herbivore vertebrates (Ong, 1995; Mouafi, 2014). High crude fat values of *Avicenna marina* compared to other halophytes confirm the findings of Zimmermann et al. (1994) that the osmoregulation processes are achieved through secretion of compounds of lipoid nature. The stakeholders ensure that animals fed on *Avicenna sp.* were healthy, higher milk and meat yield, also watering intervals increased. Random feeding on mangrove foliage by camels considered to be the main biological destructive factor to mangroves in Sudan, as confirmed by herd owners. On the other hand, the interviewed fishermen, insured that mangrove swamps were among the richest commercial fish sites along the Red Sea Coast. (Bashir, 1998; Guo, et al., 2008; Feng, et al., 2009; Sudirman, et al, 2014). Mangroves are endangered with extinction and threatened with increasing human activities, coastal development, tourism and other land use activities, as well as the climate changes due to global warming, which need more modeling research to develop suitable precautions. Delay of conservation programs execution complicates the situation (Al-Ghadban and Gharib, 2009).

Mangroves are the most hostile environment along coasts with considerable adaptation to fluctuating tidal movements and saline regime, where limited plant species can survive, consequently are extremely important to the nutrient budgets of adjoining estuaries due to decomposition of dead parts and litter in the mangrove swamp, (Rey and Rutledge, 2015).

**Phytochemical Screening:**

Novel medicines groups namely Flavonoids, Terpenoids, Alkaloids as well as Steroids, Phenol Flavonoids, Cardiac glycosides and tannins which were found (Table 2) in the plant, may justify the healthiness and the high milk and meat yield of animals grazing on *Avicenna marina* foliage ((Johanna and Jyh-Lurn, 2007; Han, 2007).
Table (2): Phytochemical Screening of *Avicenna marina*

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>A.marina</th>
<th>Biological Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>antitumor, antihypertensive, Muscle relaxant and antiprotozoal agent.</td>
<td>Aniszewski</td>
</tr>
<tr>
<td>2</td>
<td>Terpenoids</td>
<td>cough treatment and asthma</td>
<td>Edeoga et al.</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>anti-oxidant and inflammatory drug. congestive heart failure and cardiac arrhythmia</td>
<td>Moss</td>
</tr>
<tr>
<td>4</td>
<td>Cardiacglycosides</td>
<td>+</td>
<td>McMurray and feffer</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>metal ion chelators antioxidants.</td>
<td>Tukiran</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>+</td>
<td>Harborne</td>
</tr>
<tr>
<td>7</td>
<td>Saponines</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Productivity:
The mangrove forest structure, trees and shrubs height, density and canopy area could be attributed to inter-tidal action, sedimentation dynamics and hydrological pattern (Young and Harvey, 1996). Specific Leaf area indicator (SLAI) (Table 3) expressed the productivity of *A. marina*. Values range between (0.43 - 0.08 g/cm²) for mature and young leaves, respectively. Low leaf area of adult trees reflected in high (SLAI) compared with young seedlings. This could be attributed to the conservative characteristics of *A. marina* expressed in its low rate of growth (Sasitorn, *et al*; 2012).

Animals feeding on mangrove in Sudan had shown selective behavior, where the seedlings were eaten first (Bashir, 1998), so it is faithful for the grazing animals to feed on young seedlings.

Table (3): *Avicenna marina* measurements for Specific Leaf Area Indicator (SLAI)

<table>
<thead>
<tr>
<th>No</th>
<th>mLÁ/cm²</th>
<th>mLW/g</th>
<th>mSLAI g/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.0</td>
<td>6.0</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>0.22</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Note:
1: Mature Leaves ; 2: Young Leaves ; m: mean ; LA: LEAF Area; LW: Leaf weight; SLAI: Specific Leaf Area Indicator

Conclusion and Recommendation:
The present study emphasizes and creates awareness towards mangroves as potential source of nutrients and their potentiality as fodder, containing considerable amounts of carbohydrates, lipids and crude protein. In addition presence of novel medicine groups such as Flavonoids, Terpenoids, Alkaloids as well as Steroids, Phenol Flavonoids, Cardiac glycosides and Tannins may improve the performance of animal health. The Red Sea water body, gives the Red Sea State (RSS) climate its peculiarity, so the study suggested addition of *Avicenna marina* to the list of fodder plants in Sudan. It could be cultivated in estuaries where mangroves grow naturally, in experimental farms as extension for the existing mangrove stands. Seedlings could be harvested and used as fodder. Mangrove foliage could be consumed by animals, either as fresh hay (green/
dry) or in silage form. Sewage water from Port Sudan city and the seasonally natural running water during rainy season could be used for irrigation. Identification and selection of the most vulnerable areas for mangrove cultivation might be based on research recommendations, and the appropriate management strategic policies should be adopted.

REFERENCES:


