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Research Article

Nutritional and antioxidant potential of some selected edible mangrove fruits of Odisha coast

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Abstract

Keeping in mind the growing need for alternative bionutrition resources, some mangrove fruits are popularized for their edible and medicinal properties. Nutritional and antioxidant point of view, there is no concrete report on mangrove fruits. Therefore, the present study was attempted to assess nutritional parameters viz. moisture, protein, total sugar, reducing sugar, non-reducing sugar, carotenoid, fiber, ash and ascorbic acid in mangrove fruits of *Bruguiera gymnorrhiza*, *Rhizophora apiculata* and *Kandelia candel* and elemental and antioxidant analysis of the same. Fruit of *K. candel* exhibited highest level of three nutritional parameters i.e protein, total sugar and non-reducing sugar content (15.6±1.11 mg/g fwt, 396.67±4.16 mg/g fwt, 383.93±3.57 mg/g fwt. respectively) whereas lowest amount was observed in *B. gymnorrhiza* (4.4±0 mg/g fwt for protein and 108±6.9 mg/g fwt. for total sugar and 103.86±6.81 mg/g fwt. for non-reducing sugar). The reducing sugar and fiber content were ranged from 4.13±0.23 mg/g fwt. to 27.00±1.0 mg/g fwt and 0.7581±0.006 g/g dry wt. to 0.8061±0.001 g/g dry wt. respectively. Furthermore, the moisture and ash content was highest in *B. gymnorrhiza* and lowest in *R. apiculata*. It was found that the fruit with highest antioxidant activity was seen in *B. gymnorrhiza* and lowest recorded in *K. candel*. Likewise carotenoid content was highest in *R. apiculata* (3.53±0.28 mg/g fwt.) and lowest in *K. candel* (1.73±1.37 mg/g fwt.). Highest ascorbic acid content was recorded in *B. gymnorrhiza* (0.53±0.02 mg/g fwt.) and lowest in *R. apiculata* (0.35±0.03 mg/g fwt.). Fruit of *R. apiculata* was found rich in micronutrient among all the studied species. Nutrient analysis of these mangrove fruits can help us determining health benefits achieved from their use as an emergency as well as famine food and may play major role in bio-prospecting of mangroves.

Keywords: Mangroves, nutritional, micronutrient, antioxidant.

1. Introduction

The mangrove fruit species play a significant role in the food and nutrient security of the rural poor in general and coastal people community. They are nutritionally rich and provide supplement nutritional requirements for the forest dwellers and many of the marginalized rural communities since the common cultivar fruits are less familiar and not reachable for them. In view of the ever-increasing problem of human population and depleting natural resources, there is a need to exploit the role of mangrove edible fruits to the fullest extent possible. To the contrary, the mangrove fruits, which the tribes use, are not the familiar to the urban communities. Information available on the edible as well as therapeutic properties of the mangrove fruits is isolated and data on their nutrition aspect are scarcity or insufficient.

During recent years, there has been a growing interest to evaluate various mangrove fruits for their nutritional value [1-5]. There are several studies have already been carried out on the nutritive values and presence of potent micronutrient in the fruits of different plant species [6-11] but less studies have been documented with fruits of mangrove plants [10].

Besides, providing nutritional properties, these mangrove edible fruits also can serve as natural antioxidant. Fruits are rich with antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of

the ageing process [12-14]. Reports on antioxidant activity of leaf and root of some Rhizophoraceae mangroves are available [15] but research on antioxidant activity of mangrove fruits are lacking. Some reports are available i.e *Bruguiera gymnorrhiza* fruits [1], *Sonneratia caseolaris* fruit [16] but reports on *Kandelia candel* and *Rhizophora apiculata* fruit still lacking. The present piece of work explores the nutritional as well as antioxidant status of three edible mangrove fruits with a view to assess some promising species which may be considered as non-conventional bio-nutritional sources based primarily on their nutritional properties like protein, total sugars, reducing sugar, non-reducing sugar, moisture, fiber, ash, ascorbic acid, carotenoids, elemental analysis and antioxidant activity of mangrove fruits.

2. Material and Methods

2.1 Source of mangrove fruit sample

Fruits (here viviparous hypocotyls) of Rhizophoraceae mangroves viz. *Bruguiera gymnorrhiza* (Linn.) Savigny (locally called Bandari), *Rhizophora apiculata* BI (locally called Rai) *Kandelia candel* (Linn.) Druce (locally called Sinduka) were collected from mangrove forest of Bhitarkanika and Mahanadi delta of the Odisha coast (Buffer zone), India (20° 18'-20° 32' N latitude and 86° 41'- 86° 48' E longitude). Fruits of each plant species were sampled from five individual trees.

2.2 Preparation of fruit sample

All fruit samples were washed thoroughly in tap and distilled water and blotted dried. For elemental analysis, the samples were finally dried in Hot air oven at 50^0C temperature and grounded to a fine powder. Powdered samples were stored in air-tight containers at 4^0C for further analysis.

2.3 Nutritional analysis of mangrove edible fruits

2.3.1 Extraction and estimation of total protein

The fresh fruits (500 mg) were homogenized with pre-chilled mortar and pestle in ice-cold protein extraction buffer (5ml) (pH 7.9). The crude homogenate was centrifuged at 10,000 rpm at 4°C for 30 mins and pellets were washed with 10% TCA and were incubated overnight at 4^0 C. Pellets were suspended in 2 ml of 0.1N NaOH. Estimation of total protein was made according to [17]. Proteins in the unknown samples were estimated at 750 nm using bovine serum albumin (fraction V) as standard and calculated using standard curve and expressed mg per gm fresh weight basis.

2.3.2 Extraction and estimation of total sugar

Total sugar was estimated by using the method of [18]. 1 ml of alcoholic extract was taken in a test tube and chilled. After a while 4 ml of anthrone's reagent was carefully run down the walls of the test tube. The test tubes were thereafter immersed in ice water. The tubes were brought to ambient temperature and boiled in water bath for 10 min. After proper cooling, the absorbance was measured at 625 nm. Total sugar content was calculated using standard curve and expressed as mg per gm fresh wt.

2.3.3 Extraction and estimation of reducing sugar

Reducing sugar was estimated using Dinitrosalicylic acid (DNS) reagent [19]. 3 ml of DNS reagent was added to 3 ml of sample in a lightly capped test tube. The mixture was heated at 90^0 C for 5-15 minutes to attain a red brown color. Then 1 ml of Rochelle's salt solution was added to stabilize the colour. After cooling to room temperature in cold water bath, absorbance was recorded at 575 nm. Reducing sugar content was calculated using standard curve and expressed as mg per gm fresh wt.

2.3.4 Extraction and estimation of non-reducing sugar

Non-reducing was calculated by subtracting the amount of reducing sugar from that of total sugars.

2.3.5 Extraction and estimation of ascorbic acid content

Ascorbic acid was estimated following the method of [20]. Sample extraction was done by grinding 0.5g of sample material in 6% oxalic acid solution followed by centrifugation at 3000 rpm for 10 mins. Transferred the aliquot and made up the volume to 100ml. 5ml of supernatant was added to 10ml of 0.6% oxalic acid solution and it was titrated against dye solution (standard indophenols solution) till pale pink colour was seen. Standardization of dye was done with standard ascorbic acid (1mg/ml). Total ascorbic content(mg/100g) of fruits is calculated by (0.5mg/volume 1) × (volume 2/5ml) × (100ml/ wt. of the sample) × 100, where, volume 1 is burette reading of titration of dye against standard ascorbic acid and volume 2 is burette reading of titration of dye against sample.

2.3.6 Extraction and estimation of carotenoid content

Carotenoid was evaluated following standard method of [21]. 0.5 gm of the sample was weighed and homogenized in 80% acetone. The volume was then made up to 50ml. The sample was centrifuged at 5000 rpm for 20 minutes till the supernatant became transparent. The supernatant was taken and absorbance was measured at 480, 645 and 663 nm. The quantity of pigments was calculated by the formula and expressed as mg per gm fwt.

Carotenoid (mg/g fwt.) = [O.D 480+0.11(O.D 663) - 0.638(O.D 645)] x 400

2.3.7 Extraction and estimation of moisture content

The percentage of moisture content was determined using the method of [22]**.** The empty dish

was dried in the hot air oven at 105°C for 3 hours and then transferred to room temp to cool. The empty dish was then weighed. 3gms of the sample was weighed to the dish. The sample was spread to uniformity. The dish was placed with the sample in the hot air oven. It was dried for 3 hours at 105°C. After drying, the dish with the partially covered lid was transferred to room temp to cool. The dish and its dried sample were re-weighed. Percent of moisture content was calculated following method and expressed as percentage.

Moisture (%) = W1 – W2/ W1 × 100

Where, W_1 = Weight (g) of sample before drying.

W_2 = Weight (g) of sample after drying**.**

2.3.8 Estimation of fiber content

Fiber content was determined using the method of [22**].** Sample (1gm) was extracted to the extraction unit. 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a room temp and weighed (W1). The sample was then ashed at 550°C in a muffle furnace and cooled in a desiccator and reweighed (W2). Fiber content was expressed as gram per gram dry wt.

Crude fiber (%) = Digested sample (W1) – Ashed sample (W2) / Weight of sample × 100

2.3.9 Estimation of Ash content

To determine the amount of ash content, clean empty evaporating dish was heated in a muffle furnace at 600^0 C for 1 hour [22]. Cooled in desiccator and weighed as W1. One gram of sample was taken in evaporating dish (W2). The sample was ignited in muffle furnace at 550^0 C for 6 hours until it is charred. The result appeared as a gray white ash. It indicates that complete oxidation of all organic matters in the sample was occurred. The evaporating dish was cooled and weighed (W3). Percent ash was calculated by following formula:

% ash = Weight difference of ash/Weight of initial sample×100

Weight difference of ash = W3 - W1

Where: W1 = Empty evaporating dish weight after furnace heating

W2 = Initial weight of sample

W3 = Evaporating dish wt. + wt. of sample from furnace

Ash content was expressed as gram per gram dry wt.

2.4 Micronutrient analysis

Some 0.5 g of fine powdered sample of each fruit was digested following wet digestion procedures using conc. HNO3 and 30% H_2O_2. The digested samples were used for elemental analysis. Iron (Fe), Copper (Cu), Manganese (Mn) and Zinc (Zn) was determined using Atomic Absorption Spectrophotometer and Sodium (Na), Potassium (K), Calcium (Ca) using Flame photometer.

2.5 Antioxidant assay of mangrove fruits

2.5.1 Extraction of sample for antioxidant assay

Fruit samples (Fresh) were cleaned in running tap water and were dried in hot air oven (50^0C) for 12 hrs [23]**.** To 3.0 g of powdered sample, 40 ml of solvent (i.e. absolute methanol) was added in a conical flask and the mixture was stirred using stirrer for 18 h at room temperature. Each extract was filtered using Whatman No.1 filter paper. The filtrate was collected and the residue was re-extracted twice. The two extracts were then pooled out. The solvent (i.e. absolute methanol) in the extract was removed with heating at 40^0C using hot plate till the total volume reached to 10ml. The extracts were filled in the storage vial and stored in air-tight container at 4^0C until further uses.

2.5.2 Antioxidant Content through DPPH method

The antioxidant content was evaluated as described with some modifications [24]. A 50µl of extract was mixed with 2 ml of a 0.06 mg/ml methanol solution of DPPH in methanol. The mixtures were left for 15 min at room temperature and the absorbance then measured at 517 nm. The blank sample consisted of 50µl of sample with 2ml of methanol. The antioxidant content was determined using standard curve for ascorbic acid (1mg/1ml distilled water). The mean of three values were obtained and the unit was expressed as gm of ascorbic acid equivalent (AAE) per g of powder sample.

2.5.3 Ferric reducing antioxidant power (FRAP) assay

FRAP assay is used for measuring the total antioxidant capacity [25]. Freshly prepared FRAP reagent (3.0 ml) was mixed with 0.1 ml of test sample and a reagent blank was maintained with methanol. The FRAP reagent was prepared from 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L ferric chloride and 10 mmol/LTPTZ made up in 40 mmol/L hydrochloric acid. All the above three solutions were mixed together in the ratio 25:2.5:2.5. The absorbance of reaction mixture at 593nm was measured spectrophotometrically (Spekol 2000 UV-VIS, Germany) after incubation at 25^0 C for 10 min. The FRAP values were expressed in mM ascorbic acid equivalent (AAE)/g dry wt. derived from standard curve.

2.6 Statistical analysis

The values were expressed as Mean ± Standard deviation of triplicate observations. The means of all the parameters towards nutrient and antioxidant contents were analyzed for observing any significant variations following two-way ANOVA using GraphPad Prism 6.0.

3. Results and Discussion

3.1 Nutritional analysis of three edible mangrove fruits

The present piece of work explored appreciable nutritional status of mangrove edible fruits in terms of protein, sugar, moisture, fiber and ash. Results revealed that *Kandelia candel* exhibited highest level of protein content (15.6±1.11 mg/gm fwt.) followed by *Rhizophora apiculata* (14.4±0.70 mg/gm fwt.) and it is much higher than an amount compared to *Bruguiera gymnorrhiza* (4.4±0 mg/gm fwt.). The species wise differences in protein content were found significance (at P=0.0001) level. Total protein content of the fruits was also higher than those values obtained in *B. gymnorrhiza* (2.24%) fruits as reported by [2]. In a report [5], total protein content was found higher in *Ceriops tagal* than our studied species. Amount of moisture content ranged from 55.51±1.81 % in *R. apiculata* to 71.00±0.90 % in *B. gymnorrhiza*. The differential values among three species were statistically significant (at P<0.0001). Moisture (71%) and protein content (4.4 mg/g fwt.) obtained in *B. gymnorrhiza* were found higher as compared to an earlier report with moisture (66%) and protein (2.11 mg/g) [1]. On the contrary, protein and moisture content were comparatively lower than values obtained in fruits of *B. gymnorrhiza* by [4]. Among three edible fruits, *B. gymnorrhiza* revealed minimal total sugar content (108±6.9 mg/gm fwt.) which was quite lesser than *R. apiculata* (262.33±2.51 mg/gm fwt.) and *K. candel* (396.67±4.16 mg/gm fwt.). The difference in such values among three species were found statistically significant (at P<0.0001). Fruits of *R. apiculata* exhibited significantly (P<0.0001) 6.5 fold higher reducing sugar (27.00±1 mg/gm fwt.) than in *B. gymnorrhiza* (4.13±0.23 mg/gm fwt.). However, non reducing sugar content was found highest in *K. candel* (383.93±3.57 mg/gm fwt.) followed by *R. apiculata* (235.33±3.05 mg/gm fwt.) and *B. gymnorrhiza* (103.86±6.81 mg/gm fwt.) with a significant (P<0.0001) differences. The ash content ranged between 0.022±0.001 g/g dry wt. in *R. apiculata* to 0.033±0.0001 g/g dry wt. in *B. gymnorrhiza***.** A statistical significance (at P=0.0008) was obtained in ash content among all studied species. While *B. gymnorrhiza* yielded 1.15%, 1.29%, 0.34% ash content [1, 4, and 26 respectively], our study revealed 3.3% in the fruit of same species. Fruit of *Ceriops tagal* had high amount of ash content (4.30%) as reported by [5] which was higher than our studied species. The fruits of *R. apiculata* contained the highest amount of fiber content (0.80±0.001 g/g dry wt.) as compared to *K. candel* (0.77±0.009 g/g dry wt.) and *B. gymnorrhiza* (0.75±0.006 g/g dry wt.). The amount of fiber content found in this study was higher than fruit of *B. gymnorrhiza* (10.09%) reported by [2]. Fruit of *Ceriops tagal* had 15.64% of fibre content recorded by [5] and was lower than our studied species. *Rhizophora mucronata* had lowest fiber content (29.25 ± 0.4%) recorded by [27] that was twofold lower than our findings.

Table-1: Comparative analysis of nutritional parameters of three edible mangrove fruits.

Fruit Species	Nutritional Parameters						
	Protein (mg/g fwt.)	Total sugar (mg/g fwt.)	Reducing sugar (mg/g fwt.)	Non reducing sugar (mg/g fwt.)	Moisture (%)	Crude Fiber (g/g dry wt.)	Crude Ash (g/g dry wt.)
Bruguiera gymnorrhiza	4.4±0	108±6.9	4.13±0.23	103.86±6.81	71.00±0.90	0.7581±0.006	0.033±0.0001
Kanelia candel	15.6±1.11	396.67±4.16	12.73±1.10	383.93±3.57	56.41±0.38	0.7775±0.009	0.026±0.0004
Rhizophora apiculata	14.4±0.70	262.33±2.51	27±1	235.33±3.05	55.51±1.81	0.8061±0.001	0.022±0.001

Values expressed as mean±standard deviation

3.2 Micro-nutritional analysis of three edible mangrove fruits

The highest sodium content was noted in *K. candel* (700±2 mg/100g dry wt.) whereas the minimal amount was found in *R. apiculata* (690±2.64 mg/100g dry wt.). Potassium content was highest in *R. apiculata* (650±1 mg/100g dry wt.) and *B. gymnorrhiza* (280±3 mg/100g dry wt.) showed less amount of potassium content. Similarly, calcium content was found to be highest in *K. candel* (240±1 mg/100g dry wt.) and lowest in *R. apiculata* (200±2.64 mg/100 g dry wt.). Maximum iron content was exhibited in *R. apiculata* (3.06±0.11 mg/100g dry wt.) while lowest in *B. gymnorrhiza* (1.8±0.1 mg/100g dry wt.). *B. gymnorrhiza* registered highest manganese content (3.1±0.1 mg/100g dry wt.) while *R. apiculata* showed the lowest (2.36±0.05 mg/100g dry wt.). Among studied mangrove fruits, maximum Cupper

and Zinc was noted in *R. apiculata* i.e 1.96±0.11 mg/100g dry wt. and 0.31±0.01 mg/100g dry wt. respectively. Sodium is one of the essential elements that regulate the blood pressure and nerve processes for transmitting impulses through the body as well as stabilize the water in human body cells [28]. All studied fruits had high amount of Ca, Na and K content, this value was higher than the fruits of *Heritiera fomes, a non*-Rhizophoraceae mangrove species as reported by [29] but zinc and iron content was nearly equal to the present study. Potassium is one of the essential elements of human diet, and plays important role in vital cellular mechanism. Calcium helps in the development and growth of skeletal system e.g. bones and also teeth. Zinc mainly acts as a coenzyme of about 200 enzymes involved in new cells growth, acid base regulation etc. Fruits of *Sonneratia apetala* had lower amount of Ca, Fe, Na, K, Zn reported by [11]. Cupper is an essential trace element and is required as cofactor in different oxidative and reductive enzymes [28].

Table-2: Comparative study of micro-nutrient analysis of three edible mangrove fruits.

Fruit Species	Elemental Parameters						
	Sodium (Na) (mg/100g dry wt.)	Potassium (K) (mg/100g dry wt.)	Calcium (Ca) (mg/100g dry wt.)	Iron (Fe) (mg/100g dry wt.)	Manganese (Mn) (mg/100g dry wt.)	Cupper (Cu) (mg/100g dry wt.)	Zinc (Zn) (mg/100g dry wt.)
Bruguiera gymnorrhiza	700±1	280±3	200±4	1.8±0.1	3.1±0.1	1.7±0.1	0.24±0.01
Kanelia candel	700±2	420±1	240±1	1.93±0.05	3.06±0.11	1.83±0.05	0.25±0.02
Rhizophora apiculata	690±2.64	650±1	200±2.64	3.06±0.11	2.36±0.05	1.96±0.11	0.31±0.01

Values expressed as mean±standard deviation

3.3 Antioxidant content of three edible mangrove fruits

In this study, all the target species exhibited promising antioxidant content. The highest antioxidant content was recorded in *B. gymnorrhiza* (0.09±0.013 g AAE /g dry wt**.)** followed by *R. apiculata* (0.08±0 g AAE /g dry wt.) and *K. candel* (0.07±0.005 g AAE/g dry wt.) with a significant (at P=0.1705) differences. The FRAP value was highest in fruit extracts of *B. gymnorrhiza* (0.09±0 mM AAE/g dry wt.) and lowest was in *K. candel* (0.07±0 mM AAE/g dry wt.). The species wise differences in FRAP content were found significant at P<0.0001 level. *R. mucronata* contains free radical scavenging activity (83.7± 2.8 mg/ml) [30]. The potential of fruit extracts of *Avicennia marina* and *Avicennia officinalis* with antioxidant principles was reported by [31]. The antioxidant potential of methanol extracts of fruit peel of *Xylocarpus rumphii* has been reported by [32]. The methanol extracts of pericarp and seed of the fruit [33] of *S. apetala* have shown strong antioxidant activities. The antioxidant potential of acetone extract from the hypocotyls of *K. candel* were investigated by the DPPH free radical scavenging and FRAP assays reported to possess potent antioxidant activity [34]. Similarly, the carotenoid content varied from 1.73±1.37 mg/g fwt to 3.53±0.28 mg/g fwt. The lowest amount of carotenoid was noted in *K. candel* (1.73±1.37mg/g fwt.) and highest in *R. apiculata.* The species wise differences in carotenoid were found significant at P=0.1451 level. Ascorbic acid (Vitamin C) is very important in the daily food as it has a variety of roles in the life process. Highest ascorbic acid content was obtained in *B. gymnorrhiza* (0.53±0.02 mg/gm) followed by *K. candel* (0.40±0.01 mg/gm), *R. apiculata* (0.35±0.03 mg/gm). The species wise differences in ascorbic acid content were found significance (at P=0.0044) level. The study showed that all the mangrove edible fruits under investigation were good sources of ascorbic acid or vitamin C. Ascorbic acid content of the present studied fruits was also much higher than those values obtained in *A. ilicifolius* fruits as reported by [35].

Table-3: Comparative study of antioxidant content of three edible mangrove fruits.

Fruit Species	Antioxidant Analysis			
	Antioxidant content (g AAE /g dry wt.)	FRAP Content (mM AAE/g dry wt.)	Ascorbic acid (mg/g fwt.)	Carotenoid (mg/g fwt.)
Bruguiera gymnorrhiza	0.09±0.013	0.09±0	0.53±0.02	2.06±0.62
Kanelia candel	0.07±0.005	0.07±00	0.40±0.01	1.73±1.37
Rhizophora apiculata	0.08±0	0.08±0	0.35±0.03	3.53±0.28

Values expressed as mean±standard deviation. AAE-Ascorbic acid Equivalent

4. Conclusion

The results of this study demonstrated that the mangrove edible fruits contain considerable amount of nutrients which if well exploited and promoted can address many nutritional related disorders and also be useful in food industry for production of a variety of value added products and as an alternative source of bio-nutrition. The present piece of work explored superior/identical nutritional status in terms of protein, moisture, total sugar, reducing sugar, non-reducing sugar, carotenoids and ascorbic acid contents in *Bruguiera gymnorrhiza, Rhizophora apiculata* and *Kandelia candel.* Thus, the above mentioned promising species deserved to be non-conventional bio-nutritional sources based primarily on their nutritional antioxidant properties.

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