ABSTRACT

There are several types of mangrove sp. are found in Sundarban area in West Bengal. Only limited number of plant sp. thrive in mangrove ecosystem. The sp. like Avicennia alba, A. marina, A. officinalis, Sonaratia apatala, S. casiolar is, Heritiera fomes are consumed either as vegetables or beverages. Fruits of Avicennia marina is popular as vegetables and it has become very popular around Jharkhali area in Sundarban and some along coastal area of Gujarat. Main aims and objectives of the present study to evaluate the potential nutritive value and phytonutrients activity of Avicennia marina fruit. For identification of the phytonutrients in Avicennia marina fruits GC-MS analysis process is used. Several important phytonutrients are found. Among them Quinoline is very important compound which act as an anti-oxidant also. The findings of this study concluded that the fruits of Avicennia marina have high nutritive value and had potential bioactive substances that may be used as pharmaceutical ingredients for formulation of new or prospective potent drug to cure wide range of metabolic diseases.

KEYWORDS: Mangrove Fruits, Nutritive Value, Phytonutrients, Indian Sundarban.

INTRODUCTION

Avicennia marina, commonly known as grey mangrove, is a species of mangrove tree classified in the plant family Acanthaceae (formerly in the Verbenaceae or Avicenniaceae). It has smooth light-grey bark made up of thin, stiff, brittle flakes. This may be whitish, a characteristic described in the common name. THE LEAVES are thick, five to eight centimetres long, a bright, glossy green on the upper surface, and silvery-white, or grey, with very small matted hairs on the surface below. The species can tolerate high salinity
by excreting salts through its leaves. The leaf under surface has glands that excrete excess salt.\[^1\]

*Avicennia marina* species, it has aerial roots (pneumatophores); these grow to a height of about 20 centimeters, and a diameter of one centimeter. These allow the plant to absorb oxygen, which is deficient in its habitat. These roots also anchor the plant during the frequent inundation of sea water in the soft substrate of tidal systems. THE FLOWERS range from white to a golden yellow colour, are less than a centimeter across, and occur in clusters of three to five.

![Fruits of Avicennia marina.](image)

THE FRUIT contains large cotyledons that surround the new stem of a seedling. This produces a large fleshy seed, often germinating on the tree and falling as a seedling.\[^2\] In the Malaysian Northern Territory, the fruits are roasted and eaten, the leaves and bark are used for medicinal purposes, such as marine stings and skin disorders and the leaves provide flavoring when cooked with mussels.\[^3\] The fruits have several nutritive values. In Gujarat coasts it is a popular vegetable. Despite its wide spread use.

**MATERIALS AND METHODS**

The *Avicenna marina* fruit are collected from the Sundarban, in the month of August - November. The objectives and aims of this study are to determine nutritive value of the fruit of *Avicenna marina*. To investigate the nutritive value of this mangrove fruit e.g. moisture content, total fiber content, total calorie, carbohydrate, protein, fat, iron, calcium, sodium, potassium, copper, zinc, manganese, magnesium and vit- C content for which following methods are followed i.e.
(A) Methods of Nutritive Value Estimation

- Determination of moisture content.\[^{10}\]
- Estimation of fiber content.\[^{10}\]
- Gross Calorific Value Kcal/Kg Indian Standard Methods of determination of Calorific value.\[^{5}\]
- Protein % By Kjeldal method.\[^{6}\]
- Carbohydrate % by mass: Indian Standard Specification by Acid Hydrolysis Method.\[^{7}\]
- Estimation of fat by Soxhlet method.\[^{8}\]
- Determination of micro nutrients by ICP-OES.\[^{9}\]
- Estimation of Vit-C (ascorbic acid).\[^{10}\]

(B) GAS CHROMATOGRAPHY- MASS SPECTROMETRIC (GC-MS) Analysis for identification of phytonutrients:

Preparation of extract

The dried fruit of *Avicennea marina* was dissolved in absolute ethanol (1mg/ml) and extract by the ultrasonicator. Then the extracted fruit sample (10µl ) Was injected for gas chromatography- mass spectrometric (GC-MS) analysis.

Instruments and Chromatographic Conditions

GC-MS technique was used to identify the phyto-constituents present in the extract. The plant extract was analyzed using Agilent Technologies 6890 N Network GC system & interfaced to Agilent Technologies 5973 Inert Mass Selective Detector employing the following conditions: column DB-1 ms fused silica capillary column (30X0.25 I.D.X 0.10 Film, composed of 100% Dimethyl polysiloxane) chosen for improved signal to noise ratio for better sensitivity and mass spectral integrity, operating in electron impact mode; helium (5.0) was used as carrier gas at a constant flow of 1ml/min. The injector, MS Source & MS Quadrupole temperature were fixed at 250°C, 230°C & 150°C respectively and turbo Speed of the pump was 100%. The oven temperature was programmed from 50°C (isothermal for 5 minutes), with an increase of 10°C/min to 100°C (isothermal for 2 minutes), then 10°C/min to 300°C (isothermal for 5 minutes) For tuning of the MSD in EI mode Perfluoro-tributylamine (PFTBA) was used as tuning compound. Mass spectra were taken at 2235 EM Volts and fragments from 69 to 502.\[^{11}\]
RESULTS

Table. 1: Moisture and fiber content in 100gms of fruit.

<table>
<thead>
<tr>
<th>Name of the fruit</th>
<th>Moisture (gm)</th>
<th>Fiber (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina</td>
<td>66.84 ±1.54</td>
<td>4.96 ±0.72</td>
</tr>
</tbody>
</table>

Table. 2: Macro Nutrient content in 100gms of fruit.

<table>
<thead>
<tr>
<th>Name of the fruit</th>
<th>Total calorie (Kcal)</th>
<th>Carbohydrate (gm)</th>
<th>Protein (gm)</th>
<th>Fat (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina</td>
<td>226 ±5.33</td>
<td>23.75 ±1.08</td>
<td>3.71 ±0.95</td>
<td>12.15 ±1.01</td>
</tr>
</tbody>
</table>

Table. 3(a): Micro Nutrient content in 100gms of fruit.

<table>
<thead>
<tr>
<th>Name of the fruit</th>
<th>Calcium (mg)</th>
<th>Iron (mg)</th>
<th>Sodium (mg)</th>
<th>Potassium (mg)</th>
<th>Zinc (mg)</th>
<th>Copper (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina</td>
<td>0.178 ±0.716</td>
<td>0.30 ±0.021</td>
<td>0.51 ±0.43</td>
<td>0.08 ±0.106</td>
<td>0.021 ±0.07</td>
<td>9.60 ±1.79</td>
</tr>
</tbody>
</table>

Table. 3(b): Micro Nutrient content in 100gms of fruit.

<table>
<thead>
<tr>
<th>Name of the Fruit</th>
<th>Magnesium (mg)</th>
<th>Manganese (mg)</th>
<th>Selenium (mg)</th>
<th>Selenium (mg)</th>
<th>Cobalt (mg)</th>
<th>Boron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina</td>
<td>1870.12 ±159.80</td>
<td>14.55 ±0.769</td>
<td>0.449 ±0.28</td>
<td>0.449 ±0.28</td>
<td>0.1495 ±0.152</td>
<td>44.37 ±0.73</td>
</tr>
</tbody>
</table>

Table. 4: Vitamine-C content in 100gms of fruit.

<table>
<thead>
<tr>
<th>Name of the fruit</th>
<th>Vit-C (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina</td>
<td>83.3 ±0.656</td>
</tr>
</tbody>
</table>

Result of GC–MS Analysis

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST)/National Bureau of Standard (NBS) and Wiley having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST/NBS and Wiley libraries. The name, molecular weight and structure of the components of the test materials were ascertained. The GC–MS generated mass spectra. Identification of the constituents was performed by comparing the recorded mass spectra with the standard mass spectra from the National Institute of Standards and Technology (NIST) MS spectral library. Thirty-two compounds were identified following comparison with the spectral library. However, the main constituents were considered “identified” or “detected” when their mass spectral fit values were at the default value of 90% or above. Hence, 9 compounds were detected and identified using the spectral library. The GC–MS analysis of the methanol extract of the fruit is shown in Figure 1.
Table 5: From this Above Chromatogram these Peaks Identified the following Phyto Constituents.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Retention Time</th>
<th>% Peak Area</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.50</td>
<td>3.13</td>
<td>2-Furancarboxaldehyde</td>
</tr>
<tr>
<td>2</td>
<td>6.87</td>
<td>2.58</td>
<td>4(1H)-Pyrimidinone</td>
</tr>
<tr>
<td>3</td>
<td>11.42</td>
<td>0.51</td>
<td>Thiazole</td>
</tr>
<tr>
<td>4</td>
<td>12.21</td>
<td>0.75</td>
<td>Phenylethyne</td>
</tr>
<tr>
<td>5</td>
<td>13.75</td>
<td>0.85</td>
<td>2-Furanmethanol</td>
</tr>
<tr>
<td>6</td>
<td>16.86</td>
<td>4.66</td>
<td>Maleic hydrazide</td>
</tr>
<tr>
<td>7</td>
<td>18.01</td>
<td>2.95</td>
<td>Benzeneethanol</td>
</tr>
<tr>
<td>8</td>
<td>19.18</td>
<td>0.46</td>
<td>Diethyl Phthalate</td>
</tr>
<tr>
<td>9</td>
<td>23.53</td>
<td>4.37</td>
<td>Tetradecanoic acid</td>
</tr>
<tr>
<td>10</td>
<td>31.46</td>
<td>1.68</td>
<td>Quinoline</td>
</tr>
</tbody>
</table>
Fig. 3. The structures of the compounds identified in the *Avicennia marina* fruit extract.
DISCUSSION

Present study show that the mangrove fruit which is taken for proposed work has several nutritive values. The calorific values of the fruit is so high which reveals that this fruit is beneficial for those who require extra calories. e.g. under weight, burn’s patient etc. Among carbohydrate, protein, fat content this fruit contain medium amount of fat. As we all knew that protein is a very important macro-nutrient which acts as building units in our body, maintain water an p\textsuperscript{H} balance in our body and protein aids metabolic functions through enzymes (digestive and cell enzymes are protein in nature), transport agents, and hormones.\textsuperscript{[17]} On the other hand fat also have some very important role in our body. e.g. provide high energy value (approx 9.3 kcal/1gm of fat), carry fat-soluble vitamins and aid in their absorption, in diet it add flavor to foods and contributes to feeling of satiety or satisfaction after meal.\textsuperscript{[17]}

Sodium and potassium content are not so high. So at a glance it reveals that this fruit is beneficial for those who are suffering from kidney disease, syndrome of edema etc. These apart in humans, sodium is an essential nutrient that reduce blood pressure and sodium concentration in the kidney result in the production of rennin, which in turn produces aldosterone and angiotensin, retaining sodium in the urine. Because of the increase in sodium concentration, the production of renin decreases and the sodium concentration returns to normal.\textsuperscript{[18]} Sodium is also important in neuron function and osmoregulation between cells and the extracellular fluid, their distribution mediated in all animals by Na\textsuperscript{+}/K\textsuperscript{+}-ATPase,\textsuperscript{[19]} hence, sodium is the most prominent cation in extracellular fluid.

Potassium is the major electrolytes inside cells (intracellular) the Osmotic effect holds water inside cells & counter Balance the osmotic effect of Sodium (Na).It helps conversion of Blood glucose $\rightarrow$ stored glycogen, Synthesis of muscle protein & energy production. Potassium ions also play a role in nerve impulse transmission to stimulate muscle action. Equal amount of Na & K help to prevent the development of hypertension, this diet is called DASH (Dietary approaches to stop Hypertension).\textsuperscript{[20]}

This fruit contain below 1mg/100gm of zinc. Zn plays important structural role as a component of several proteins and function as an intra- cellular Signal in brain Cells. Metallothionein, is the most abundant, non-enzymatic zinc containing protein which has low-molecular weight & rich in cysteine. Zn is abundant in the nucleus where it stabilized RNA &
DNA structure and for the activity of RNA polymerase. Zn also functions in chromatin protein involves in transcription & replication.[20]

Cupper (Cu) content is quite good for *Avicennea marina*. Cu has frequently been called the “IRON TWIN” because both Cu & Fe are metabolized in much the same way & both are Components of cell enzyme. Cu protein complex known as ceruloplasmin which plays an important role in the transport of Fe in transferrin for hemoglobin synthesis. Cu plays a part in preventing anaemia by stimulating the Synthesis of the hemo or globin functions of the haemoglobin molecules. Cu is the part of the enzyme tyrosinase which helps to convert tyrosin -> melanin.[21]

This fruit contain high amount of magnesium which plays as catalyst in many metabolic reactions. Mg is required to activate the enzyme in the oxidative phosphorylation of ADP to ATP & also for the return of ATP to cyclic AMP which turns regulate parathhormone secretion.[23]

Mg is involves in conduction of nerve impulse that stimulate muscle contraction and also help to prevent Cardiovascular disease by maintain normal heart beat & blood pressure.[22]

The Manganese content is also better. Manganese is an essential trace nutrient in all forms of life. The classes of enzymes that have manganese co-factors are very broad and include oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins and integrins. The reverse transcriptases of many retroviruses (though not lentiviruses such as HIV) contain manganese. The best-known manganese-containing polypeptides may be arginase, the diphtheria toxin, and Mn-containing superoxide dismutase[Mn-SOD].[24] *Avicennea marina* contain little amount of selenium. Se is essential to maintain glutathione Peroxide (GHS – PX) which acts together with other anti-oxidants & free radicals scavengers. The anti-oxidant effects of Se & vitamine-E may reinforce each other by the overlap of their protective actions against oxidative damage.[20]

Boron content is 44.37 mg/100gm of the fruits which is quite good. Boron has several important functions in our body. Its deficiency alters brain function and reduces bone composition, structure & strength. Boron is also thought to complete with some enzyme for the co-enzyme NAD. Though cobalt content is below 1 mg for all fruits but the well Known essentials role of cobalt is a component of Vit-B12 (Cobalamin). This Vitamin is essential for the maturation of red blood Cells & the normal function of all cells. Methionine amino
peptidase, an enzyme involved in the regulation of translation (i.e. of DNA to RNA), is the only enzyme in humans known to have an established requirement of this trace element.\textsuperscript{[20]}

Vitamin – C is an essential water soluble vitamin in our daily life. It has several critical important functions on our body. e.g. act as a protective agents as antioxidant to protect our body from free radicals damage which are associated with increased risks of developing cancer and heart disease. Also necessary to build and maintain strong tissues by its involvement in collagen synthesis and plays a role in many metabolic and immunological activities. The main important role is to prevent SCURVY. In present study, fruit of \textit{Avicennia marina} contain a little high amount of vitamin- C which is approx. 83.3mg/100gm.\textsuperscript{[25]}

The GC–MS analysis of the fruit extract revealed the presence of 10 compounds. These 10 compounds were mostly fatty acids or fatty acid esters. Some compounds have great antioxidant activity also.2-Furancarboxaldehyde acts as an Antioxidant, anticancer 12’-therapeutic agents toward breast cancer cells. The acute toxicity of 2-furancarboxaldehyde,5-(hydroxymethyl)-(5-HMF) is very low. An acute Oral LD50 of 3100 mg/kg was observed in a study on rats.\textsuperscript{[14]} \textbf{Thiazole} act as an Anti-breast cancer activity.

\textbf{Quinoline} has an anti-oxidant activity itself. Oxidative stress is defined as an imbalance between pro-oxidants and antioxidant systems. In fact it is characterized by an increase in the reactive species (RS) production and/or a decrease in the antioxidant defenses. RS are a normal product of cellular metabolism, and they are involved in important biological functions. However, excessive production of RS may generate oxidative stress, causing tissue damage. Furthermore, several diseases are related to oxidative stress, such as such as cardiovascular and neurodegenerative diseases, cancer, atherosclerosis, rheumatoid arthritis, hypertension, ischemia and diabetes mellitus. In this sense it is important to use antioxidant molecules that decrease oxidative stress, reducing the incidence of various diseases. It is therefore important to highlight organo selenium compounds, which exhibit different pharmacological properties, such as antioxidant action. Parallel to organo selenium compounds are quinoline derivatives. Quinolines are synthetic or natural heterocyclic compounds with interesting biological activities. Although several research groups have studied the mechanisms involved in the pharmacology of organochalcogens), the study of a quinoline derivative containing selenium could be an alternative to search for new compounds with antioxidant properties for the treatment of diseases related to oxidative...
stress. In addition, oxidative stress has been proposed as a mechanism involved in the athophysiology of inflammation and pain. Pain and inflammation are a major clinical problem, and several undesirable side effects caused by the use of analgesic and anti-inflammatory agents occur. In view of this, the development of new drugs with antinociceptive effects for the control of several painful conditions would be very useful as a therapeutic source.\textsuperscript{[12]} Tetra-decanoic acid is also used in medicine industry as Antioxidant, Cancer preventive, Nematicide, Lubricant, Hypocholesterolemic agents.\textsuperscript{[16]} **Maleic hydrazide** is a synthetic compound which has a plant growth regulating action. It is used as a foliar treatment of potatoes to prevent volunteer formation and sprouting during storage. It can also be used pre-harvest to suppress sprout formation in onions.2-Furanmethanol is a flavouring ingredient 2-Furanmethanol belongs to the family of Furans. These are compounds containing a furan ring, which is a five-member aromatic ring with one oxygen atom, four carbon atoms.

The antimicrobial and cytotoxic effects related to these substances are due to the fact that fatty acids, fatty acid ester and aliphatic chains (long chain alkanes and alkenes) normally accumulated in the lipid layer of the cell membrane and mitochondria. Consequently, they disturb the integrity of cell structure which becomes permeable.\textsuperscript{[13,15]} Unsaturated fatty acids are also known to lower blood cholesterol levels. This phenomenon results from the ability of these compounds to alter efflux pumping and, thus, pH, increase membrane permeability.\textsuperscript{[4]}

**CONCLUSION**

This study provides valuable preliminary data on the nutritive value profile of *Avicennia marina* fruit. Further investigations involving pre-clinical and clinical studies of the extract will be necessary to determine the safe dose before it is prescribed as a drug, in order to protect the population from the possible toxic effects of this fruit. Thus it perpetrates from the above observation that fruits of *Avicennia marina* are on the whole lot beneficial to the human being because of its presence of several phytonutrients (detect by GC-MS analysis) and non-toxic to the human. Therapeutic mechanism of a plant can be better understood with a proper investigation of its active ingredients. The compounds identified by the GC-MS analysis of ethanolic extract of *Avicennia marina* fruits relate their applications in folklore medicine. It envisages propagation of more such plants in the home - stead land of Sundarban people.
ACKNOWLEDGMENT

I appreciate the contribution of all those who participated in this study. I sincerely thankful to Dr. Pranabea Sanyal (Jadavpur university) and Dr. Suniti Ghosh Chatterji(Calcutta University). I am beholden to Dr. Utpal Roychowdhery (SD) scientist chemical, National Test House, kolkata for his advice, encouragement and necessary suggestions especially for pharmacognostic identification of the fruit samples for this work.

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