



ASSESSMENT OF MICRO-NUTRIENT POTENTIALITY AND IDENTIFICATION OF BIO ACTIVE COMPONENTS OF THE *AVICENNA ALBA* FRUIT (MANGROVE) IN SUNDERBAN, INDIA

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ABSTRACT

The Sundarban is a natural region in the Bengal region comprising Eastern India and Bangladesh. It is the largest single block of tidal halophytic mangrove forest in the world. Only a limited number of plant sp. thrive in mangrove eco-system. In Indian Sundarban the mangal vegetation comprises of 94 intertidal sp. but less than 10 sp. are utilized by the locals as food & fodder materials. *Avicenna alba*, is one of them. Local people of Sundarban use this fruit as vegetable. The present study was aimed to identify the various micro-nutrients and phytochemical constituents by ICP-OES analysis and GC-MS analysis. The results for the first time revealed the presence of several bioactive components in the fruit of *Avicenna alba*.

KEY WORDS: Mangrove fruits, Indian Sundarban, phytochemical, bioactive components.



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INTRODUCTION

The Sundarban is the largest single block of tidal halophytic mangrove forest in the world¹. The Sunderban is an UNESCO World Heritage Site (1997), most of which situated in Bangladesh and the remaining in India. While most of the mangroves in other parts of the world are predominantly characterized by members of the *Rhizophoraceae*, *Avicenniaceae* or *Combretaceae*. Several mangroves have local medicinal use, but less than 5% fruits are edible. If edible fruits proved non-toxic then they can be included in regular diet for poor Sundarban local peoples. There are several studies have already been carried out on the nutritive values and presence of potent micronutrient in the fruits of different mangrove plant species^{2,3,4} as well as safety evaluation by acute and chronic toxicity studies after in-vivo exposure in animals but less studies have been documented with fruits of mangrove plants^{5,6}. Recently, the fruit of *Avicennea alba* has increased in popularity by local people in and around Sundarban, not only because of its green coloration and economic value as a food product, but also because of its health benefits. Knowing the valuable effect of these mangroves, the main aim and objectives of the present project to estimate the micronutrient values of the mangrove fruit of *Avicennea alba* and to evaluate the pharmaceutical roles of this fruit through GC-MS analysis and also increase the awareness among the people to protect the mangroves. *Avicennea alba* forms a low, dense bushy crown often

branching near the base of the trunk. The shrub does not grow more than about 20 meters (66 ft) high. Few descriptions about *Avicennea alba* tree are given below:

- **Root:** The roots are shallow and send up a large number of pencil-shaped pneumatophores. These aerial roots help with gas exchange and also play an important part in the exclusion of salt from the plant's vascular system⁸.
 - **Trunk:** The trunk has smooth, greenish black bark which is finely fissured and does not flake.
 - **Leaves:** The dark green leaves 15 cm (6 in) long and 5 cm (2 in) wide have a silvery grey underside and grow in opposite pairs.
 - **Flowers:** The small, orange yellow flowers, borne in a racemose inflorescence, have 4 petals and a diameter of about 4 millimeters (0.16 in) when expanded.
 - **Fruit:** The fruits are greyish-green capsules and conical in shape with an elongated beak up to 4 centimeters (1.6 in) long. Each contains a single seed⁹.
- **Scientific classification**
- Kingdom: Plantae
 - Division: Magnoliophyta
 - Class: Magnoliopsida
 - Order: verbenales
 - Family: Avicenniaceac
 - Genus: *Avicennia*
 - Species: *A. alba*
 - Binomial name : *Avicennea alba*⁷
Local name: kalobani



Figure no. 1
Fruits of Avicennea alba

- Uses: *Avicennea alba* is a fast growing species and is sometimes planted, along with *Sonneratia* and *Rhizophora*, to help prevent coastal erosion. The timber from *Avicennea alba* does not make good firewood or charcoal but is used in the smoking of rubber and of fish. An extract of the heartwood is used in herbal medicine to make a tonic and the resin has been used in birth control. The seeds are boiled and eaten as a vegetable and are sometimes available in local markets⁹.

METHODS & MATERIALS

Procurement of plant materials

The tubers of *Avicennea alba* were collected from the Jharkhali area of Sundarban, West Bengal (India). Authentication was confirmed by BOTANICAL SURVEY OF INDIA (BSI) West Bengal, (India).

A. ESTIMATION OF MICRONUTRIENT VALUE

Evaluation of the micronutrient values of given mangrove fruits through the Determination of micro nutrients by ICP-OES¹⁴

B. GAS CHROMATOGRAPHY- MASSSPECTROMETRIC (GC-MS) ANALYSIS

Preparation of extract

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

RESULTS

Table 1(a)
Micro Nutrient content in each 100gms of fruit

Name of the fruit	Calcium (mg)	Iron (mg)	Sodium (mg)	Potassium (mg)	Zinc (mg)	Copper (mg)
<i>Avicennea alba</i>	0.93±0.43	0.27±0.40	2.62±0.51	1.294±0.85	0.012±0.62	14.098±0.74

Table 1(b)
Micro Nutrient content in each 100gms of fruit

Name of the fruit	Magnesium (mg)	Manganese (mg)	Chromium (mg)	Cobalt (mg)	Boron (mg)
<i>Avicennea alba</i>	1144.78±237.05	5.04±0.763	0.195±0.59	0.0717±0.441	43.04±0.642

Results of GC-MS analysis

Gas chromatography was used to know the other components which are presents in those fruits and few of them should be beneficial for human. The results pertaining to the GC-MS analysis leads to the identification of twenty pharmacologically important compounds from the GC fractions of the ethanolic extracts of *Avicennea alba* respectively. These compounds were identified through mass spectrometry

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 Quadrapole 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 502¹⁵.

Identification of components

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.

attached with GC. The various components present in the ethanolic of *Avicennea alba* as detected by GC-MS analysis and among them twelve compounds are presented in Table 2, respectively, along with their pharmacological activity. Accordingly, figure 1A represents the Total Ion Chromatogram of ethanolic extracts of *Avicennea alba*. Moreover it is also seen that themajority of the said compounds showed greater peak area % in the ethanolic extract.

Table 2
Phytoconstituents present in Ethanolic Extract of *Avicennea alba*¹³

Sl. No	Retention Time	% Peak Area	Compound	Pharmacological Activity
1	9.609	0.79	Aldicarbsulfoxide	Anti-oxidant
2	10.112	9.33	Acetamide	Antihypertensive agent
3	11.068	2.56	Ethanone,	Anti microbialactivity, antoconvulsant
4	14.404	0.91	Diethyl Phthalate	Antimicrobial activity
5	18.089	0.24	Hexadecanoic acid, methyl ester	Antimicrobial activity, antifungal activities
6	19.422	0.37	Diphenidol	Antiemetic agent used in the treatment of vomiting and vertigo
7	19.891	3.64	Phytol	Antimicrobial, antinociceptive and antioxidant activities
8	20.131	1.32	Oleic acid, 3-hydroxypropyl ester	Anti-inflammatory, Antiandrogenic Cancer preventive, DermatitigenicHypocholesterolemic, AnemiagenicInsectifuge
9	25.933	0.43	Corydine	Sedative
10	27.393	12.67	Vitamin E	Antiageing, analgesic, antidiabaticantiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic.
11	28.474	3.47	Stigmasterol	Antihypercholesterolemic
12	28.983	7.10	beta-Sitosterol	Anti-bacterial, anticancer (breast, cervix and lung), antiinflammatory, antioxidant, epatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic

Figure 2(a)

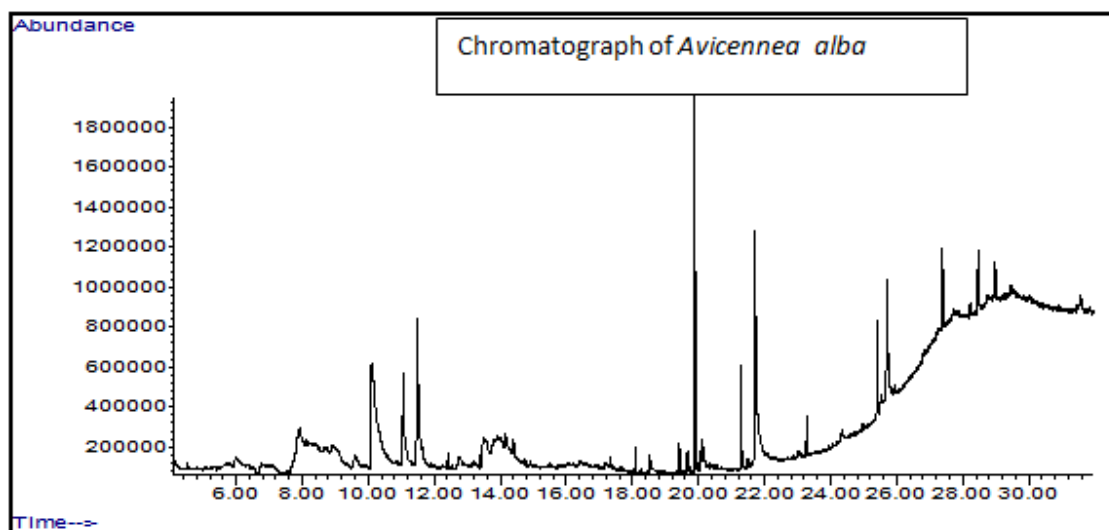
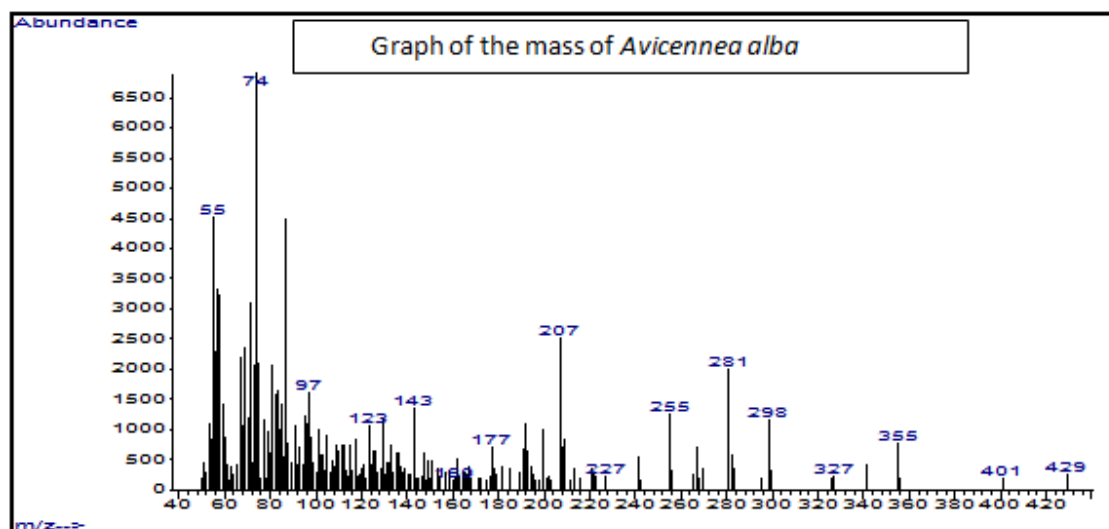


Figure 2(b)



DISCUSSION

It is evident from the Present study that the mangrove fruit which was taken for proposed work has several importance. Sodium and potassium content are not so high for this fruit. So at a glance it reveals that these fruits are 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 normal¹⁶. Sodium is also important in neuron function and osmoregulation between cells and the extracellular fluid, their distribution mediated in all animals by Na^+/K^+ -ATPase;¹⁷ 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)¹⁸. 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 contains 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 & replication¹⁸. Copper (Cu) content is quite good for *Avicennia alba*.

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 transferring 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 \rightarrow melanin.¹⁹ 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 turn regulate parath-hormone secretion¹¹. Mg is involves in the conduction of nerve impulse that stimulate muscle contraction and also help to prevent Cardiovascular disease by maintain normal heart beat & blood pressure²⁰. 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]¹². Boron content is approx 43.05 mg/100 gm of each 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 essential 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 element¹⁸. Plants have formed the basis for traditional medicinal system for thousands of years. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques traditional knowledge system has given clues to the discovery of valuable drugs.⁹ Here in this study we have actually focused on the presence of the phyto-constituents that contribute to the plant versatile use. The different components identified possess diverse medicinal value and are also an important component of other medicinal plants. Among the major components identified Acetamide, Ethanone, Hexadecanoic acid, methyl ester, Diphenidol Oleic acid, 3-hydroxypropyl ester, beta-Sitosterol, Stigmasterol are of immense medicinal value. Aldicarb sulfoxide, phytol, vitamin- E are responsible for the free radical scavenging activity due to their redox properties and can thus attribute to the anti-oxidant property¹⁰ of the fruit of *Avicennia alba*. Besides the study also reveals the fact that the ethanol serves a more potent solvent extractor than others.

CONCLUSION

Thus it perpetrates from the above observation that fruits of *Avicennia alba* are on the whole lot beneficial to the human being and it envisages further propagation of more such plants in the other mangrove areas of India. The present work is a first time determination of micronutrients and phyto-constituents assessment of the

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mangrove fruit from a major ecological hot-spot of West Bengal, Sundarban-the famous mangrove forest in INDIA. The plant *Avicennia alba* (Kalobani) are popular vegetables in mangrove areas of Gujrat now it should also be popularized in Sundarban. Several recipes now may be suggested to Sundarban dwellers in order to popularize & encourage planting along barren patches of the creeks of reclaimed Sundarban. Therapeutic mechanism of a plant can better understood with a proper investigation of its active ingredients. The compounds identified by the GC-MS analysis of ethanolic extracts of *Avicennia alba* fruit's relate their application in folklore medicine. This active principles provide inspiration for further investigation to achieve lead molecules in the discovery of novel herbal drugs. Furthermore, as this fruit is popular in a certain geographical location, more insight into the isolation of active components may lead to low cost drug development against oxidative stress as well as other killer diseases. It can turn out to be a promising nutraceutical in future.

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