REASEARH ARTICLE

Study on the morphology and nutritional status of Roktogota (*Haematocarpus validus)* - an important medicinal fruit plant of hilly areas of Bangladesh

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Abstract: Cross-sections of ethnic group people live in the hilly regions of Bangladesh and their livelihood is directly and indirectly dependent on forest and forest resources. Along with different food items, they traditionally use different ethnic fruits. Roktogota (*Haematocarpus validus*) is a very promising ethnic fruit plant grows in the remote areas of Chittagong Hill Tracts (CHT) and it is endemic in these natural habitats. Roktogota is an evergreen perennial dioecious creeping woody climber belonging to the family Menispermaceae. Fruits are slightly acidic sweet taste. A detail study on the morphology and nutritional status of this fruit was conducted to popularize this ethnic fruit.

Keywords: Roktogota, *Haematocarpus validus*, morphology, nutritional status, ethnomedicinal fruit

INTRODUCTION

Bangladesh is one of the unique countries of the world with the assemblage of natural habitat, biodiversity, heritage and culture. Among the 45 smaller groups of indigenous people 13 ethnic groups have been dwelling in the Chittagong Hill Tracts [CHT] which is rich in natural biological resources. This area is the repository of many medicinal (Snigdha *et el*., 2008; Animesh *et el*., 2010), fiber, ornamental, fodder, vegetable and fruit plant though some ethnic fruits are threatened and endemic. The ethno-people are aware and careful for the better utilization of plant resources around them. Bangladesh is also rich in various underutilized fruit species, most of which contain considerable amount of minerals and vitamins. Among them Roktogota (*Haematocarpus validus)* is an antioxidant rich endemic promising ethnomedicinal fruit in Bangladesh. It is one of the endemic species still existing in Bangladesh territory. It was first time collected from wild and planted in Germplasm Center, Bangladesh Agricultural University in 2010 by Prof. Dr. M.A. Rahim, Director, BAU-GPC. During the current study, it was also noticed that *H. validus* is distributed throughout the hilly ecosystem of CHT in Bangladesh. However, its population suffers from serious environmental problems such as deforestation, soil degradation, loss of biodiversity, land slides etc. So, its conservation status is highly threatened and they need critical morphological documentation, and also deserve special attention from the conservation point of

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view.

Nutrient composition is a term that is used to describe an analysis of the vitamins, minerals and other nutritive substances in a given food. In addition to vitamins and minerals, many food composition reports include information on and analysis of phytonutrients and macronutrients within a food. Nutrient composition of foods is important for various uses including food based dietary guidelines, health and nutrition assessment formulation of therapeutic diet, nutrition education, nutrition planning etc. To explore this treasure an attempt has also been taken to estimate nutrient composition of Roktogota fruit.

MATERIALS AND METHODS

The study area was phytogeographical region in the district of Khagrachari, Chittagong Hill Tracts of Bangladesh.

Freshly collected Roctogota (*Haematocarpus validus)* from different parts of Khagrachari was used for ethnobotanical study and phyto-chemical analysis.

Botanical identification and collection

Roctogota (*Haematocarpus validus)* used as experimental material, was collected from Krisnomohanpara and weekly Hat (Bezza) of Khagrachari sadar, Shotokheda and local market of Ramgorh and Baradom bazaar of Diginala in Khagrachari district through an initial ethno botanical survey. This fruit was collected during April, 2012. The collected fruit was placed in a polythene bag to prevent loss of moisture during transportation to the laboratory. Efforts were made to collect these plants in flowering and fruiting conditions for the correct botanical identification. Detailed ethno botanical knowledge was recorded while collecting voucher specimens. Vouchers of wild edible fruit plant species were collected with knowledgeable local participants and the botanical identification and authentication ware made by the supervisor using the various volumes of the Encyclopedia Flora and Funa of Bangladesh and expert taxonomist in Bangladesh, National Herbarium, Dhaka and checking against identified specimens found in the National Herbarium, Mirpur, Dhaka. The study was conducted at Plant Ecology Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh. Nutrition analysis was done at Postharvest Technologies Division and Soil Science Division of Bangladesh Agriculture Research Institute, Gazipur, Dhaka.

Sampling Protocol

A multiregional sampling plan (Fig. 1) was employed to collect fruit samples. Every two samples were pooled together to make three analytes (test sample) which were analyzed for their nutrient profile. The ethnic fruit sampling plan is depicted in Figure-1.

Specimen collected and examined by: i) M. A. Rahim, from Bandarban on 10-05-2010, ii) M.J.M. Khatun & M. Mahfuzur Rahman, from Khagrachari on 26-4.2011, iii) M.J.M. Khatun, from Rangamati on 21.04.2012. iv) M.J.M. Khatun, Ramgarh, from Khagrachari on 28.05.13.

Preparation of fruit sample

In laboratory, the freshly collected sample was washed with deionized water to eliminate visible dirt and removed the water quickly with a blotting paper. Then the sample was cut into small pieces, homogenized and accurate amount was weighed as required for different analysis. Three test samples from each fruit were selected for measurement of various parameters.

All chemicals and reagents used in analysis were of analytical grade and purchased from

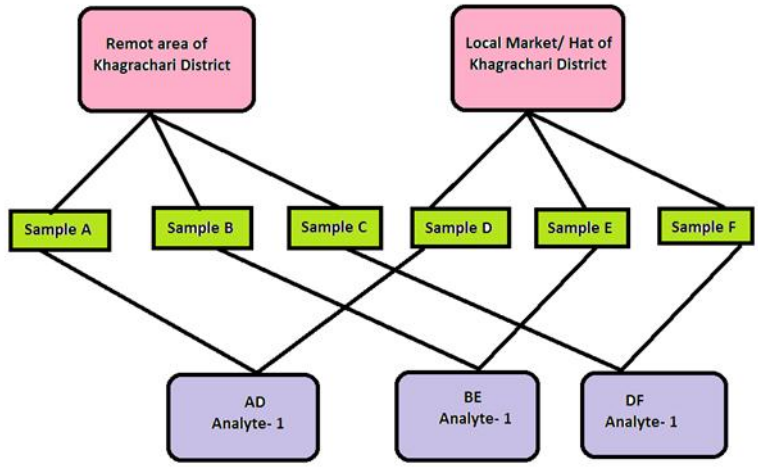

Figure 1: Sample Analysis Plan

Merck (Darmstadt, Germany, BDH, UK). Ascorbic acid and minerals standards, 2,4 di-nitrophenyl hydrazine were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Nutrition Analysis

Fresh fruits were used for nutritional value analysis. Physico-chemical analysis of different parameters was done following the methods in Table 1.

Table 1: Methods of Nutrition analysis

Nutrient class	Nutrients	Methods
Macronutrients	Moisture	digital moisture analyzer
	PH	pH meter (Ibrahim, 2002)
	TSS	Hand refractometer (Gofur *et al*., 1998)
	Total sugar	Lane and Eynon method (Ranganna, 1986)
	Protein	Micro-Kjeldahl method (AOAC, 1998a)
	Fat	Soxhlet extraction (Raghuramulu *et al*., 2003a)
	Crude fiber	Gravimetric method (Raghuramulu *et al*., 2003b)
	Ash	Muffle furnace (AOAC, 1998b)
	Carbohydrate	By calculation (Rand *et al*., 1991)
Micronutrients:		
Vitamin	Carotenoids	Spectrophotometer (Roriguez-Amaya and Kimura, 2004; (Rahman *et al*., 1990)
	β-carotene	HPLC (Roriguez-Amaya and Kimura, 2004)
	Vitamin C	Spectrophotometer (AOAC, 1998c)
Mineral	Cu, Zn, Fe, Mn, Ca, Mg, Na, K, P	Atomic Absorption Spectrophotometer (Petersen, 2002)

Estimation of P^H, Moisture, TSS and Total sugar

Weight of Roktogota was estimated by digital weight balance. The pH was determined with a digital pH meter (Ibrahim, 2002) and moisture content was determined by digital moisture analyzer. The Total Soluble Solid (TSS) was determined with a hand refractometer (Gofur *et al*., 1998). Total sugar was determined by Lane and Eynon method (Ranganna, 1986).

Estimation of protein

Protein content in the fruit was determined by indirect method estimating total nitrogen in the food. It was calculated by multiplying the total nitrogen using the respective factor as estimated by Micro-Kjeldahl method (AOAC, 1998a).

Estimation of fat

Powdered fruit was subjected to extraction with mixture of chloroform and methanol (Raghuramulu *et al*., 2003).

Estimation of crude fiber

Crude fiber was estimated by gravimetric method as described by Raghuramulu *et al.* (2003). The dried and fat free fruit sample was treated with boiling sulphuric acid at constant volume, cooled, filtered, washed with hot water, made alkaline, boiled, filtered and washed with water followed by ethanol and ether wash. The residue was then heated in a muffle furnace at 600^{o}C for 3 hours. Crude fiber was finally calculated from the weight difference.

Calculation of carbohydrate and energy

The content of available carbohydrate in the fruit sample was determined by difference. Carbohydrate was calculated by subtracting the sum percentage of moisture, protein, fat, ash, crude and dietary fibre (Rand *et al*., 1991). The energy content in the food sample was calculated by the sum of protein, fat and carbohydrate using respective Atwater factors (Rand *et al*., 1991).

Analysis of vitamin-c

Ascorbic acid in fruit sample was estimated by spectrophotometer method (AOAC, 1998c). The fresh food sample (fruit) was homogenized in a mortar with pestle using met phosphoric acid, filtered, treated, and incubated at 60 ^{0} C for 60 minutes with 2, 4-dinitrophenyl hydrazine. The reaction was stopped by adding 85% sulphuric acid and absorption was read at 520nm in a spectrophotometer (UV-1601, UV-Visible, Shimadzu).

Analysis of carotenoid

All-trans-β- carotene (Sigma Chemical Co., USA) was used as the standard. Total carotenoid content in the fruit sample was determined by acetone-petroleum-ether extraction followed by spectrophotometric measurement (Roriguez-Amaya and Kimura, 2004). Extraction of carotenoid was performed by *grinding* of processed food sample in mortar and pestle, filtration through sintered glass filter under vacuum and *separation* from acetone to petroleum ether. The petroleum eluent adjusted to specific volume was read at 450nm in a spectrophotometer (UV-1601, UV-Visible, Shimadzu, Tokyo, Japan) for concentration of total carotenoids.

Analysis of β-carotene

Reverse phase HPLC (Shimadzu PC based Binary Gradient HPLC Prominence System with PDA Detector, SPD-M20A; Solvent delivery System, LC-20AT; LC Solution Multi workstation Software) was used to determine the β-carotene (Roriguez-Amaya and Kimura,

2004). The nitrogen dried carotenoid was reconstituted with mobile phase (acetonitrile: methanol: 2-propanol-) and 50ul reconstituted sample was injected into the VYDAC reverse phase C column (5um particle size). The column was reequilibrated with the mobile phase for at least five minutes before the next injection. β-carotene was purchased from Sigma Chemical Co. USA and was used as standard analytes.

Analysis of mineral profile

Mineral content in the food sample was analyzed by Atomic absorption spectrophotometer method (Petersen, 2002). Dried food sample was subjected to wet digestion with nitric acid and perchloric acid in an auto-digester at 325^{o}C. The digested sample after appropriate dilution and treatment was aspirated into the spectrophotometer where it was burned into atomic components and absorbance was read at their respective wavelength. Sigma standard elements were used as standard analyses.

Data Analysis

Values were expressed as mean from three replications.

RESULTS AND DISCUSSION

Morphological Identification

Taxonomic enumeration

Family : Menispermaceae
Haematocarpus validus Bakh.f. ex Forman (authority). heptapleuri Karny, 549. aematocarpus Miers, 365, 383. comptus Miers, 383. subpeltatus 383. Haematopinus eurygaster Nitzsch, 184. eurysternus Nitzsch, 172. spinulosus Denny, "31753002580584_0700.txt".

Vernacular name

Blood fruit (English), Roktogula, Lalgula (Bengali), Rosco (Chakma), Ranguichi (Marma), Thoyphal (Tripura) and Khun phal (Hindi).

Biology

H. validus is dioecious. In Bangladesh, the vines produce flower in Mid November-January and the fruits mature in April-May. The fruiting season is May to August. Propagated by sexually and asexually (cutting).

Habitat

Haematocarpus validus grow dry land in hill forest.

Distribution

It mostly found in hilly forest of Chittagong Hill Tracts in Bangladesh.

Description

Haematocarpus validus is an evergreen perennial creeping woody climber capable of growing under extreme conditions, from very dry environments to highly acidic soils. It is up to 1000 m and more in height with dark green glabrous profusely branched. It creeps supported by big banyan or other long tree. Alternate leaves obovales elliptiques or ovales elliptiques , base obtuse. Length of leaf 11.8 cm and breath 5.6 cm, dark green, smooth and glossy. Petiole glabrescent (long : 12-35 mm) (Figure 2). Three distinct margin entire. The tiny, odorous, greenish-white flowers unisex

(a) Young Seeding

(b) Tree climbing on other tree

(c) Fruit bearing plants

(d) Leaves

(e) Green fruits

(f) Ripe fruits

(g) Longitudinal section of a fruit

(h) Seeds

(i) Ethno people eating fruit consumption

Figure 2 : Photographs showing the different parts of the Roktogota plant

(dioecie), and sepals ovales and elliptiques, petales oblongs (long : 1-2 mm), groups in panicules and pseudo-racemes axillaires (long : 9 cm). Fruits are drupes ovoid's ellipsoids, rouges and glabres. The ripe fruits are dark red. The average fruit is around 28 g, peel wt. 18g, flesh wt. 6g, peel thickness 2.01mm and long : 4-5 cm, diam : 2-2,5 cm, TSS 18. The fruits are single seeded and each seed weight is 4g, seed length 1.80cm, breadth 1.51cm and thickness 1.11cm. The round or ovoid fruits are borne in grapelike, pendent clusters (often paired) which are extremely showy because the berries ripen unevenly, the pale yellowish-green, bright- dark red stages present at the same time. The skin is very thick and tough but yields an abundance of bright-red juice which left a purple stain on fabrics. When unripe, whole fruits are very acidic, ripe fruits; are sub acid, slightly sweet much like Burmese grape. Some tasters detect a bitter principle or "unpleasant aftertaste" which is unnoticeable to others. Fruit was available in April to August. Mainly in Chittagong Hill Tract. It is mainly consumed by different ethnic communities. Roktogotas (*Haematocarpus validus*) are uses in various forms (Table 2). Fruits and vegetables are known to be nutritionally rich with vitamins and minerals. The nutritional composition of Roktogota fruit (*Haematocarpus validus)* has been discussed below.

Macronutrient/ Proximate Composition

This fruit contents 90.12% moisture of fresh weight. It is noted that fruits contain 10% to 30% dry matter and the rest is water (Ranganna, 1986). Most fruits and vegetables are composed of 70% to 90% water (Haque *et.al.*, 2009). The amount of p^H, TSS and Total sugar content of this fruit are 2.77, 21%, 11.07% respectively. Ceggara (1964) found that the pH range of ripe fruits was 4.5 to 5.35. Generally higher TSS indicates more sugar in the pulp. The more ripe the fruits contained the more amount of sugar in fruits. According to Norman (1976), the sugar content of fresh fruits ranges between 2% and 30%. This range is similar to the present study.

For most of the studies the fiber and protein content are considered as the main determi-

Table 2: Uses of Roktogota (*Haematocarpus validus*)

Purpose of uses	Mode of uses	Parts uses	Consumer
Economic uses: As a fruits, food colour and organic fertilizer.	Fruits of *H. validus* are slightly acidic sweet taste and are eaten in raw. The fruit can be used as natural and organic source of food colour. The fruit extracts of Roktogota can be used in colouring of soft drinks and deserts. Leaves are used as organic matter.	Pulp, fruit extract and leaves	All ethnic community of CHT
Medicinal uses for Jaundice, anemia and itching.	Tender shoot extract is used as curative measure of Jaundice. Fruits and seeds are used as curative measure of anemia and root mash is used to get relief from itching.	Fruits, seeds, tender shoot and root	Tribal people like Chakma and Marma tribe in CHTs

Table 3: The nutritional composition per 100 g of fruits of *Haematocarpus validus*

Item	Amount per 100 g of fruits of *H. validus*	Item	Amount per 100 g of fruits of *H. validus*
Moisture (g)	90.12	Iron (g)	0.57
Protein (g)	0.6	Copper (µg)	129.57
Carbohydrate (g)	6.99	Zinc (µg)	0.14
Fat (g)	1.44	Manganese (µg)	152.04
Crude fibre (g)	1.22	Calcium (g)	9.16
Ash (g)	1.23	Magnesium (g)	6.86
Energy (Kcal)	50	Sodium (g)	0.42
Vitamin C (g)	13.15	Potassium (g)	255.70
Total carotenoids (µg)	1170	Phosphorus (g)	39.50
β- carotene (µg)	9.0		

nants of food type and less is known about elemental composition of various wild edible species Anonymous, 1970-1988). Protein and crude fibre content of Roktogota ware 0.6% and 1.22% respectively in dry

weight basis. In general, protein content of different fruits is not greater than 3.5% in accordance with Norman (1976). The *Haematocarpus validus* as an excellent source of carbohydrate 6.99% in dry weight basis. Carbohydrate of fruit is less concentrated than cereals because of their high water content. Fruits rich in carbohydrate provides high amount of energy. This fruit showed the high amount of energy, 50 kcal/100g due to its high carbohydrate content. The fat and ash content ware 1.44% and 1.23% respectively. Due to generally low level of fat in the fruits, their consumption in large amounts is a good dietary habit and may be recommended to individuals suffering from overweight or obesity.

Vitamin and antioxidant

Vitamins, carotenoids and β- carotene, are naturally present in fruits, vegetables, herbs and spices (Ali *et al.* 2008). carotenoids and β- carotene play a very important role in human health and nutrition and can reduce the risk of cancer and coronary disease (Yahia & Ornelas-Paz, 2010). *Haematocarpus validus* contents 13.15 mg vitamin C, 1170 (µg) Carotenoids and 9.0 (µg) β- carotene respectively. Roktogota fruits content (13.15 mg/ 100 gm edible portion) vitamin C which is higher than jackfruit (11.08mg), litchi (7

mg), papaya (7.48mg) and mango (10.88mg) (Islam, 2012). The micro and macro mineral content in the Roktogota fruits are as follows (Table 3).

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