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Total phenolic compound, moisture and total carotenoid content of selected ten vegetables consumed by tribal people of Bangladesh

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ABSTRACT

The current experiment was conducted for the simultaneous determination of Total Phenolic Compound and Total Carotenoid Content in ten highly consumed tribal vegetables named as Dodder (CuscutareflexaRoxb.) (local name Torulota, Jigro) (common name Shornolota), Fodder Radish (Raphanussativus var. oleiformis) (local name Mulachi) (common name Pahari Mula), Banana inflorescence (Musa acuminataColla.) (local name Kolattur, Feufu) (common name Pahari/Bazraikolar Thor), Bronze Banana (Musa ornataRoxb) (local name Chera Kola) (common name Jongli Kola), Common red Stem–fig (FicusvariegataBlume) (local name JogunaGola) (common name Unknown), Indian coral tree (Erythrinavariegata L.) (local name Matar) (common name Mandar), Pigeon pea (CajanuscajanMillsp.) (local name Dumursumi, Crakchi) (common name orhorshak), Pigeon pea Leaves (CajanuscajanMillsp.) (local name Dumursumi Shak, Crakchi Shak) (common name orhor), Spiny Amaranth (AmaranthusspinosusL.) (local name Katamaris) (common name Kantanotey) and unknown (Leucasaspera(Roth) Spreng) (local name Thorinlodi, Shetodhron) (common name Dhondokolos). The analyses were performed by acetone-petroleumether extraction followed by Spectrophotometric measurement for total carotenoids, moisture and total phenolic compounds were done spectrophotometrically according to the Folin–Ciocalteu's method with slight modification.

Keywords: Spectrophotometry, Phenolic Compound, Carotenoids, Vegetables, Tribal people.

INTRODUCTION

Plants have sustained human populations by providing food, cloth, shelter, fuel and medicine. The agricultural revolution that began more than 10,000 years ago created a dramatic shift in the human food supply [1, 2 and 3]. It consequences a significant change in human food pattern where they becoming more prone to cultivated food rather wild species used as prime source of food once.

Bangladesh is a densely populated country of South East Asia, rich in about 58 different tribes with approximately 1.2 million tribal people, which is just above 1 percent of the total population. The use of wild plants is integral part of their strong traditional and cultural systems and practice that have developed and accumulated over generations. Wild edible plants not only supplement the food quantity but also make significant contribution to the populations' nutrition throughout the year [4, 5 and 6]. Besides, These vegetables are indiscriminately grown without much care at anywhere like at homesteads, forests, roadsides and near railway, are cheaper and can easily consumed by mass people with much less cost and effort [7].

Evidence found that forest people lead healthier and disease free life compared to rural and urban one. Moreover, the current chronic or non-communicable diseases are no longer the "disease of the affluent". It is currently emerging both in poorer countries and poorer population groups in the richer countries [8]. Moreover, like other developing countries, malnutrition is a severe health problem in Bangladesh and more than 80% of the people are suffering from malnutrition (BBS, 2006) [9]. Vitamin A deficiency in Bangladesh is one of the Public health concerns [10]. It is a major cause of preventable blindness in children; about 10 million children become completely or partially blind every year in Bangladesh (BBS, 2006). On the otherhand, Phenols are one of the major groups of nonessential dietary components appearing in vegetable foods. They are a wide chemical compounds group that are considered as secondary plant metabolites, with different activity and chemical structure, including more than 8,000 different compounds. Phenols, has traditionally been considered as antinutritive compounds due to the adverse effect of one of their main components, tannins, on protein digestibility [11]. However, actually there is an increased interest in these compounds because they have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolics may be related to their antioxidant behaviour, which is attributed to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [12]. In order to overcome this situation, much attention has been centered on staple foods like vegetables [13]. In the light of above facts, an effort was made to document some of the cultivated as well as wild plant parts consumed by selected tribal groups, which are used for meeting their nutritional requirements. Only frequently utilized plants were further investigated for determining the nutrient composition.

This research will provide information of total phenolic compound and total carotenoid content of selected ten vegetables consumed by tribal people of bangladesh (more importantly the bioactive compounds) for calculating nutrient intakes. Its Results have the potential to meet increasing demands from health professionals providing dietary guidance, nutrition policy makers and researchers studying the relationships between food and health.

Purpose of the Study

The main purpose to conduct this study were to document some rare foods commonly consumed by tribal people but not so familiar in mass people and carry out a nutritional analysis and thus come up with recommendations for researchers and food policy programmer.

MATERIALS AND METHODS

2.1. Reagents

The analytical grade acetone, petroleum ether, and butylatedhydroxytoluene (BHT) were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu Reagent (FCR) and gallic acid solutions were purchased from Sigma Chemical Co. (St. Louis,MO, USA).

2.2. Sampling protocol

A multi-regional sampling plan (fig.1) was employed to collect representative vegetable samples. Food items were collected from local weekly markets at Rangamati, Bandarban, Khagrachari, Gazipur and some places of Dhaka. Three food samples for each food items were collected from the market as well as from the field directly (when possible). Foods were divided into two categories: 1) modified tribal

Multi-regions sampling plan

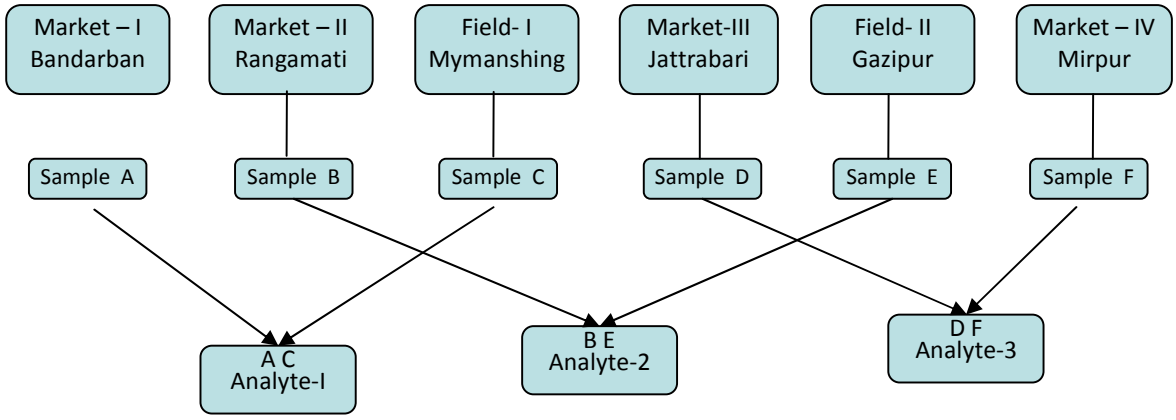
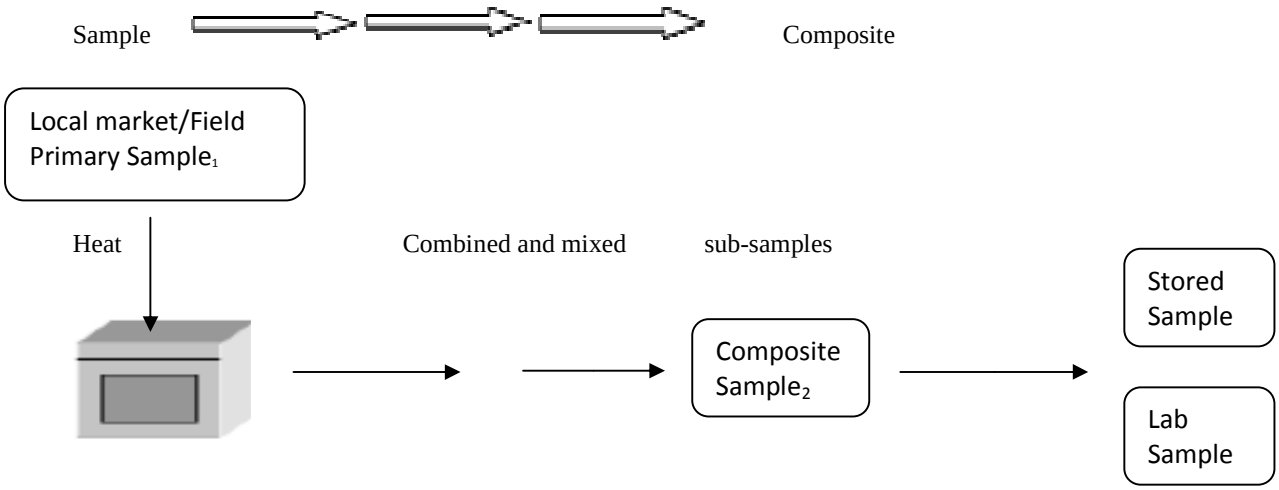


Figure 1. Multi-regions sampling plan

foods, consumed by mainstream populations, which were collected from supermarkets and takeaways, and 2) authentic tribal foods (mainly consumed by tribal minority groups) which were either home-made or obtained from Tribal food stores. Every two food samples were pooled together to make three analytes (test sample) which were prepared in edible form and stored at -20˚C prior to analysis.

Figure 2: Sampling procedure and Processing plan



2.3 Collection and preparation of vegetable sample

The representative vegetable samples were collected during May 2012 and January 2013 from the selected sampling areas and processing procedure (Figure 1 & 2).The vegetable items that were collected from distant points such as from fields were well packed in clean dark plastic poly bags to prevent water loss and damage by light, and transported to laboratory within shortest time span. The fresh vegetables were washed with tape water followed by with distilled water and then the surface water was removed using blotting paper. The cleaned air-dried vegetable samples were cut into small pieces (peeled and removed seeds where needed) by hand, using a clean knife on a clean cutting surface. In case of carotenoid, these operations were performed very fast in dim light to avoid any degradation.

2.4. Identification of sample
The collected food samples were identified and authenticated by an expert taxonomist from the department of Botany, University of Dhaka, Bangladesh.

2.5.1. Estimation of moisture content
The moisture content of food was determined by estimating the amount of water removed from the food upon heating [14]. Approximately 10g of fresh sample was taken in a crucible (pre-washed and dried at 105°C). It was then kept 100-105°C temperature in an oven for 5 hours and cooled in desiccators and weighted again. Heating, cooling and weighing were continued until a constant weight was obtained.

Calculation

$$\text{Moisture \%} = \left[\frac{\text{Initial weight} - \text{final weight}}{\text{weight of sample}}\right] \times 100$$

Here,
Initial weight = Sample weight + crucible weight (before heating).
Final weight = Sample weight + crucible weight (after heating).

2.5.2 Analysis of total carotenoids
It was done by acetone-petroleumether extraction followed by Spectrophotometric measurement [15]. Extraction of carotenoids was performed by grinding of processed food sample in mortar and pestle, filtering through sintered glass filter under vacuum and separation from acetone to petroleum ether.

If the colour of eluent was orange like, the absorbance was taken at 450nm in a spectrophotometer (UV-1601, UV-Visible, Shimadzu, Tokyo, Japan) for concentration of total carotenoids. But if the eluent was green, that contains chlorophyll then the extract was passed through a column packed with activated Alumina and Sodium anhydrous (1:1) to remove green pigment. The column eluent then read at 450 nm. To avoid photosensitive damage total preparative and extractive procedure were performed in dim light.

2.5.3 Analysis of Total phenolic compounds:
Total phenolic content was determined spectrophotometrically according to the Folin–Ciocalteu's method [16] with slight modification. Briefly, 0.5g of the dried sample was subjected to extraction by Hexane: Dichloromethane (1:1). After shake overnight and centrifuge then supernatant was discarded and the precipitate was dried to evaporate the left over solvent. Then AWA (Distilled Acetone: Distilled Water: Glacial Acetic Acid =70:29.5:.5) was added and sonicated for disrupting the cell matrix of the sample for maximum extraction. After sonication, the samples were again centrifuged at 5000-7000 rpm for 15 minutes. The supernatant was then separated from the precipitate. Distilled water, Folin-Ciocalteu Reagent (FCR) and 2 % Sodium carbonate solution was added to the supernatant.

This colorimetric method is based on the reduction of a phosphotungstate - phosphomolybdate complex by phenolics to blue reaction products in alkaline conditions. Since different types of polyphenols react similarly with Folin-Ciocalteau (FC) reagent, it is more easily quantifiable. Finally, the absorbance of blue coloration was measured at λ = 750nm against a blank sample. The measurements were compared to a standard curve of prepared gallic acid solutions. Gallic acid, in varying concentrations (0, 50, 100, 150, 250 and 500 mg/l), were used to prepare the standard curve in Figure 3. This curve is used to relate the absorbance of the unknown samples to Gallic acid equivalents (GAE).

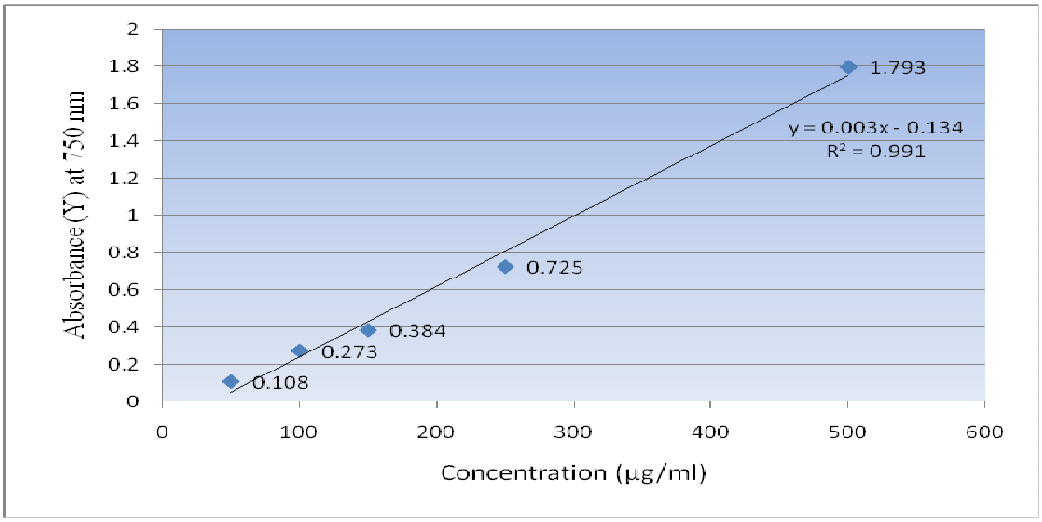


Figure 3: Gallic Acid Standard Curve

The result was reported as milligrams of Gallic acid equivalents (GAE) per 100 g ± SD. All measurements were performed in triplicate.

2.6. Data quality

Data quality was maintained by precisely and accurately carried out the intra- and inter-laboratory analysis in and between the laboratory at the Institute of Nutrition and Food Science, University of Dhaka and Center for Advance researches (CARs), University of Dhaka; Bangladesh using internal standard [17].

2.7. Data analysis

SPSS software package (version 12.0 SPSS Inc. Chicago, IL, USA) was used to analyze the nutrient data. Descriptive statistics were used for all of the variables. Values were expressed as mean and standard deviation.

RESULTS AND DISCUSSION

3.1 Moisture content:

Moisture content (Table 1) in Mulachi was 92.71-92.87 % with an average of 92.79 %. Arhar seed had the lowest moisture content of about 58.6%. Other vegetables contains moisture content of around 86-90% while 90.99%, 90.91%, 88.94% in Mandar, pahari kolar thor and Dondokolos, respectively. High moisture content of vegetable in turn considers the vegetables as low calorie food

Table 1: List of Moisture contents of selected 10 vegetables

Common Name	English Name	Local Name	Scientific Name	Moisture (%)
Shornolota	Dodder	Torulota, Jigro	*Cuscutareflexa*Roxb.	89.84±0.88
PahariMula	Fodder Radish	Mulachi	*Raphanussativus var. oleiformis*	92.79±0.08
Pahari/ Bazraikolar Thor	Banana inflorescence	Kolattur, Feufu	*Musa acuminata*Colla.	90.91±0.62
Jongli Kola	Bronze Banana	Chera Kola	*Musa ornata*Roxb	77.1±0.04
Unknown	Common red Stem –fig	JogunaGola	*FicusvariegataBlume.*	90.85±0.38
Mandar	Indian coral tree	Matar	*Erythrinavariegata* L.	90.99±0.20
Orhorshak	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	86.34±0.31
Orhor	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	58.6±0.63
Kantanotey	Spiny Amaranth	Katamaris, Khuriakanta	*Amaranthusspinosus*L.	86.77±0.06
Dhondokolos	Unknown	Thorinlodi, Shetodhron	*Leucasaspera*(Roth) Spreng	88.94±0.58

3.2 Total carotenoids

From list of carotenoid contents of all vegetables (Table 2), it was found that except Banana inflorescence and Bronze Banana all other samples contained appreciable amount of carotenoids ranged from 1301 to 4200 µg/100g whereas Pigeon pea leaves contained higher carotenoids of about 4199.16 followed by Spiny Amaranth leaves and

Pigeon pea which contain 3962.78 µg/100g and 4124.64 µg/100g carotenoids, respectively. Banana inflorescence and Bronze Banana contained 191.70 and 513.23µg/100g of raw weight. Among the other vegetables Pigeon pea leaves, Fodder Radish, Dodder, Common red Stem –fig leaves contained about 2514.16, 2414.51, 2238.69 and 2005.24 µg/100g carotenoids, respectively.

Table 2: List of total carotenoids contents of all vegetables

Common Name	English Name	Local Name	Scientific Name	Carotenoid (µg/100g)
Shornolota	Dodder	Torulota, Jigro	*Cuscutareflexa*Roxb.	2238.69±4.5
PahariMula	Fodder Radish	Mulachi	*Raphanussativus var. oleiformis*	2414.51±17.71
Pahari/ Bazraikolar Thor	Banana inflorescence	Kolattur, Feufu	*Musa acuminata*Colla.	191.70±6.3
Jongli Kola	Bronze Banana	Chera Kola	*Musa ornata*Roxb	513.23±1.02
Unknown	Common red Stem –fig	JogunaGola	*FicusvariegataBlume.*	2005.24±16.8
Mandar	Indian coral tree	Matar	*Erythrinavariegata* L.	1901.02±6.9
Orhorshak	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	4199.16±5.4
Orhor	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	3962.78±29.6
Kantanotey	Spiny Amaranth	Katamaris, Khuriakanta	*Amaranthusspinosus*L.	4124.64±5.4
Dhondokolos	Unknown	Thorinlodi, Shetodhron	*Leucasaspera*(Roth) Spreng	1928.90±5.1

The rare vegetable Pigeon pea leaves (4199.16±5.4µg %) and Spiny Amaranth (4124.64±5.4µg %), Pigeon pea seeds (3962.78±29.6µg %) and Fodder Radish (2414.51±17.71µg %) contained higher amount of fiber as compared to very commercial and nutritive leafy vegetables like cabbage (120µg %), cauliflower (35µg %), Lettuœ (990µg %) and Pumpkin leaves (1940µg %) [17].

It indicates that these rare vegetables could also be a rich source of pro-vitamin A to prevent the night blindness in the children.

3.3 Total Phenolic Compound Content

Total phenolic compound of different vegetables included in the present study was shown in **Table 3.** Obtained results showed that, except Pigeon pea TP content varies from 37.63±1.16 to 63.46±0.61 mgGAE/100g on fresh weight basis. Data presented in the table showed that Pigeon pea contained the highest TP (91.76 ±3.56mgGAE/100g), whereas Banana inflorescence contained the lowest (37.63 mgGAE/100g) among the ten vegetables in fresh weight basis. Among the others Common red Stem –fig, Spiny Amaranth, Dhondokolos, Pigeon pea leaves contained 55.73±0.88, 62.10±1.27, 56.58± 0.50 and63.46±0.61 mgGAE/100g fresh. Moreover, it can be said that the TPC of GLVs varies widely depending on the variety and the environmental conditions. Several factors could be added to be responsible for differences in total phenolic content of food stuffs of same or similar origin. They include variation in fruit cultivars, harvest and post-harvest handling and storage conditions, processing techniques during analytical determinations.

Table 3: Total phenolic (TP) content of all analyzed food items (in fresh & dry basis)

Common Name	English Name	Local Name	Scientific Name	TP content (mgGAE/100g) Dry weight basis	TP content (mgGAE/100g) Fresh weight basis
Shornolota	Dodder	Torulota, Jigro	*Cuscutareflexa*Roxb.	443.87±0.67	44.79±0.32
PahariMula	Fodder Radish	Mulachi	*Raphanussativus var. oleiformis*	675.96±1.92	48.56±1.38
Pahari/ Bazraikolar Thor	Banana inflorescence	Kolattur, Feufu	*Musa acuminata*Colla.	414.31±1.84	37.63±1.16
Jongli Kola	Bronze Banana	Chera Kola	*Musa ornata*Roxb	472.06±2.23	43.15±1.76
Unknown	Common red Stem –fig	JogunaGola	*FicusvariegataBlume.*	668.23±0.91	63.46±0.61
Mandar	Indian coral tree	Matar	*Erythrinavariegata* L.	456.66±2.36	50.74±1.78
Orhorshak	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	407.94±1.23	55.73±0.88
Orhor	Pigeon pea	Dumursumi, Crakchi	*Cajanuscajan*Millsp.	490.64±5.15	91.76±3.56
Kantanotey	Spiny Amaranth	Katamaris, Khuriakanta	*Amaranthusspinosus*L.	469.48±1.49	62.10±1.27
Dhondokolos	Unknown	Thorinlodi, Shetodhron	*Leucasaspera*(Roth) Spreng	509.22±0.77	56.58± 0.50

Total phenolic compound in current study in dry basis was 490 mgGAE/100g whereas [18] reported the value as 250-391 mgGAE/100g on different varieties on dry weight basis.

CONCLUSION

The rare vegetables analysed in this study are rich in carotenoid as well as with phenolic compound. Making awareness for mass use of these vegetables would help to prevent suffering from many nutritional deficiency diseases. To ensure dietary diversification and also to maintain the biodiversity by protecting them from extinction, these rare vegetables should cultivate like other vegetables commonly consumed by mass people. Besides, it is believed that tribal consumed plants species may contain some potentially important bioactive components. Thus, by quantifying the important active ingredients of the potentially important plants they were introduced as future medicinal plants. Moreover, the nutrient values contained in these fruits will enrich the food composition database for Bangladesh which is essential for health, nutrition and food policy program planning.

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