EDXRF Quantification of Elements in Mangrove Plants with Medicinal and Anti-Microbial Properties

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The inorganic elemental concentrations of eleven mangrove species collected from the Coringa mangrove forest near Kakinada Bay were determined using EDXRF spectra. Leaves and barks of seven species and leaves of four other species were used as samples. The obtained spectra were used to determine the concentrations of macro elements such as Na, P, S, Cl, K, Ca, as well as micro or trace elements, namely Zn, Cu, V, Mn, Fe, Ni, Co, Sr, Se and Rb. The computed ratios of Na:K and Ca:P were used to interpret the results in terms of understanding their role in traditional medicine. The obtained chemical or elemental profiles of the leaves and bark of the different species of mangroves of Coringa forest provide baseline data on the nutritional status of the Coringa mangrove forest that supports the medicinal and nutritional usage of these species. This may promote the determination or identification of new phytochemical compounds resulting in the development of new drugs or useful applications.

Key words: Mangrove plants; macro elements; medicinal; micro elements; Coringa

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Mangrove forests can be considered as one of the most productive ecosystems in the world in terms of environmental significance and socioeconomic value [1]. These forests are distributed globally along the Ameri-can, Australian, African and South-East Asian coasts. Out of these, nearly half of these ecosystems in Asia are located mainly in Indonesia, Brazil, Bangladesh and India. A major part of the mangrove wetlands in India exists on the country's eastern coast, as there are no major west-flowing rivers. Periodicity of freshwater inflow from north to south on the east coast decreases with tidal amplitude. Correspondingly the diversity of species and the area of mangrove wetlands also decreases towards the south showing the impact of freshwater inflow and the relationship between mangrove wetlands and tidal amplitude [2]. Sundarbans (West Bengal) is the largest mangrove forest in India while Coringa represents the biggest mangrove forest located in the Bay of Kakinada, East Godavari district, Andhra Pradesh. Mangrove plants are a resource for the local inhabitants in the treatment of different ailments such as coughs, colds, dental and skin problems, etc. along with chronic diseases namely diabetes, hypertension and cancer. Ayurvedic medicine is increasingly popular with the public because of its minimal side effects. The chemical constituents of these plants contribute to their physicochemical properties. Bioactive compounds play a paramount role in curing diseases, and also have antimicrobial properties. Secondary metabolites such as alkaloids, steroids, terpenoids and phenolic compounds are responsible for their medicinal and pharmacological properties. For the preparation of drugs, medicinal plants are used as raw materials either singly or in combination [3].

Medicinal plants contain both organic and inorganic chemical constituents, which are responsible for their medicinal and anti-microbial behaviour. But most of the investigations so far were carried out on organic components such as glycosides, essential oils, amines and other compounds, resulting in the evaluation of a variety of compounds facilitating the understanding of their therapeutic impact on different diseases. Bandaranayake et al [4] reported the traditional and medicinal uses of bark, leaves, fruits, roots and stems of a variety of mangroves. Gitishree Das et al [5] reviewed and discussed the potential of certain mangrove plants with suitable bioactive compounds for the preparation of drugs for cancer treatment. Valentin Bhimba et al [6] reported the antibacterial properties of leaf extracts of Avicennia officinalis which can be used for obstructing the growth of cancer cells. A review on the Rhizophora mucronata and Avicennia marina mangroves showed that the methanolic extracts of the leaves from these plants contained anti-diabetic agents [7]. Kanika Arora et al [8] documented the anti-diabetic properties of twenty-two different mangrove plants. Investigations relating to antioxidant, anticancer and anticoagulant activities of the leaves of Lumnizera racemosa and roots of Acanthus ilicifolius were performed by Tanvira Paul et al [9]. They dis-cussed the response of the extracts of those plants to Hep G2 cancer cells, and total phenolic content relative to total flavonoid content. Vijai Lakshmi et al [10] reported anti-malarial activity along with some other medicinal advantages exhibited by the chloroform fraction of Xylocarpus granatum fruit. Earlier researchers [11] observed a strong relationship between phenolic content in the extracts of the leaves and bark of mangrove plants belonging to X. granatum species and their capacity to obstruct alpha-glucosidase because of their protein-binding nature. The secondary metabolites derived from the seed of the Derris trifoliate species was found to be useful in the treatment of a variety of diseases through traditional or Ayurvedic medicine [12]. The leaves of this plant were found to have several therapeutic uses for rheumatism, chronic paralysis, dysmenorrhea, and as an antispasmodic. Phytochemical studies of leaves taken from the Dalbergia spinosa species were carried out by earlier investigators [13]. They mentioned the medicinal usage of these leaves for the treatment of fever, pain, and skin and urinary tract infections.

Extracts of different species of mangrove plants, namely Aegiceras corniculatum, Excoecaria agallocha, R. mucronata and X. granatum have shown anti-fungal activity towards certain strains [14]. Raviku-mar et al [15] studied five Indian mangrove species (Rhizophora apiculata, R. mucronata, Bruguiera cylindrical, Ceriops decondra, A. marina) to shed light on the role of their antibacterial activity toward some bacterial pathogens. Margaret Beula et al [16] concluded that the leaf extracts of A. marina could be used as a potential antiviral drug. Somayeh Rastegar et al [17] evaluated the antifungal activities of both aqueous and ethanol extracts of A. marina and R. mucronata leaves. Shanmugapriya et al [18] studied and clearly expressed the antimicrobial activity of A. marina, C. decondra and A. officinalis by well diffusion assays. Investigations on the antibacterial activity of A. officinalis and A. ilicifolius extracts showed the methanol extract had greater antimicrobial activity towards bacterial and fungal human pathogens, followed by ethyl acetate and chloroform extracts [19]. Shahbudin Saad et al [20] observed the antimicrobial activity of Lumnitzera littorea. Selvam et al [21] investigated the antifungal activity of mangrove plants against Candida albicans and C. glabrata. Oliveira Silva et al [22] evaluated the antifungal potentiality of Avicennia schau-eriana, Laguncularia racemosa, Combretaceae and Rhizophora mangle extracts against yeasts and derma-tophytes of clinical importance.

The leaves of Sonneratia apetala Buch.-Ham. were found to contain antioxidant properties consistent with their role in folk and traditional medicine. This species is also a nutritious vegetable [23]. Phytochemical and elemental analyses of the extracts of S. apetala Buch.-Ham. leaves were carried out to understand the chemical profile of these leaves. Ashfaque Ahmed et al [24] noted that the mineral nutrition data of man-grove species were not available and that this information could be useful as mineral concentration levels are of great importance to herbivores; hence they studied the chemical composition of the leaves of S. apetala from the coastal zone of Bangladesh and found that the correlation of the obtained results with some soil variables was weak. Fathullah Abdullah et al [25] mentioned that very limited work was available in the literature on the elemental content of mangrove plants; therefore, they attempted to study macro and microelements along with non-essential elements of R. apiculata from the Matang mangrove forest in Ma-laysia. Their studies provided baseline data for the industry in Malaysia. Badarudeen et al [26] carried out an analysis of heavy metals in the leaves and stems of some mangrove species with the sediment substratum from three environments: Veli, Kochi and Kannur. All the elements showed higher concentrations in the vegetal (leaves & stems) parts relative to the sediments. Haritha et al [1] described the utilization of the Coringa mangrove forest for commercial, traditional and subsistence purposes, as well as its medicinal and environmental benefits. However, its inorganic chemical or elemental information is not available in the literature. This information provides their nutritional status and is needed to understand the medicinal properties of the respective mangrove plants. Essential trace elements are involved as co-factors in the production of enzymes, and are also important for cell regulation, gene and membrane functions. The leaves and barks of plants are commonly used in traditional medicine. Traditionally, leaf paste is used for the treatment of joint pains, wounds, skin diseases etc., while the bark is used for treating colds, coughs and fever [27]. In Sharavathi Valley traditional medicine centres, leaves and barks of plants are used for medicinal purposes in around 29% and 23% of cases respectively [27]. As mentioned, information on both organic and inorganic chemical profiles is essential for the cumulative understanding of a particular species and to utilize it for the development of new drugs. Based on the above studies, significant attention has been given in the literature to organic components relative to inorganic chemical components. The latter also needs to be investigated to better understand the medicinal usage of mangrove plants. Investigations on secondary metabolites in mangrove plants correlating to their therapeutic usage through leaf and bark parts are shown in Table 1, along with the related references. The inorganic elemental concentrations of the majority of these species are not available in the literature. To obtain the aforesaid information, we used energy-dispersive X-ray fluorescence spectrometry (EDXRF) as it has been found to accurately determine inorganic elemental concentrations.

EDXRF is an analytical technique for the evaluation of elemental concentrations that exhibits several ad-vantages over other methods and is capable of detecting a wide range of elements simultaneously. It is a non-destructive, fast, highly accurate and environmentally friendly technique. EDXRF can be used on bulk, liquid and powder samples. It can also detect particles in the air. By counting the number of photons emitted from a sample, the corresponding elements present may be identified and quantified for each energy. The identification of elements is possible due to their characteristic radiation emitted from the inner electronic shells of the atoms under certain conditions. The emitted quanta of radiation are X-ray photons whose specific energies permit the identification of their source atoms. Therefore, the present work aimed to quantify macro and micro (trace) elemental concentrations in the leaves and bark of the mangrove species listed in Table 1. These were collected from the Coringa mangrove forest of the Eastern Ghats in Andhra Pradesh, India. The obtained results were correlated with their traditional therapeutic usage as given in Table 1. This facilitated obtaining baseline data on their inorganic chemical profiles, indicating the nutritional status of Coringa mangrove plants.

MATERIALS AND METHODS

1. Study Area Description

The present study area, Coringa mangrove forest (Lat. 160 441 to 160 531 N and Long. 820 141 to 820 221 E) is located on the southern side of Kakinada Bay, around 150 km from the southern side of Visakha-patnam city. The name Coringa comes from a river called "Corangi". Coringa mangroves receive fresh water from Corangi and Gaderu rivers, distributaries of the Godavari River and backwaters of Kakinada Bay. Numerous creeks and canals traverse this ecosystem. The Coringa reserve forest area consists of 3,156 hec-tares of land and an extension of the Coringa forest spreads over 9,442 hectares including waterways. The ecosystem of the Coringa forest contains a rich biodiversity of flora and fauna. This

Table 1. 11 Coringa mangrove plant species and their respective medicinal (traditional) uses

S. No.	Scientific name	Family name	Vernacular name	Medicinal use (traditional)	References	Plant Part
1	*X. granatum*	Koen Meliaceae	Senuga	Anti-malaria; anti-cholera; anti-diabetic; anti-cancer; anti-oxidant	5,10, 11	Leaf bark
2	*R. mucronata*	Rhizophoraceae	UppuPonna	Anti-diabetic activity against hyperglycemia	7, 8	Leaf Bark
3	*E. agallocha*	Euphorbiaceae	Tilla	Increases anti-tumor activity	5	Leaf Bark
4	*B. gymnorrhiza*	Rhizophoraceae	Kandriga	Lewis; Anti-tumor activity against Sarcoma 180	5	Leaf Bark
5	*A. officinalis*	Acanthaceae	Nallamada	Human leukemic cell line HL-60; Ehrlich ascites carcinoma cells	5, 6	Leaf Bark
6	*Avicennia alba*	Acanthaceae	Vilavamada	Anti-cancer	5	Leaf Bark
7	*C. decandra*	Rhizophoraceae	Thoraga	Malignant ulcers, buccal pouch carcinogenesis	5	Leaf Bark
8	*D. spinosa*	Fabaceae	Chillinga	Fever, pains, skin and urinary tract infections,	13	Leaf
9	*D. trifoliata*	Fabaceae	Nalla theega	Stimulant, antispasmodic, wounds, anti-malarial, anti-diarrhea, counter-irritant, rheumatism, chronic paralysis, dysmenorrheal, asthma	12	Leaf
10	*L. racemose*	Combretaceae	Thanduga	Anti-oxidant; anti-coagulant; anti-cancer; asthma; blood purification; skin allergy; snake bites; rheumatism	8, 9	Leaf
11	*S. apetala*	Lythraceae	kalinga	Treatment of hepatitis; cataract, diarrheal; hyperlipidemic	13	Leaf

ecosystem consists of mangroves (15 species), phytoplankton (137 species), zooplankton (81 species), microbenthos (126 species), macrobenthos (114 species) and meiobenthos (37 groups) as recorded by the Department of Ocean Development report [28] in 2001.

2. Sample Collection and their Preparation

The leaves and bark were collected from eleven and seven different species, respectively, of mangrove plants in the Coringa mangrove forest, as shown in Fig 1. These samples were handpicked and washed thoroughly with tap water to remove dust and sand particles. They were transported to the laboratory and washed thoroughly with tap water again and then sterilized with distilled water. The samples were spread on blotting paper to remove excess water and dried at room temperature for about 4 h, minimising the possibility of contamination from the surrounding environment. Dried samples were cut into small pieces and oven-dried at 60°C for 20 h. The concentration of potassium (K) has been reported to change if the drying temperature is higher than 70°C [29]. Therefore, to avoid this effect, the oven temperature was pre-set to 60°C. The samples were ground to homogeneity using a clean agate mortar and pestle. 200 mg of the powdered sample was used to form a thin pellet, 13 mm in diameter, of uniform thickness under a pressure of 100 – 110 kg/cm2 for two minutes using a die and pelletizer. The die surface was cleaned with acetone before pelletization to avoid the possibility of contamination from previous samples. X-ray intensity increases with pressure, such that beyond a certain pressure, the X-ray intensity becomes saturated [30]. This effect can be minimized by keeping the pelletization pressure constant for each sample. If the required pressure is achieved speedily, the sample may crack due to the expansion of the air trapped in the sample when pressure is released. Hence, the sample pressure was released several times before the target pressure was reached to avoid sample breakage. Without a binder, fine powder particles may fall off or scatter from the pellet surface and cause contamination of the spectrometer's sample chamber in vacuum mode [30] and thus special care was taken to avoid this problem.

3. Experimental Method and Validation

Energy-dispersive X-ray fluorescence (EDXRF) spectrometry was available at NCCCM, Hyderabad. The instrument consisted of a SPECTRO XEPOS, EDXRF spectrometer and an X-ray tube with a binary cobalt/palladium alloy as an anode, which provides extra sensitivity and lower LODs for specific element groups. This binary alloy anode emits radiation due to palladium excitation that gives the best results for sodium to chlorine, iron to molybdenum and hafnium to uranium, while cobalt emits radiation by its excitation for potassium to manganese. The SPECTRO XEPOS silicon drift detector had higher resolution with low spectral interference, possessing an enlarged surface and maximized active area (20 mm^2). Its high-speed read-out system provided an ultrahigh-count rate of up to 1 million counts per second. This contributed to the system's improved peak to background ratios with extremely low LODs and ultra-high sensitivity. The system used SPECTRO XRF Analyzer Pro operating software.

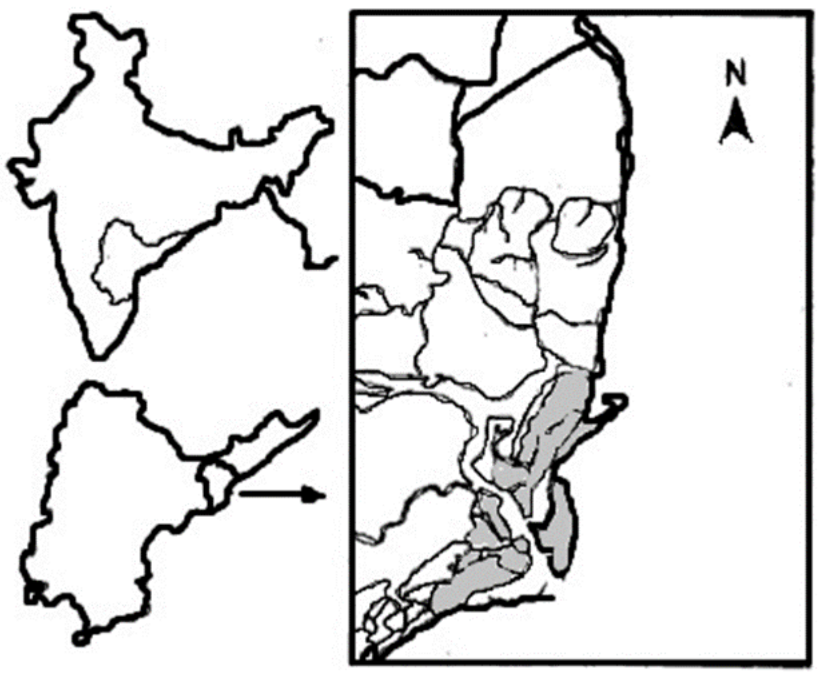


Figure 1. Coringa mangrove area in the Kakinada coast.

Table 2. Concentration of elements (ppm) obtained from NIST (SRM 1515) Apple Leaves and Oriental Tobacco Leaves (CTA-OTL-1)

	NIST (SRM 1515)		(CTA-OTL-1)	
Element	Certified value	Present work	Certified value	Present work
Ca	15260.00	15581.52	3.17±0.12*	3.038*
K	16100.00	15878.04	1.56±0.05*	1.36*
S	18000.00	18780.64	0.732±0.12*	0.684*
P	15900.00	15278.01	2892±134	4723.83
Zn	12.5	14.5	49.9±2.4	46.69
Cu	5.64	7.08	14.1±0.5	12.57
Ni	0.91	0.73	6.32±0.65	6.68
Fe	83.00	71.70	989	1000.34
Mn	54.00	47.98	412±14	421.12
Cr	0.30	1.15	2.59±0.32	2.13
Ba	49.00	67.97	84.2±11.5	82.26
Sr	25.00	29.29	201±20	205.27
Rb	10.20	9.85	9.79±1.27	10.61
Br	1.80	4.26	9.28±1.06	11.78
Se	0.05	0.11	0.153±0.018	0.11

*Concentration in (%)

The qualitative analysis depended on the assurance of the top right position of each peak (centroid) in the spectrum of a sample. Peak centroid channels change of energies was performed with the support of the energy calibration curve. Therefore, distinctive standard reference elements with known energies were secured to cover the interesting energy region of 1–25 KeV for the present energy calibration. The spectra of all the standards were analysed by spectrum analysis software to attain the energy–channel relationship by utilizing the least squares fitting method. Quantitative analysis of the desired samples relied on the curve established by standard specimens. The measured intensity value of the unknown element was then fed into the standard curve to obtain the elemental content. The validity of the EDXRF set-up was performed by analysing standard reference materials (SRM) obtained from the National Institute of Standards and Technology (NIST). Apple Leaves (SRM 1515) and Oriental Tobacco Leaves (CTA-OTL-1) were used for quantification of the elements as shown in Table 2, and to verify the reliability of the data obtained by the present system.

RESULT AND DISCUSSION

From the obtained EDXRF spectra, the concentration values of macro and micro (trace) elements were determined. The concentrations of six macro elements, Na, P, S, Cl, K, and Ca are shown in Table 3. Concentrations of the microelements Zn, Cu, Mn, V, Fe, Ni, Co, Sr, Se and Rb are presented in Table 4. These were detected in all the mangrove plant samples. Some of the trace elements known to be essential to human beings include I, Zn, Se, Cu, Mo, Cr, Mn, Fe, Co, Sr, V. Different trace elements present in the various mangrove plants have therapeutic roles in the treatment of different diseases as well as for the smooth functioning of the human body. The roles of these elements in curing certain ailments are elucidated in the present study.

1. Macro Elements

The observed concentrations of macroelements in the leaves as well as the bark of the Coringa mangroves are presented in Table 3. Ca was found to be present at the highest concentration among the elements in the leaves and bark of all the studied species except in the leaves of *E. agallocha*, *A. officinalis, A. alba and D. trifoliate*. K was found to be the highest among the other elements observed in the leaves of *E. agallocha*, *A. officinalis*, *A. alba* and *D. trifoliate*. The concentration of K was greater than that of Na in the leaves of *E. agallocha*, *A. alba*, *C. decandra*, *D. spinosa*, *D. trifoliata*, *L. racemosa* and *S. apetala*, as well as in the bark of *X. granatum*, *E. agallocha*, *A. alba* and *C. decandra*. The concentration of Ca was found to be higher than that of P in all the samples studied. The order of these elemental concentrations were consistent with results obtained by earlier investigators [23, 25] for mangroves from Myanmar and Malaysia respectively. The concentration levels of Ca in the bark were found to be higher relative to the leaf, as observed in previous studies [25, 32]. The concentration levels of Ca in the bark were approximately 2 to 5 times higher relative to the leaf but significantly, in *A. officinalis*, it was nearly 20 times higher in the bark than in the leaf.

Ca is required for plant cell wall formation; thus, all plants need Ca for proper growth and development. Usually, absorption of elements takes place through the roots, via stems and leaves leading to their accumulation in the respective tissues. Variation of Ca concentrations in different species might be understood on the lines of individual or respective biogeochemical cycles. Ca levels were found to be very high (64,810 ppm) in A. officinalis bark relative to all other samples in the present study. The Ca concentration in X. granatum bark was 36,500 ppm while its value in E. agallocha (bark) was 28,920 ppm. These species are highly useful for cancer treatment [5] and the present results support the development of these species for the production of calcium-rich food items and drugs. Lower elemental concentrations in leaves compared to bark may be due to the excretion of some Ca-based compounds from leaves and their temporal behaviour. As the present samples have higher Ca, these may be more useful as food supplements for elderly people, particularly women at the menopause stage.

Table 3. Concentrations of macro elements (ppm) in 11 different mangrove plants (leaf & bark)

S. No	Scientific name of the plant	Part	Na	P	S	Cl	K	Ca	Na: K	Ca: P
1	*X. granatum*	Leaf	5040± 102.5	501.6± 52.3	888.8± 78.4	734.3± 78.1	3852±9 6.3	12370± 776.9	1.308	24.661
1	*X. granatum*	Bark	1100± 14.7	1458± 127.4	262.7± 26.8	1319±1 45.1	1790±1 28.8	36500± 3102.5	0.614	25.034
2	*R. mucronata*	Leaf	3900± 125.4	410.2± 45.6	452.5± 57.6	6434±4 82.1	2147±4 0.7	10180± 682.0	1.816	24.817
2	*R. mucronata*	Bark	3010± 541.1	369.9± 35.8	179± 15.9	527.3± 71.3	583.8± 40.2	20350± 1058.2	5.160	55.015
3	*E. agallocha*	Leaf	5500± 123.4	562± 12.7	631.8± 54.7	805.7± 78.4	9221±3 50.3	5624±3 76.8	0.596	10.007
3	*E. agallocha*	Bark	3100± 12.3	1672± 241.3	1541± 59.4	3195±2 57.4	7267±6 61.2	28920± 2342.5	0.427	17.300
4	*B. gymnorrhiza*	Leaf	5170± 451	448.8± 51.2	809.7± 57.9	6857±5 24.8	2276±1 09.2	12440± 360.7	2.271	27.718
4	*B. gmnorrhiza*	Bark	1010± 25.1	1767± 261.3	1911± 124.1	6321±2 41.8	763.6± 17.5	21370± 1111.2	1.310	12.094
5	*A. officinalis*	Leaf	4760± 524.7	558.5± 14.8	487.3± 58.7	6015±2 45.8	7055±3 24.5	3210± 308.1	1.483	5.548
5	*A. officinalis*	Bark	64700± 253.1	3081± 23.4	709.1± 25.6	11660± 124.7	9372±4 12.7	64810± 2268.3	6.904	21.035
6	*A. alba*	Leaf	2104± 152.7	530.5± 67.2	348.7± 56.9	4712±2 51.3	8120±2 76.0	4397±2 11.0	0.260	8.288
6	*A. alba*	Bark	4100± 12.4	1782± 12.4	720.2± 56.1	2615±1 23.7	6545±3 01.0	24080± 1468.8	0.624	13.513
7	*C. decandra*	Leaf	3000± 45.9	451.4± 27.4	732.8± 23.4	4928±2 45.7	3724±1 93.6	6863±3 15.6	0.806	15.204
7	*C. decandra*	Bark	2750± 124.3	445.7± 21.5	730.4± 25.1	4609±2 3.4	3759±1 57.8	6705±2 54.7	0.732	15.043
8	*D. spinosa*	Leaf	3110± 11.5	3026± 123.8	2049± 451.3	3361±1 24.9	5174±2 54.5	12830± 590.1	0.601	4.240
9	*D. trifoliata*	Leaf	4720± 321.8	515.1± 21.3	603.8± 51.6	6553±1 53.9	8260±3 79.9	3312±3 04.7	0.792	6.430
10	*L. racemosa*	Leaf	5920± 245.7	4570± 120.8	518.4± 45.7	8116±5 24.7	7521±3 23.4	8666±6 23.9	0.787	1.896
11	*S. apetala*	Leaf	3380± 451.2	483.9± 21.7	1141± 12.8	4823±2 3.8	4069±1 46.4	7894±4 89.4	0.831	16.313

Interestingly, K values seemed to be higher in leaves relative to bark except in the case of *A. officinalis*. As mangrove plants grow in a saline ecosystem, potassium is required in a higher quantity for osmotic regulation purposes [31] and becomes useful for water uptake in the salt gradient environment [32]. Several metabolic and physiochemical activities such as protein synthesis, enzyme activation, photosynthetic metabolism and intracellular electric neutrality are performed with the support of K [33]. An earlier study also observed higher levels of K in leaves compared to bark [25]. K deals with muscle and nerve function as well as functioning as a major component of intracellular fluid. It regulates heartbeat, maintains fluid balance, reduces blood pressure and helps muscles contract [34]. The highest content of K was found in *A. officinalis* (bark) (9,372 ppm) while the lowest (583.8 ppm) was found in *R. mucronate* (bark). The recommended average intake of K is 2,300 mg/d for adult women and 3,100 mg/d for adult men. The upper intake level (UL) for this mineral is unknown, but taking a lot of K supplements can cause hyperkalaemia as a too much K in the blood is a health hazard [35]. The result of the present study indicated that the selected mangroves were found to be rich in K and thus may be used in the development of K-rich products to reduce high blood pressure.

The presence of Cl and Na as macro elements in mangroves is expected as these plants grow in salt water and similar results were reported earlier [23]. Na and Cl are important for life; they are present as positive and negative ions in cell fluids and are the two most highly abundant ions in seawater. Therefore, Cl values in the present study were found to be in the range of 527.3 to 11,660 ppm. Previously, higher concentrations of these elements were observed in *R. mangle* mangroves grown under glasshouse conditions with constant salinity [36]. Thus, higher values of Na and Cl can be understood in line with earlier studies [36].

S was also detected in the present study, exhibiting a minimum value of 179 ppm in *R. mucronate* while the highest value of 2,049 ppm was found in *D. spinosa*. S was also detected in the mangroves from three islands in the coastal zone of Bangladesh, and concentrations of this element were found to vary between the islands [24].

P is essential for the growth of plants; usually, it is absorbed from the soil by the roots. P plays an important role in reproduction and is also required for biological energy transfer processes. It also enhances the use of other nutrients. Among the present selected mangrove plants, the highest concentration of P (4,570 ppm) was detected in *L. racemose* followed by *A. officinalis* (bark) (3,081 ppm) and *D. spinosa* (3,026 ppm) while the lowest concentration (369.9 ppm) was found in *R. mucronate* (bark). The recommended intake of P is 700 mg/d, and the tolerable upper intake level (UL) is 4,000 mg/d for adults, although for those over 70 years, 3,000 mg/d is sufficient. In the present study, the P content of the samples was found to be relatively high, and thus, these mangrove plants may be used as a staple food source for P and also for the primary prevention of osteoporosis.

2. Micro (trace) Elements

The concentrations of micro or trace elements in the leaves and bark of the Coringa mangrove plants are displayed in Table 4. Trace elements play a vital role in several biological activities of living organisms. Ten microelements, Zn, Cu, V, Mn, Fe, Ni, Co, Sr, Se, Rb were observed in the studied mangrove species. These elements were also observed by earlier investigators in mangroves from Myanmar and Malaysia, respectively [23, 25]. Mn was found in high concentrations in most of the species and with nearly two to six-fold higher concentrations in leaves than in bark. Sr and Fe were found at high levels in the majority of the samples. Zn and Cu were present at moderate concentrations which did not exhibit much variation between leaves and bark within the obtained error limits. Non-essential elements such as V, Ni and Co were also detected.

Ranju Chowdhury et al [37] observed a higher concentration of Mn in mangrove plants from the Sundarban mangrove forest. Mn is essential for the reduction of nitrates in green plants, and also for their growth. Along with other elements, Mn is also widely distributed in the soil causing it to be absorbed by different organs of plants. Enrichment of Mn can be ascribed to the weathering of rocks upstream in the Coringa and Gaderu rivers while organic sources arise from leaves and debris of mangrove plants. High levels of Fe were also observed in the present study. This can be attributed to the geochemical oxidant that is formed due to the oxygen released by the roots of the mangrove plants [38]. It facilitates the oxidisation of soluble Fe and Mn ions namely Mn^{2+} and Fe^{2+} into insoluble compounds such as MnO_2 and $Fe(OH)_3$ [31]. Moderate accumulation of both Cu and Zn in the present study may be used for several metabolic processes. These moderate concentrations may be due to a similar type of absorption process [39] as well as limited or restricted mobility. The presence of Fe, Cu, and Zn can be interpreted based on the requirements of these essential micro or trace elements for different metabolic roles such as protein synthesis, chloroplast reactions, enzyme systems, growth hormones and carbohydrate metabolism [40, 41].

Mn is essential for proper bone structure, reproduction, and the proper functioning of the central nervous system. Mn functions as a Lewis acid and catalyst for oxidation. Mn exists in several oxidation states, of which Mn (II) is the predominant form in biological systems. The concentration of Mn in foodstuffs varies considerably but mostly lies below 5 mg/kg. This trace element is also connected with the synthesis of proteins and nucleic acids. A certain interrelation is assumed between Mn deficiency and the development of lupus erythematosus. Mn-rich food is useful in preventing diseases such as diabetes mellitus. According to previous scientific findings, the rate of growth of children largely depends on Mn consumption. Mn was detected in the present samples such as *A. officinalis* (leaf) (777.2 ppm), *D. spinosa* (leaf) (645.4 ppm) and *A. officinalis* (bark) (627.6 ppm); these have a rich concentration of Mn when compared to other mangrove species. The lowest content of Mn (5.7 ppm) was detected in *X. granatum* (bark). In the present work, the concentration of Mn in some of the samples was found to be higher than the permissible limit set by FAO/WHO [42]. The daily required minimum concentration of Mn for adults is approximately 2 to 3 mg, but an intake of 2 to 9 mg/d results in optimum performance.

Iron is required for the production of haemoglobin, myoglobin and certain enzymes. It is necessary for red blood cell formation, and improves brain function in children and women of reproductive age who most often suffer from Fe deficiency. In children, Fe deficiency may be caused by improper nutrition. In women, it arises from the constant loss of blood during menstruation. Fe deficiency is dangerous, particularly during pregnancy. Anaemia arising from Fe deficiency can cause the death of the foetus due to a shortage of oxygen. The necessary intake of Fe is 10 to 30 mg per day. However, a dose of 200 mg per day produces toxic effects. The permissible limit of Fe as set by FAO/WHO [42] is 425.5 ppm. Fe concentrations in the mangrove samples are shown in Table 4. The highest level (890.6 ppm) was observed in *A. alba* (bark), while the lowest (1.4 ppm) was found in *C. decandra* (bark). RDAs for Fe are 10 mg/d for children of 4–8 years, 11 mg/d for males of 14–18 years, 15 mg/d for females of 14–18 years, 8 mg/d for male adults of 19–50, 18 mg/d for female adults of 19–50, and 8 mg/d for all adults >50 years of age. In the present study, the highest concentrations of Fe were found in *A. alba* (bark), *A. officinalis* (bark) and *D. trifoliata* (leaf) which

Table 4. Trace elements and their concentrations (ppm) in 11 different mangrove plants (leaf & bark)

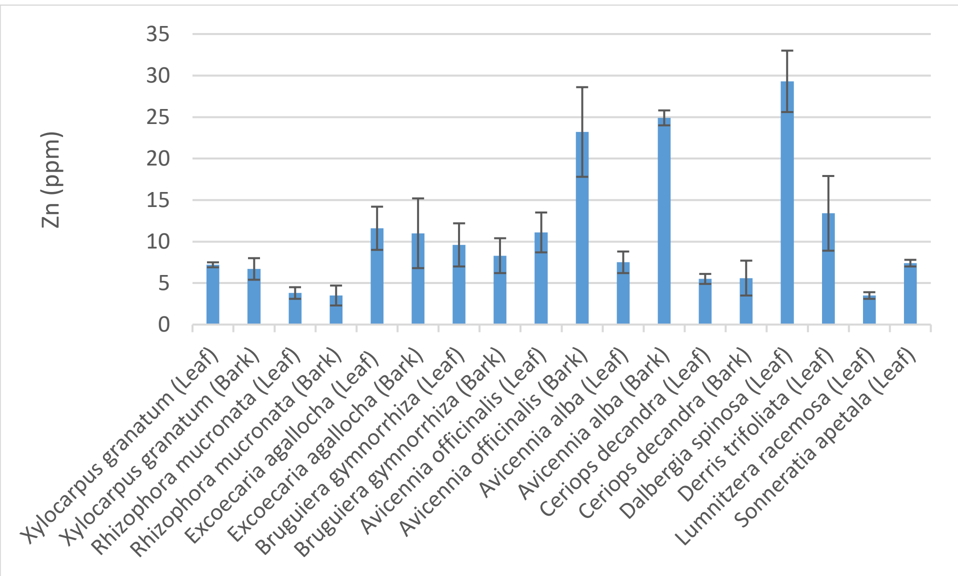
S. No	Species	Part	Zn	Se	Cu	V	Mn	Fe	Ni	Co	Sr	Rb
1	*X. granatum*	Leaf	7.2±0.3	0.5±0.2	2.5±0.2	8.4±1.2	14.8±0.7	91.4±2.6	1.7±0.2	3±0.7	98.6±8.2	0.5±0.1
1	*X. granatum*	Bark	6.7±1.3	0.5±0.8	4±0.07	11.4±2.3	5.7±0.3	10.4±0.7	2.6±0.1	3±0.2	212.4±12.2	0.5±0.5
2	*R. mucronata*	Leaf	3.8±0.7	0.5±0.8	15.6±1.2	14±2.3	349.2±12.3	10.7±02	2.3±0.8	3±0.9	79.8±4.5	0.5±0.2
2	*R. mucronata*	Bark	3.5±1.2	0.5±0.1	1.9±0.2	9.2±2.1	58.6±0.2	17.5±1.4	1.7±0.4	3±0.6	233.5±12.4	0.2±0.3
3	*E. agallocha*	Leaf	11.6±2.6	0.5±0.4	3.3±0.2	9±2.3	96.4±6.3	10.8±0.4	1.8±0.4	3±0.4	43.1±3.4	1.5±0.7
3	*E. agallocha*	Bark	11.0±4.2	0.2±0.4	2.4±0.2	15.6±3.4	51.9±0.6	13.5±1.6	2.1±0.7	2.1±0.1	191.9±15.1	1.1±0.4
4	*B. gymnorrhiza*	Leaf	9.6±2.6	0.5±0.3	21.3±0.3	13.5±2.4	101.6±2.7	11.7±1.2	2.8±0.5	3±0.3	104.9±4.6	0.5±0.6
4	*B. gymnorrhiza*	Bark	8.3±2.1	0.5±0.6	16.9±2.3	18.4±3.4	39.2±2.7	55.2±3.2	3.7±0.6	2.7±0.8	179.4±8.9	0.5±0.7
5	*A. officinalis*	Leaf	11.1±2.4	0.5±0.4	5.7±0.8	8.8±1.1	777.2±10.4	72.9±8.1	2±0.1	1.6±0.4	26.7±0.2	0.5±0.4
5	*A. officinalis*	Bark	23.2±5.4	0.5±0.5	7.3±0.7	29.4±4.1	627.6±20.4	396±14.2	3.5±0.4	3±0.4	367.8±21.4	0.3±0.1
6	*A. alba*	Leaf	7.5±1.3	0.1±0.2	11.9±1.1	11.6±4.6	182.3±14.2	40±0.2	1.7±0.9	1.8±0.2	33.1±2.7	0.6±0.4
6	*A. alba*	Bark	24.9±0.9	0.5±0.6	45.5±2.1	21.3±4.2	108.5±7.3	890.6±9.8	3.3±0.6	3±0.5	179.3±7.4	1.3±0.7
7	*C. decandra*	Leaf	5.5±0.6	0.5±0.5	11.7±1.2	11.3±3.4	83.5±2.6	3.5±0.1	2±0.7	3±0.4	45±2.1	0.2±0.1
7	*C. decandra*	Bark	5.6±2.1	0.5±0.4	7.4±0.7	9.6±4.2	79.7±4.2	1.4±0.1	2±0.4	2±0.4	48.8±3.2	0.2±0.1
8	*D. spinosa*	Leaf	29.3±3.7	0.5±0.2	17.0±1.4	15±3.1	645.4±42.1	116.1±10.1	3.8±0.6	2.3±0.4	44.5±2.6	1.2±0.1
9	*D. trifoliata*	Leaf	13.4±4.5	0.5±0.4	5.7±0.5	9.3±2.3	259.1±2.4	130.6±4.3	2.3±0.4	1.4±0.4	17.8±2.1	0.2±0.1
10	*L. racemosa*	Leaf	3.5±0.4	0.5±0.7	4.3±0.2	5.5±4.3	19.6±1.8	26.5±0.3	2.2±0.4	3±0.7	49.1±3.2	0.5±0.4
11	*S. apetala*	Leaf	7.4±0.4	0.5±0.1	4.3±0.4	9.4±0.7	353±21.6	85.5±6.4	1.7±0.2	3±0.4	58.3±4.7	0.4±0.1

signifies that these mangrove plants may be used for the preparation of Fe-rich foodstuff items for persons with iron deficiency syndrome.

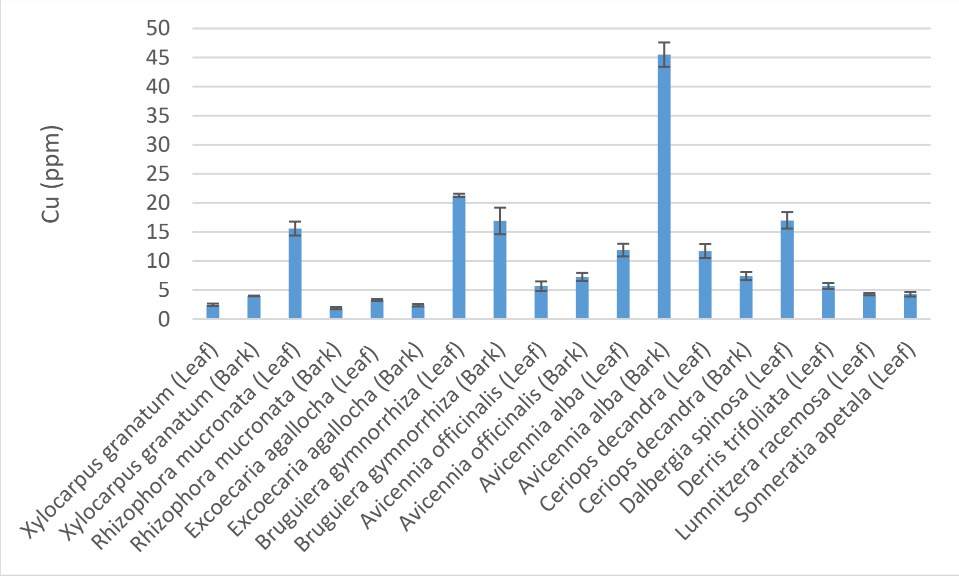
Zn is essential for proper digestion, metabolism, reproduction and wound healing; these processes involve more than 200 enzymes. Zn deficiency is characterized by recurrent infections. The content of Zn in the present samples was found to be in the range of 3.5 ppm – 158.5 ppm, as shown in Table 4. The observed variation in the Zn content of different mangrove plants might be due to changes in the environment, their growing area etc. Zn accumulation in the present study was found to be in the order of *D. spinosa* (leaf) > *A. alba* (bark) > *A. officinalis* (bark) > *D. trifoliata* (leaf) and so on. The RDA value of Zn is 4 mg for children ranging from 4–8 years, 8 mg for 9–13 years, 11 mg for males 14 years and above, 9 mg for female children of 14–18, and 8 mg for female adults above 19. A direct association has been observed between Zn deficiency and cancer [43]. Zn plays a protective role against carcinogenesis and the presence of considerable amounts of Zn in all these samples may be used in the treatment of cancer.

Cu acts as a catalyst to store and release Fe for the formation of haemoglobin and contributes to the function of the central nervous system. It is involved in the production of normal red blood cells and connective tissues. As shown in Table 4, the level of Cu ranges from 1.9 ppm to 45.5 ppm in the present samples. In the present study, the maximum concentration (45.5 ppm) of Cu was obtained in *A. alba* (bark) while the lowest (1.9 ppm) concentration was found in *R. mucronate* (bark). The Cu concentrations in the present samples were lower than the required maximum limit set by FAO/WHO. Adequate intake (AI) levels of Cu for infants i.e., 0 to 6 months and from 7 to 12 months have been set at 200 and 220 g/d respectively. The RDAs for 1–3 years, 4–8 years, 9–13 years, 14–18 years and 19–50+ years of age are 340, 440, 700, 890, 900 and 900 mg/d respectively. The RDAs during pregnancy (14 through 18 years and 19 through 50 years) and lactation (14 through 18 years and 19 through 50 years) are 1000 and 1300 g/day respectively. Although Cu is indispensable for good health, excessive consumption may result in serious health problems such as kidney and liver damage. Consequently, 10 mg/d was established as the upper intake level (UL) for adults over 19 years of age [42]. The levels of Cu found in the present study are lower than the UL set by FAO/WHO; therefore, these mangrove plants can be consumed safely.

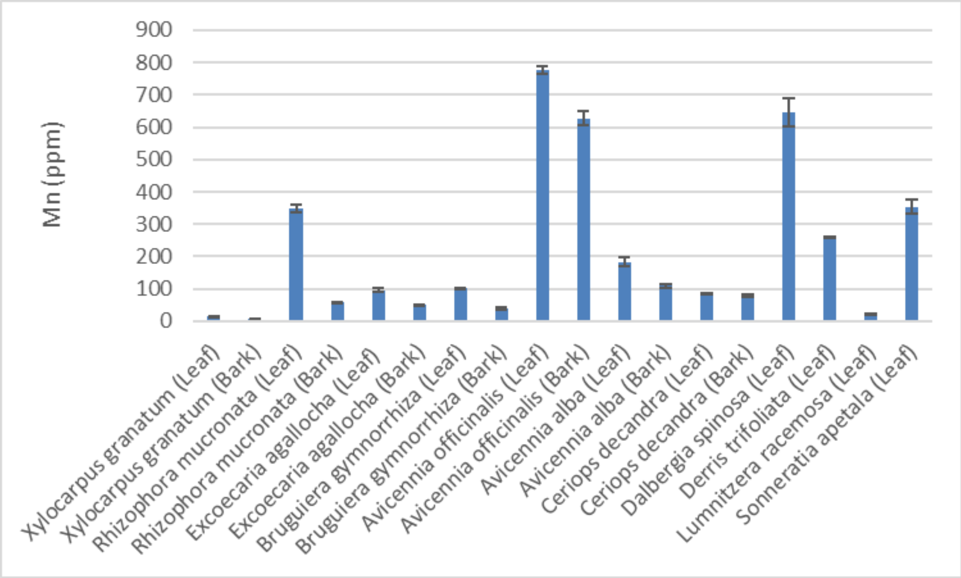
Limited quantities of non-essential elements such as Co and Ni are accumulated in the leaves of the present mangrove species as observed earlier [37] in the Sundarban mangrove forest, due to acropetal movement of these metals through translocation with the advancement of the age of leaves. Co is a heavy metal that has toxic effects beyond a certain concentration. However, at lower concentrations it has some advantages. It is necessary for the formation of normal red blood cells. Co is an essential component of vitamin B12. It is observed at low levels in samples in the present study. An excess intake of Co may cause overproduction of red blood cells which can be hazardous. Co concentrations obtained in the present study (Table 4), were found to be low in the selected mangrove plants, with values ranging from 1.4 ppm to 3 ppm. The RDA of Co is 1-2 mg for a normal male adult of 22 years of age. Red blood cells form improperly due to Vitamin B-12 deficiency as a consequence of carrying insufficient oxygen to different parts of the body, resulting in anaemia. Very limited and mostly fixed levels of Se and Rb concentration values were obtained in the present study. These function as antioxidants and boost the immunity of an individual.



(a)



(b)



(c)

Figure 2. Variation in (a) Zn, (b) Cu and (c) Mn concentrations (ppm) for different mangrove plant species (leaf & bark)

In the present study, the intervariation of a few trace elements such as Zn, Cu and Mn are shown in Fig. 2. Intravariation of elemental concentrations in the same species as shown in the tables can be understood in a variety of ways. These plants are capable of accumulating a variety of elements in different organs/components. Variation in climatic parameters, external resources, anthropogenic activities, soil geo-physiochemical properties and nutrients contained in substrate etc. may affect the uptake of some elements, resulting in differences in their concentration. Some elements are transported to the upper parts of the plants [44] while other elements may be immobile. Depending upon the chemical and physical response of a particular element, its concentration varies from one species to another as well as in different components of a particular species. Seasonal effects and the physiological age of tissues also influence the accumulation of elements [45].

Statistical analysis with a linear correlation of macro and micro (trace) elements was performed. The results indicated an association between Na, Cl and Ca in macro elements. In the case of microelements, this relationship was observed between Zn and V as well as Fe and Ni. The highest positive significant correlations were found for Fe-Zn (r=0.9) and Ni-V (0.9). V had a positive and significant correlation with Zn (r=0.8) and Fe (r=0.8). Ni was found to have a significant positive correlation with Zn (r=0.8) and Fe (r=0.8). A correlation between two elements indicates the relative behaviour or impact of one element on the other based on the correlation value.

3. Role of Elements in the Medicinal Usage of Mangroves

K plays an important role to control lipid metabolism, nerve impulse transmission etc. The Na:K ratio has a significant role in controlling blood pressure, as reported by earlier investigators [46]. This ratio needs to be less than one to effectively manage hypertension and cardiovascular problems [47]. K works to attenuate the reactivity of blood platelets. With daily consumption of salt, Na is available in considerable quantities in the human body. Hence to balance the Na:K ratio, consumption of a higher quantity of dietary K is required; this in turn enhances the bioavailability of Ca, which promotes bone health. In the present study the Na:K ratios for seven species were found to be less than one, indicating their suitability for controlling hypertension, while the remaining four species had values greater than unity, which make them unsuitable for the said purpose. A Ca:P ratio of less than 0.5 was first identified in an animal protein-rich diet [48]. It enhances the loss of Ca through the excretion of salt, reflecting its lower content in the bones. In the present study all obtained values of Ca:P were found to be very high, indicating the availability of larger quantities of Ca for the development of healthy bones. As mentioned above, higher consumption of K enhances the bioavailability of Ca. Some of the essential micronutrients such as Ca, Zn, Fe and P are referred to as phytates as they form insoluble salts, resulting in their bio-unavailability and hence the non-absorption of these nutrients by the body. However, Ca and P are present in higher quantities in bones and structural tissues.

Based on earlier studies [5, 7, 8, 10, 11] as shown in Table 1, *X. granatum* and *R. mucronate* species were found to have anti-diabetic properties. In the present study, the obtained values for Mn and Zn in these species are given in Table 4. These elements promote anti-diabetic activity, as reported by earlier investigators [49]. Zn plays a significant role in regulating blood glucose levels through insulin function and boosts the immune system as well. This may be due to Zn's role as a co-factor for more than 300 enzymes, including antioxidant enzymes [49]. Mn helps various enzymes in the process of gluconeogenesis where non-carbohydrate food items can be built to burn fuel, turning digested fats into sugars. These species were also found to contain Cu, which regulates immune systems along with cholesterol and glucose metabolisms. Therefore, these elements may provide the above-mentioned mangrove species with anti-diabetic properties. Chavpil et al [50] explained the inhibiting role of Zn in the growth of cancer cells in terms of cell membrane stabilization. It is noteworthy that Zn masks specific receptors related to malignant cells located on the surface of the membrane, obstructing the growth of cancer cells. Bhuloka Reddy et al [51] observed lower quantities of Mn and Se in cancer-affected tissues of the stomach relative to normal tissue; on the other hand, inhibition of cancer growth due to Mn was explained by Balo and Bingo [52]. An inverse relationship between death rates due to cancer and Se concentration was reported by Allaway et al [53]. V is usually present in most drugs used for cancer treatment. Mn and Zn at considerable concentrations along with smaller quantities of Se and V were observed in all species of mangroves analysed in the present study. Hence, one can corroborate the usage of the *L. racemosa, E. agallocha, B. gymnorrhiza, A. officinalis, A. alba, C. decandra* and *X. granatum* mangrove species mentioned in Table 1 in cancer treatment with the presence of trace elements such as Mn, Zn, Se and V in those species.

The medicinal properties of the *S. apetala* mangrove species, used for the treatment of hyperlipidemia (a cardiovascular problem), may be due to its Na:K ratio, which was found to be less than unity as shown in Table 3. It was also found to contain Mg, which is related to cardiovascular etiologies and a limited concentration of Se, which prevents the development of diabetes, cancer and heart diseases [54]. The anti-oxidant property of *L. racemose* may be due to its low levels of Se as shown in Table 4, making it suitable for the treatment of rheumatism. The presence of Zn and Cu also supports its usage for the treatment of skin allergies and asthma [54]. *D. spinosa* was found to contain considerable quantities of Cu, Fe, Mn, and Sr, each of which has its medicinal advantages. A deficiency of Cu leads to Mo toxicity causing an increased quantity of Cu excretion in urine [55]. Hence the usage of *D. spinosa* for skin and urinary tract infections can be interpreted due to the presence of trace elements such as Cu, Fe, Mn, and Sr. An investigation of the chemical organic components of *D. trifoliate* found it had therapeutic properties for different ailments such as wounds, rheumatism, asthma, malaria, chronic paralysis and diarrhoea [56]. Almost no research related to inorganic components is available in the literature. However, a study [57] related to trace elements suitable for curing rheumatism indicated the requirement of Zn, Fe, Mn, Mg, etc. WHO also recommended the use of Zn tablets in association with ORS for its anti-diarrhoea activity. The observed amounts of Mn (259.1±2.4 ppm?); Fe (130.6±4.3 ppm); Zn (13.4±4.5 ppm) and Cu (5.7±0.5 ppm) in the *D. trifoliate* species support the use of this mangrove plant for the treatment of diarrhoea.

Among the Coringa mangrove plants, *A. officinalis, A. marina, E. agallocha, S. apetala, R. apiculata and A. corniculatum* were found to be the six main species [28]. Hence comparison of nutrient accumulation in the leaves of these species provide baseline data that might be useful to researchers, as nearby inhabitants use the leaves for food and medicinal purposes [24]. Therefore, an attempt has been made to compare the present results with data from earlier investigators on the same species of mangrove plants from three different locations in Kerala, India [26]. A comparison of trace elements concentrations (ppm) in the leaves of *A. officinalis* and *E. agallocha* from Veli, Kochi and Kannur was carried out with the present results, as shown in Table 5. Values for Fe, Mn, Cu, Zn obtained in the present study were lower than those from the Kerala study. This variation may be due to climatic conditions, geophysical-chemical conditions, properties of saline water that interact with soil, mangrove species, and natural sources. However, the Mn content of the Coring samples was found to be higher than those from Veli and Kochi, but not Kannur. Similarly, results for *S. apetala* samples from Myanmar [23] and Bangladesh [24] were also compared with data from the present study, as shown in Table 6. The observed variations among mangrove species could be due to the role of microbes in the release of nutrients from organic matter, as mentioned by earlier investigators in their study of a mangrove island in Florida [58].

The results obtained from the Coringa mangrove species and the information mentioned in section 3.3 support the usage of these species for traditional (folk) medicinal purposes, as shown in Table 1. These mangrove species contain important inorganic elements that are required for good health. Therefore, the present data may be useful in the research and development of new drugs for curing a variety of diseases.

CONCLUSIONS

1. Six macro elements, Na, K, P, Ca, Cl and S, and ten microelements were detected by EDXRF in the mangrove species analysed in this study.
2. he role of the observed elements in the growth and development of mangrove plants, and their importance as inorganic nutrients for human health has been discussed in detail.
3. The use of these mangrove plants in traditional (folk) treatment of numerous diseases is corroborated with the inorganic chemical components found in this study. The relationships between various elements were also evaluated using linear correlations.
4. The obtained results were compared with previous results or data reported by other investigators. The observed variations in elemental concentrations are believed to be due to differences in climatic conditions, natural sources, and the role of microbes and organic matter etc.

CONFLICT OF INTEREST

Authors of this paper have no conflict of interest with regard to the publication of this paper.

Table 5. Comparison of trace element concentrations (ppm) in mangrove plants (leaf) collected from different locations in India

S. No	Location	Elements in *A. officinalis* (ppm)				Elements in *E. agallocha* (ppm)			
A	Kerala State (Ref -26)	Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
1	Veli	6900	495	140	169	-	-	-	-
2	Kochi	5500	510	068	146	4300	864	67	230
3	Kannur	2700	1749	047	127	4300	3628	87	262
B	AP State	Data from the present study							
1	Coringa	72.9±8.1	777.2±10.4	5.7±0.8	11.1±2.4	10.8±0.4	96.4±6.3	3.3±0.2	11.6±2.6

Table 6. Comparison of trace elements concentrations (ppm) in *S. apetala* (leaf) from other countries

. No	Location	Elements in *S. apetala* (ppm)						
		K	Ca	S	Fe	Mn	Cu	Zn
1	Rangabali, Bangladesh (Ref-24)	18000 (1500)	ND	ND	ND	1076 (494)	10.21 (4.48)	23.62 (4.11)
2	Sittway township, Rakhine state, Myanmar (Ref-23)	6690	3030	2360	680	130	10	20
3	Coringa forest, AP State, India (Present studies)	4069± 146.4	7894± 489.4	1141± 12.8	85.5±6.4	353±21.6	3±0.4	7.4±0.4

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AUTHOR CONTRIBUTIONS

D.Ch. Rajubabu (PhD scholar): Procurement of mangroves leaves and bark, preparation of samples for experimental work, performing experimental work, evaluation of spectra, computation of data, results analysis, preparation of manuscript.

A. Srinivasulu: (PhD Co-Scholar): Analysis of data and interpretation of results

A D P Rao: (Professor): Problem design, guiding entire work, revision of the prepared manuscript and finalization for submission

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