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Assessment of anti-nutritional properties in four edible fruits of mangroves of Odisha coast

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ABSTRACT

Though some mangrove fruits are known for their medicinal and edible properties, their anti nutritional aspect has not yet been evaluated. Mangrove fruits provide innumerable direct and indirect benefits to human beings. However, scientific report on anti nutrients in edible mangrove fruits is still lacking. The present study was aimed to determine anti-nutritional properties in four edible mangrove fruits viz. *Bruguiera gymnorrhiza*, *Rhizophora apiculata*, *Kandelia candel* and *Xylocarpus granatum* found in Bhitarkanika and Mahanadi delta (buffer zone) of Odisha. The fruit of *X. granatum* exhibited highest level of oxalate content (1.68 ± 0.25 mg/g dry wt.) whereas lowest amount was observed in *K. candel* (1.02 ± 0.12 mg/g dry wt.). Highest phytate content was noted in *K. candel* (0.057 ± 0.002 mg/g dry wt.) and lowest in *B. gymnorrhiza* (0.052 ± 0.001 mg/g dry wt.). Tannin content was highest in *X. granatum* (0.89 ± 0.01 TAE/g dry wt.) and lowest in *R. apiculata* (0.57 ± 0.03 TAE/g dry wt.). Saponin content was highest in *K. candel* (0.0301 ± 0.007 g/g dry wt.) and lowest in *R. apiculata* (0.022 ± 0.001 g/g dry wt.). The present findings revealed that all the studied fruits can be considered as ample sources of antinutrients. Thus, excess consumption of these fruits contained high anti-nutrients may be avoided.

Introduction

Mangroves are specialized group of salt tolerant plants that grow in the intertidal regions of tropic and sub tropic along the coastlines (Basak *et al.*, 2000). They provide innumerable direct and indirect benefits to human beings. Keeping in mind, the edibility properties of mangrove fruits, they have a good potentiality to become a food source by the coastal people. Mangrove fruits are considered as famine food (Patil and Chavan, 2013). But mangrove fruits are utilized for food as well as bioactive compound such as tannin, phenol etc reported by (Bandaranayake, 1998). However, scientific evidences regarding anti nutritional properties are still lacking.

Anti-nutritional factors including oxalate, tannins, phytic acid, saponins (Makkar and Singh, 1993; Gupta *et al.*, 2005) whose presence greatly impair the digestion of various nutrients, therefore, reducing the nutritional value of such plants and limiting their utilization as food (Gidamis *et al.*, 2003; Prathibha *et al.*, 1995). Oxalic acid binds to calcium and is found naturally in a variety of fruits, vegetables, nuts, grains and legumes. It has also been reported earlier that oxalates causes irritation and

swelling in the mouth and throat (Ladeji *et al.*, 2004). Phytic acid constitutes many essential elements like iron, calcium, phosphorous etc. are combined by phytic acid to form insoluble phytate salt, which are not absorbed by the body and thus limiting the bioavailability of these elements (Weaver and Kannan, 2002). Saponin is naturally occurring glycosides and occurs in wide varieties of plants. Tannins have the ability to precipitate certain proteins. They combine with digestive enzymes thereby making them unavailable for digestion (Binita and Khetarpaul, 1997; Abara, 2003).

There is no comprehensive report on anti-nutritional properties of edible mangrove fruits of Odisha coast. Considering their extensive edibility and therapeutic uses, four mangrove species viz *Bruguiera gymnorrhiza*, *Rhizophora apiculata*, *Kandelia candel* and *Xylocarpus granatum* were subjected to analysis for evaluating antinutrient properties with an aim to create awareness on legitimate consumption of mangrove fruits.

Materials and Methods

Collection of mangrove fruit sample



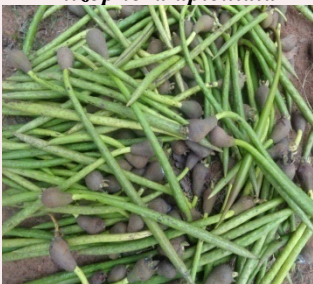
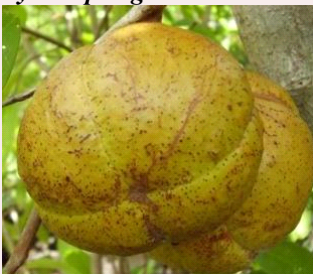
The fruits (at edible stage) of the species viz. *Bruguiera gymnorrhiza* (Linn.) Savigny (Bandari) (Fam.-Rhizophoraceae), *Rhizophora apiculata* BI (Rai) (Fam.-Rhizophoraceae), *Kandelia candel* (Linn.) Druce

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Table - 1: Ethno medicinal and Edible properties of four mangrove fruits

<u>MANGROVE SPECIES</u>	<u>EDIBLE/MEDICINAL PROPERTIES</u>	<u>REFERENCES</u>
<p><i>Bruguiera gymnorhiza</i></p> 	<p>EDIBILITY: Fruits (Propagules) are consumed regularly during the fruiting season. They are peeled, soaked and boiled three or four times in water and eaten. Sometimes they are cooked with salt, dried and then consumed.</p> <p>Medicinal: Medicinal on eye ailment. Treatment of diarrhoea.</p>	<p>Singh and Odaki (2004)</p>
<p><i>Kandelia candel</i></p> 	<p>EDIBILITY: The fruits of <i>Kandelia candel</i> contain starch and if sliced, soaked in water to rinse out tannins and then ground to a paste can make excellent cakes or sweetened stuffing for pastry.</p> <p>Medicinal: Treatments of diabetes</p>	<p>Banerjee and Rao (1990)</p>
<p><i>Rhizophora apiculata</i></p> 	<p>EDIBILITY: Cooked as vegetables, a famine food.</p> <p>Medicinal: Human consumption</p>	<p>Agoramoorthy <i>et al.</i> (2008) Bandaranayake., 1998, Beula <i>et al.</i> (2012)</p>
<p><i>Xylocarpus granatum</i></p> 	<p>Edibility- Fruits are scented and aromatic and chewed with betel leaves.</p> <p>Medicinal- Seed oil is used for illumination and grooming hairs, seed-paste is used to cure breast tumour</p>	<p>The wealth of India (1990).</p>

(Sinduka) (Fam. - Rhizophoraceae), *Xylocarpus granatum* Koenig (Susumara) (Meliaceae) (Table-1) were collected from mangrove forest of the 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 at least five individual trees.

Processing of the fruit sample

Fruit samples (Fresh) were cleaned in running tap water and were dried in hot air oven (50°C) for 12 hrs (Khamsah *et al.*, 2006). The dried samples were pulverized and stored in freezer in airtight containers for further extraction.

Analysis of Samples for anti-nutritional factors

Estimation of oxalate content

Total oxalate was determined through titration methods according to (Day and Underwood, 1986). One gram

powdered sample was weighed into 100 ml conical flask. 75 ml of 3 M H₂SO₄ was added and stirred for 1h with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. The filtrate (25 ml) was then titrated while hot against 0.05M KMnO₄ solution until a faint pink colour appeared and persisted for at least 30 sec. The oxalate content was then calculated by taking 1 ml of 0.05 M KMnO₄ as equivalent to 2.2 mg oxalate (Ihekoronye and Ngoddy, 1985; Chinma and Igyor, 2007). Finally, oxalate content was expressed as milligram per gram dry wt.

Estimation of Phytate content

Phytate content was determined by the method of Wheeler and Ferrel (1971) with minor modification. Three grams sample was mixed in 25 ml of 10% TCA in a 125 ml flask and shaken the same with mechanical shaker for 2 hrs. This sample mixer was centrifuged at 3000 rpm for 20 min. To a 50 ml centrifuge tube, 10 ml of the supernatant

Table - 2: Anti nutritional factors in four edible mangrove fruits

Parameters	Fruit Species			
	<i>Bruguiera gymnorrhiza</i>	<i>Kandelia candel</i>	<i>Rhizophora apiculata</i>	<i>Xylocarpus granatum</i>
Oxalate Content (mg/g dry wt.)	1.02±0.25	1.02±0.12	1.39±0.12	1.68±0.25
Phytate Content (mg/g dry wt.)	0.052±0.001	0.057±0.002	0.055±0.001	0.054±0.002
Tannin Content (TAE g/g dry wt.)	0.61±0.01	0.59±0.01	0.57±0.03	0.89±0.01
Saponin Content (g/g dry wt.)	0.0235±0.004	0.0301±0.007	0.022±0.001	0.022±0.01

Values are mentioned with mean ±SD values of three replicates; TAE-Tannic acid equivalent

Figure - 1: Oxalate content of four edible mangrove fruits

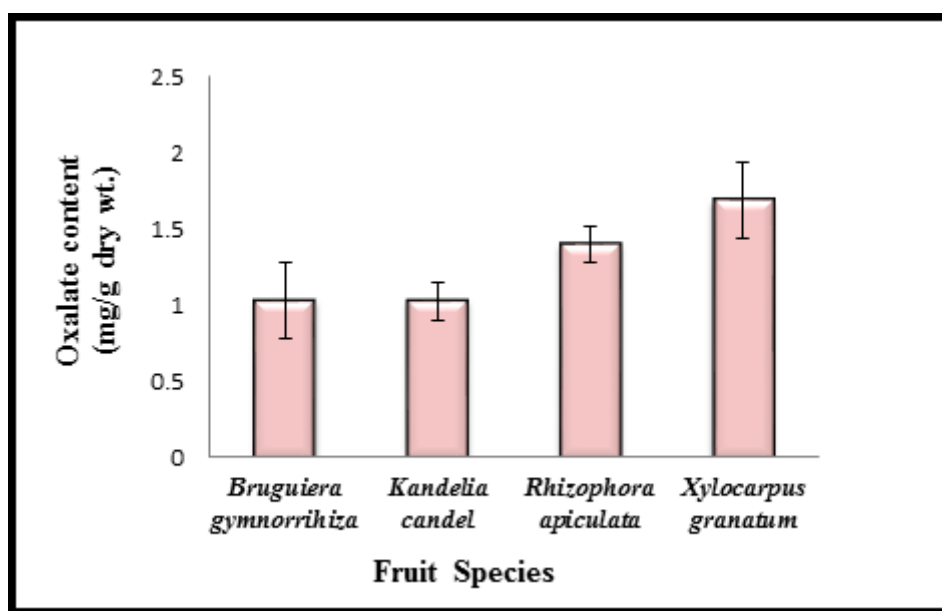


Figure - 2: Phytate content of four edible mangrove fruits

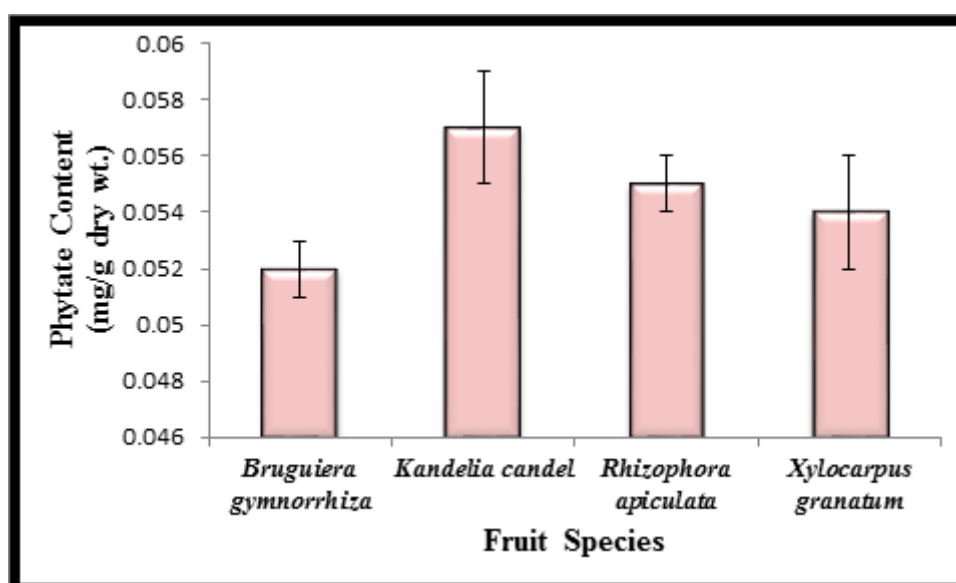


Figure - 3: Tannin content of four edible mangrove fruits

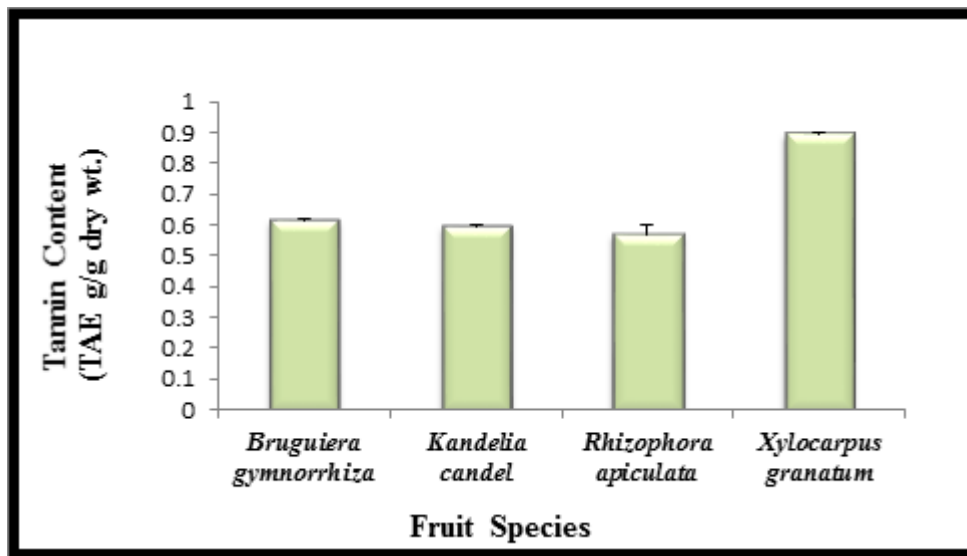
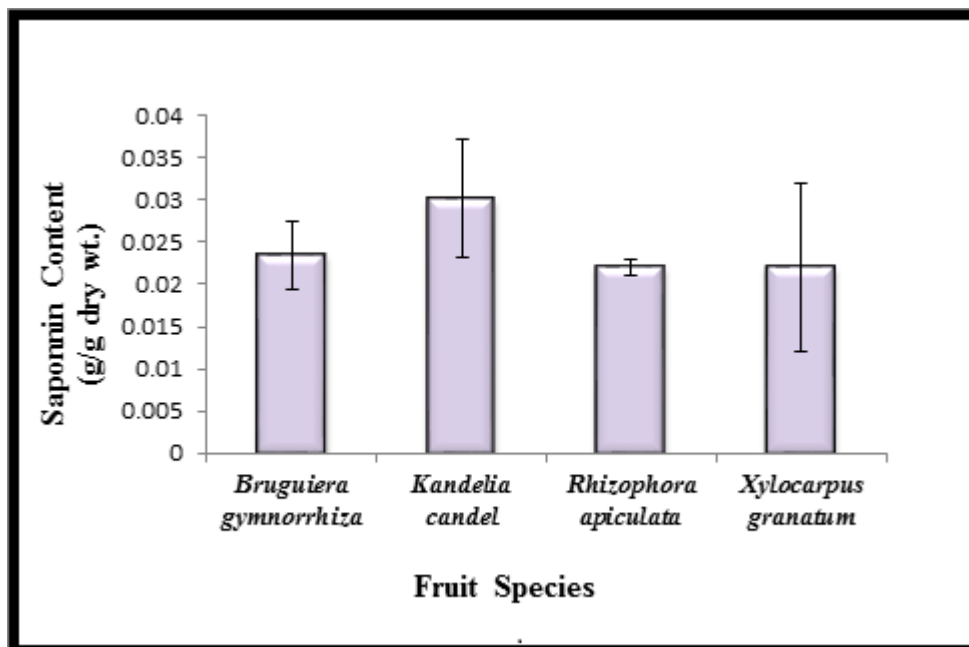


Figure - 4: Saponin content of four edible mangrove fruits



was mixed with 4 ml of FeCl₃ solution by rapid blowing from the pipette. The solution was heated then in boiling water bath for 45 min. To make the supernatant clear, one or two drops of 3% sodium sulphate in 10% TCA was added and continued heating; then centrifuged for 10 to 15 min at 3000 rpm and finally the clear supernatant was decanted. The precipitate so obtained was washed twice by dispersing in 25 ml 10% TCA and heated again in boiling water for 10 min and centrifuged after cooling to room temperature. The precipitate was again dispersed in a few ml of water followed by addition of 3 ml of 1.5 N NaOH and made the volume up to 30 ml with distilled water. After heating in boiling water for 30 min, the solution was filtered with Whatman No 2 paper; the precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate obtained on the filter paper was then dissolved with 40 ml hot HNO₃ (3.2 N) into a 100 ml volumetric flask. A 5 ml aliquot taken in 100 ml volumetric flask was diluted to 70 ml with dist. Water followed by addition of 20 ml 1.5 M potassium thiocyanate (KSCN). The pinki-red colour so obtained was measured immediately (within 1 min) at 480 nm in a spectrophotometer (Specord 2000, Analytik Jena, Germany) with reference to the Ferric nitrate as standard. The phytate content was expressed as mg/g dry wt.

Estimation of tannin content

Tannin was analyzed using the method of Schanderi (1970). Powder sample (0.25 g) was extracted with 37.5 ml distilled water and heated the flask gently and boiled for 30min. The sample mixer was centrifuged at 2000 rpm for 20min and the volume of the supernatant was finally made up to 37.5 ml with distilled water in a 100 ml flask. An aliquot of 500 µl of the sample was treated with 1 ml of Folin-Denis reagent followed by 2 ml of sodium carbonate and allowed to stand for color development. The absorbance of the reaction mixture was measured at 700 nm in a spectrophotometer (Spekol 2000, Analytik Jena, Germany). Tannic acid used as standard. Tannin content was expressed as Tannic acid equivalents (TAE) in gram per gram dry wt.

Estimation of Saponin content

Saponin was determined using the method of Obadoni and Ochuko (2001). The powder sample (3 g) was dispersed in 30 ml of 20% aqueous ethanol. The suspension was stirred for 12hrs with constant stirring at about 55°C on a hot plate (Spinot, Tarson make). The mixture was filtered and the residue was re-extracted with another 30 ml of 20% aqueous ethanol. The combined extracts (filtrates) were reduced to 15 ml over water bath at about 90°C. The concentrated sample extract was transferred into 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated twice. To the combined aqueous, 20 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the concentrated sample was dried in dry bath to a constant weight and saponin content was calculated as detailed below.

% Saponin = $\frac{W2 - W1}{Wt. \text{ of Sample}} \times 100$

Where, W1 = Weight of evaporating disc

W2 = Weight of disc + Sample

Saponin content was expressed as gram per gram dry wt.

Statistical analysis

All the values are expressed as Mean±SD (n=3). The results were analyzed statistically through two-way ANOVA using Graph pad Prism 6.0.

Results and Discussion

Anti nutritional analysis of four edible mangrove fruits

Analysis of the data is presented in the (Table-2). It was revealed that the oxalate content of the *X. granatum* was maximum (1.68±0.25 mg/g dry wt.) followed by *R. apiculata*, *B. gymnorrhiza* and *K. candel* (1.02±0.12 mg/g dry wt.) (Figure-1). The results showed significant variation at P=0.0274 among four species. Maximum amount of Phytate (0.057±0.002mg/g dry wt.) was observed in *K. candel* followed by *R. apiculata* (0.055±0.001 mg/g dry wt.), whereas minimum amount was found in *B. gymnorrhiza* (0.052±0.001 mg/g dry wt.) (Figure-2). The results showed significant variation at P=0.0383. Likewise, maximum tannin content was estimated in *X. granatum* (0.89±0.01 TAE g/g dry wt.) followed by *B. gymnorrhiza* (0.61±0.01 TAE g/g dry wt.) with minimum amount in *R. apiculata* (0.57±0.03 TAE g/g dry wt.) (Figure-3). The results showed significant variation at P<0.0001. It was observed that *K. candel* had maximum saponin content (0.0301±0.70 g/g dry wt.) and *R. apiculata* with minimum saponin (0.022±1.058 g/g dry wt.) (Figure-4). The results showed significant variation at P=0.0352. There are so many reports available regarding anti nutritional properties viz. tannin, saponin of mangrove fruits but quantitative point of view, scientific reports are still lacking. Mangroves are promising source of several bioactive compounds. They have a potential source of alkaloids, flavonoids, saponin, steroids, tannins and terpenoids (Bandaranayake, 1995). Phytochemical screening of ripe *R. mucronata* fruit showed the presence of tannin, saponin, flavonoid, and steroid (Hardoko *et al.*, 2015). Ghosh *et al.*, (1985) reported that *R. mucronata* contained steroid, triterpenoid, alkaloid, flavonoid, tannin, catechin, quinon and anthocyanidin. Patra *et al.* (2009) and Odom *et al.* (2013) also reported that some of the mangrove fruits contained alkaloid, tannin and saponin. Sudirman *et al.* (2014) determined that fruits of *Bruguiera gymnorrhiza* as good source of bioactive compound.

Conclusion

Though all the four studied edible mangrove fruits contained oxalate, phytate, tannin and saponin at varied concentrations, consumption in large quantity of these fruits may be discouraged. The findings of the present study may encourage to exploit these fruits for preparation of natural drugs for the treatment of various diseases with appropriate pharmaceutical approaches.

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