http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (21): 1019-1026, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.1019.1026

Biochemical Analysis of Two Varieties of Water Chestnuts (*Trapa* Sp.)

[1]M. Omar Faruk, [2]M. Ziaul Amin, [1]Niranjan Kumar Sana, [1,3]Ranajit Kumar Shaha and [1]Kamal Krishna Biswas
[1]Deptartment of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh
[2]Deptartment of Genetic Engineering and Biotechnology,
Jessore Science and Technology University, Jessore-7408, Bangladesh
[3]Faculty of Agroindustry and Natural Resources, University of Malaysia, Kalantan, Malaysia

Abstract: In this study, two varieties (Green and red) of water chestnuts (*Trapa* sp.) have been selected for their biochemical analysis as well as nutrient composition using standard methods. The proximate composition of green water chestnuts revealed moisture 62.5, ash 1.04, crude fiber 2.13%, total soluble sugar 0.92%, reducing sugar 0.33%, non-reducing sugar 0.59%, starch 8.7%, lipid 0.84%. One hundred gram of green variety contained water soluble protein 0.275 mg, β-Carotene 60 μg, vitamin-C 1.1 mg and total phenol 0.5 mg. The minerals contents of green variety were potassium 5.22%, sodium 0.64%, calcium 0.25%, phosphorus 6.77%, sulpher 0.38%, and iron, copper, manganese and zinc 200, 430, 90 and 600 ppm, respectively. The red variety contained moisture 62.7%, ash 1.30%, crude fiber 2.27%, total soluble sugar 0.90%, reducing sugar 0.30%, non-reducing sugar 0.60%, starch 8.2%, lipid 0.83%. The red variety contained water soluble protein 0.251 mg, β-Carotene 92 μg, vitamin-C 0.9 mg and total phenol 0.60 mg per 100 g. The red variety contained potassium 5.32%, sodium 0.59%, calcium 0.26% phosphorus 6.77%, sulpher 0.32%, Iron 200 ppm, copper 450 ppm, manganese 110 ppm and zinc 650 ppm. The free amino acids, glutamic acid, tryptophan, tyrosine, alanine, lysine and leucine were commonly found in both varieties. In addition, green and red variety contained cysteine, arginine and proline and glutamine and asparagines, respectively. Thus, the present study sheds light on the nutrient contents of the two varieties of water chestnuts and suggests that water chestnuts may play a crucial role in human nutrition.

Key words: Aquatic plant, *Trapa*, fruit, red variety, green variety, nutritional composition, total titratable acidity

INTRODUCTION

Water chestnut is an annual aquatic dicotyledonous plant commonly known as singhara or paniphol in Bangladesh. Several varieties are grown in Bangladesh among which *Trapa bispinosa* Roxb. and *Trapa natans* L. are important. It grows well in shallow fresh water basin in the tropical, sub-tropical and temperate zone of the world (Daniel *et al*., 1983; Kumar *et al*., 1985). *Trapa bispinosa* has two varieties, one is red (Leaf, petiole and fruit) and the other is green (Leaf, petiole and fruit), each of the fruit is large in size having four dull spines.

It has been commercially cultivated in India, Japan, China, Pakistan, Srilanka and different parts of Southeast Asia (Kumar *et al*., 1985; Mazumdar, 1985). Recently, the cultivation of water chestnut is becoming more popular in Bangladesh due to its easy grow, low cost of production, good profit and to meet up the demand for more food production. This plant is extensively cultivated in fresh water tanks, beels, ditches and ponds (Khan and Halim, 1987).

A balanced diet is essential to maintain a sound health. A balanced diet should contain sufficient fruits and vegetables in order to supply vitamins and minerals, deficiency of those cause specific diseases. Bangladesh is abounding with diverse fruits. Water chestnut is a seasonal fruit that grows well everywhere in Bangladesh. Recently a large number of people have been eating this delicious fruit for its rich nutrient contents (Pandit and Quadri, 1986) as well as low price. According to a report by Kusum and Chandra (1980) the nutritive value of the fruit is not less than that of wheat.

For human consumption water chestnut is eaten in many countries as raw, boiled or roasted and stem or leaves as vegetables or flavor or is added in other dishes. Fruits are used as a substitute for cereals in the Indian sub-continent, especially during certain important festivals. It is used also to make colored powder and dye (Kusum and Chandra, 1980).

The fruit has also been used in medicinal purposes (e.g., rheumatism, sunburn) (Kosuge *et al*., 1985). They are also useful in burning sensation, dyspepsia,

Corresponding Author: Kamal Krishna Biswas, Deptartment of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh Tel: +88-0721-711109 Fax : +88-0721-750064

intermittent fever fatigue, inflammation, bronchitis and debility. Flour made from fruit is suitable for textile sizing and is a good substitute for cornstarch in ice cream manufacture.

Importance of fruits in the human diet is well recognized because they are rich in essential contents like vitamins and minerals which help in the maintenance of proper health and resistance to specific diseases. Fruits also provide carbohydrates, proteins, fats as well as dietary fiber (fruit skin). Presently, it is well accepted that dietary fiber appears to lower the risk of colon cancer. According to the recommendation of the National Cancer Institute, USA, 20 to 35 g of dietary fiber should be present in our everyday meal (Kowtaluk and Kopan, 1997). Therefore, eating fruits with their skins daily may help us to maintain a good health.

Some research is going on in several institutes of our country with the emphasis on the callus induction and *in vitro* organogenesis and thus plant regeneration, higher production, development of new varieties, control of the diseases and thereby higher production of fruits. However, data available on the physicochemical parameters, enzyme and antimicrobial activities of water chestnuts produced in Bangladesh is quite scanty. Only a limited work was done on the physicochemical properties of different varieties of water chestnuts (Majid, 1986; Irfanullah, 2002). Recently, Alfasane *et al.* (2008, 2009) have studied the biochemical composition of the seeds of *Euryale ferox* Salisb and *Nelumbo nucifera* Gaertn. The natural habitat of the plant has also been decreasing at a high rate.

Water chestnut may contribute a lot in the nutrition of our Bangladeshi people by providing essential nutrients. Therefore, the details research works including biochemical and biological investigations are very important to release a good variety of water chestnut, which would be nutritionally rich as well as high yielding. Keeping all these in mind in this study, an attempt was made to investigate in details of the water chestnut fruits using locally available two varieties (green and red). This study comprises the determination and comparison of physiochemical characteristics such as pH, Total Titratable Acidity (TTA), moisture, ash, free sugar, starch, reducing sugar, non-reducing sugar, β-carotene, protein, minerals, free amino acids etc. Finally, the purpose of this study was to increase existing knowledge on aquatic plants by investigating the chemical and mineral composition of the plant and to explore its suitability as a source food.

MATERIALS AND METHODS

Freshly harvested fruits of Water chestnut (*Trapa* sp.) were collected on July 2010-September 2010 from the Fruit Research Substation, Shyampur, Rajshahi, Bangladesh for experimental purpose. The physical and chemical parameters were studied from July 2010-January 2011 by the following methods. The chemicals and reagents used in this study were of analytical grade.

Determination of pH:

- **Extraction of juice from water chestnut (*Trapa* sp.):** About 70-90 g of water chestnuts (*Trapa* sp.) was taken in a mortar. The fruits were crushed thoroughly in a mortar with a pestle and then filtered through two layers of muslin cloth. The filtrate was then centrifuged for 10 min at 3000 rpm and the clear supernatant was collected
- **Preparation of standard buffer solution:** Buffer tablets (BDH Chemicals Ltd. Poole, England) of pH 7.0 or 4.0 was dissolved in distilled water and made up to the mark of 100 mL with distilled water
- **Procedure:** The electrode assembly of the pH meter was dipped into the standard buffer solution of pH 7.0 taken in a clear and dry beaker. The temperature correction knob was set to 28°C and the fine adjustment was made by asymmetry potentially knob to pH 7.0. After a wash the electrode assembly was then dipped into a solution of standard pH 4.0 and adjusted to the required pH by fine asymmetry potentially knob. The electrode assembly was raised, washed twice with distilled water, rinsed off with the juice of the cultivars and then dipped into the juice of the water chestnut (*Trapa* sp.). The pH of the juice was noted

Determination of total titratable acidity (TTA): TTA was determined by the Folin method (Oser, 1965).

Determination of moisture content: The moisture content was determined by the conventional procedure (ICOMR, 1971).

Determination of dry matter: Dry matter content was calculated from the data obtained from percentage of moisture content.

Determination of ash: Ash content was determined as followed by the method of AOAC (1980).

Determination of water-soluble protein: Water-soluble protein concentration was determined following the method of Lowry *et al.* (1951) using BSA as standard. The extraction was carried out with distilled water.

Determination of lipid: Lipid content of the water chestnuts (*Trapa* sp.) was determined as described by Bligh and Dyer (1959).

Determination of total sugar: The total sugar content of water chestnuts (*Trapa* sp.) was determined colorimetrically by the anthrone method as described in Laboratory Manual in Biochemistry (Jayarayamau, 1981).

Extraction of sugar: Extraction of sugar from water chestnuts (*Trapa* sp.) was done as described by Loomis and Shull (1937).

Determination of reducing sugar: Reducing sugar content of the water chestnuts (*Trapa* sp.) was determined by dinitrosalicylic acid method (Miller, 1972).

Determination of non-reducing sugar or sucrose: Non-reducing sugar or sucrose content was determined by using the formula (Ranganna, 1979).

Determination of starch: The starch content of the water chestnuts (*Trapa* sp.) was determined by the Anthrone method, as described in Laboratory Manual in Biochemistry (Jayarayamau, 1981; Clegg, 1956).

Determination of vitamin-C: Vitamin-C content of water chestnuts (*Trapa* sp.) was determined by the titrimetric method (Bessey and King, 1933).

Determination of β-Carotene: β-Carotene content of water chestnuts (*Trapa* sp.) was determined according to the procedure reported in methods of Biochemical Analysis (Metcalf, 1957).

Column preparation: A column was prepared by using alumina as a packing material. Ten precent acetone in petroleum ether was used as eluent buffer.

Procedure: The 5.0 g of fresh water chestnuts (*Trapa* sp.) and about 4.0 g of ammonium sulphate were taken in a mortar and rubbed to an even paste with pestle. The extraction was carried out with acetone and small amount of hexane. Extraction was continued until the acetone extract became colorless. Potassium hydroxide solution (10 mL, 5.6%) was added to the extract and it was kept in a dark place for half an hour. The mixture was then transferred to a separating funnel, then 20 mL of petroleum ether few mL of hexane and 10 mL of water were added to the process was repeated until the petroleum ether layer became colorless. The petroleum ether, was concentrated by gentle heating, the concentrated extract (1-2 mL) was applied on to the top of the alumina column and eluted with 10% acetone in petroleum ether. The absorbance of the eluent was taken at 440 nm in a Coleman Junior II spectrophotometer.

Construction of standard curve of β-carotene: A standard curve (data not shown) was prepared by taking 0.0, 0.1, 0.2, 0.4, 1.6, 0.8 and 1.0 mL standard solution of β-carotene and the volume was made up to 5 mL with petroleum ether and mixed well. The absorbance of the solutions was taken at 440 nm in a Coleman Junior II spectrophotometer and a standard curve of β-carotene was prepared by plotting the data. The amount of β-carotene content in each variety of water chestnut (*Trapa* sp.) was calculated by using the standard curve (data not shown).

Determination of total phenol: Total phenol content of Water chestnuts (*Trapa* sp.) was determined colorimetrically by Folin-Ciocalteau's method (Bray and Thorpe, 1954).

Extraction of phenol: Extraction of phenol from Water chestnuts (*Trapa* sp.) was done according to the methods described by Loomis and Shull (1937).

Five to six gram of water chestnuts (*Trapa* sp.) were cut into small pieces and immediately plunged into boiling ethyl alcohol and allowed to boil for 5-10 min. (5 to 10 mL of alcohol was used per g of fruits). The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract filtered through two layers of muslin-cloth and re-extracted the tissue for three min. in hot 80% alcohol, using 2 to 3 mL of alcohol for each g of tissue. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Both the extracts were filtered through Whatman No. 41 filter paper. This alcohol extract was used for the estimation of total phenol.

Determination of crude fiber: Crude fiber was determined by the following method (AOAC, 1980).

Determination of minerals: Minerals compositions were determined as described by Blake (1965).

Preparation of plant samples for analysis

Drying: A clean container (dish or beaker) was placed in an oven at 105°C overnight and allowed the container to cool in desiccators and weigh it. The samples were put in to the container and weight was taken. Then the container was placed in the oven at 105°C for 24 h and allowed the container to cool in a desiccator and weight was taken again. Drying cooling and weighting were repeated until

the weight becomes constant. The dried sample was stored in an airtight container. The moisture content in the sample was calculated.

Grinding: The dried plant material was cut into small pieces with a knife or scissors. The sample was grinded in a plant grinder fitted with a suitable screen. When the grinding takes a long time, the sample were absorbed moisture and then it necessary to dried the sample again in the oven at 105°C overnight.

Digestion: After grinding, the organic matter was digested and Ca, K, Na, Fe, Cu and P were released by digestion with nitric acid. In digestion 0.5 g dried plant material was weighed into each of 38 nitrogen digestion tubes. The two remaining tubes were blanks. The 5 mL 68% nitric acid was added to each of all 40 tubes. The content in each tube was mixed and left overnight. The tubes in the digester were placed and covered the tubes with the exhaust manifold. The temperature was set to 125°C. The digester was turn on and continued the digestion for 4 h after boiling has started. Precaution was taken so that no tubes became dry. After cooling, the digestion mixture was transferred with distilled water to a 100 mL volumetric flask. The flask was made up to volume with water and mixed and filtered on a dry filter into a dry bottle, which was closed with a screw cap. The filtrate was kept in the closed bottle. Ca, K, Na, Fe, Cu and P contents in the filtrates were determined.

Estimation of minerals: The minerals Ca, Fe and Cu were estimated by atomic absorption spectrophotometer. For the estimation of desired mineral, 20 mL of filtrate was transferred to a 100 mL volumetric flask. The flask was made up to volume with distilled water and was mixed well.

Estimation of Ca: For the estimation of Ca, 20 mL of diluted filtrate was transferred into a 50 mL volumetric flask and the flask was made up to volume with distilled water and mixed. The content of Ca was measured by Atomic Absorption Spectrometer (AAS).

Estimation of K and Na: Diluted filtrate (10 mL) was transferred into a 50 mL volumetric flask using a pipette. The flask was made up to volume with water and mixed. The content of K and Na were measured by flame photometer.

Estimation of P: For the estimation of P, diluted filtrate (5 mL) was transferred to a 50 mL volumetric flask. Water (approximately 30 mL), added to it and mixed well followed by addition of 10 mL ammonium molybdate-ascorbic acid solution. The flask was then made up to volume with water and mixed well. The content of P was measured by Atomic Absorption Spectrometer (AAS).

Estimation of Fe and Cu: The content of these elements were measured by Atomic Absorption Spectrometer (AAS) directly in the undiluted filtrate.

Identification of free amino acids: Free amino acids present in both of the examined varieties were determined as described by Jayarayamau (1981).

Procedure: Water chestnuts (1 g) was taken in a morter and pasted with distilled water. The pasted mixture was filtered through filter paper (Whatman No. 40). The filtrate was centrifuged at 8000 rpm for 10 min and the clean solution was used for the experiment. The free amino acids were separated from the filtrate by two-dimentional paper chromatography following the conventional procedure (Mazumdar, 1985) and identified by ninhydrin spray. The following solvents were used during chromatographic separation. First dimension: n-Butanol: acetic acid: water (3:1:1) second dimension:phenol:water (4:1).

RESULTS AND DISCUSSION

We demonstrated for the first time the pH as well as TTA content (Table 1) of different varieties of water chestnuts (*Trapa* sp.). The pH of water chestnuts (*Trapa* sp.) was found in the acidic range of both varieties. From the results of TTA it was observed that the acidity of the water chestnuts (*Trapa* sp.) was decreased in green variety. This decrease in TTA was consistent with the increase in pH at green variety.

Moisture plays an important role in the growth activities of trees. Water is indispensable for the absorption and transport of food, to carry out photosynthesis, to metabolize materials and to regulate moisture in plants, as in all other living systems. It contributes as much as to the essential properties of life as do the other constituents like carbohydrate, protein. Moisture is also essential for most of the physiological reactions in the plant tissues and in its absence life do not exist. Therefore, in this present study emphasis was also

Table 1: pH and total titratable acidity (TTA) of two varieties of water chestnuts (*Trapa* sp.)

	Values	
Parameters	Green	Red
pH	5.88±0.04	5.11±0.03
TTA (mL of 0.1 N NaOH required/100 g)	7.1	7.3

Table 2: Nutrient content of two varieties of water chestnuts (*Trapa* sp.)

	Amounts	
Parameters	Green	Red
Moisture (%)	62.5±1.16	62.7±1.19
Dry matter (%)	11.5±0.15	17.3±1.22
Ash (%)	1.04±0.02	1.09±0.03
Water soluble protein (mg/100 g)	0.275±0.03	0.251±0.04
Total lipid (g %)	0.84±0.02	0.83±0.02
Total sugar (g %)	0.92±0.03	0.9±0.05
Reducing sugar (g %)	0.33±0.02	0.3±0.03
Non-reducing sugar or sucrose (g %)	0.59±0.01	0.6±0.03
Starch (g %)	8.7±0.03	8.2±0.02
Vitamin-C (mg)	1.1±0.02	0.9±0.02
β-Carotene (μg)	60±2.06	92±3.73
Total Phenol (mg)	0.5±0.01	0.6±0.02
Crude fiber (%)	2.13±0.03	2.27±0.05

given to determine the moisture contents of the two varieties of water chestnuts. The moisture contents were found to be 62.5 and 62.7% in the green and red varieties of water chestnuts, respectively (Table 2). These results are closely related to a recent report by Alfasane *et al.* (2011) who demonstrated that water chestnuts (*Trapa bispinosa* Roxb.) contained moisture 70.35%.

Dry matter content analysis (Table 2) revealed a marked increase of dry matter as derived from percentage of moisture content in red variety as compared to green variety. The increased in dry matter content showed good correlation with the decrease in moisture content.

The ash content of water chestnuts (*Trapa* sp.) is shown in the Table 2. Most of the inorganic constituents or minerals are present in ash. The ash content was observed 1.04 and 1.09% in green and red varieties, respectively. The results suggesting that the ash content is almost similar in both of the varieties of water chestnuts.

Protein plays an important role in all the biological processes. The protein constituents of fruits and vegetables, although occurring in low concentration, are of primary importance not only as component of nuclear and cytoplasmic structures, but also including, as they must the full complement of enzymes involved in metabolism during growth, development, maturation of fruit and vegetables (Hansen, 1970). So, in this present study we focus on to determine the protein contents into the two variety of water chestnuts. Water soluble protein content was determined by Lowry method as described in the materials and methods. The results presented in Table 2 indicated that water chestnuts (*Trapa* sp.) contained 0.275 and 0.251 mg (per 100 g) of water soluble protein in green and red varieties, respectively.

Lipid is more useful in animal body. Fats serve as efficient source of energy and insoluble material. Dietary fat helps in the absorption of fat soluble vitamins, lipoproteins are important cellular constituents. Lipids are also essential components of cell membrane, source of metabolic energy for cell maintenance, reproduction and embryogenesis in insects. As lipid is very important for our body, so in this study we have determined the lipid contents in water chest nuts and it was found that water chestnuts contain a very low amount of lipid i.e., 0.84 and 0.83% in green and red varieties, respectively (Table 2). These results are consistent with a report by Alfasane *et al.* (2011).

The total soluble sugar content of water chestnuts (*Trapa* sp.) was analyzed and the result is summarized in Table 2. The amount of total soluble sugar was estimated to be 0.92% (green) and 0.90% (red). Table 2 also showed the reducing sugar content of water chestnuts (*Trapa* sp.). It was found that water chestnuts (*Trapa* sp.) contained low amount of reducing sugar and the content of reducing sugar was estimated to be 0.33 and 0.30% in green and red varieties, respectively. It is reported that in case of water chestnuts (*Trapa* sp.) the reducing sugar content is affected by several factors including variety, growing conditions, maturity and the storage environment (Mazumdar, 1985).

In this study, we also determined the non-reducing sugar or sucrose content of two variety of water chestnuts (*Trapa* sp.). From Table 2, it was observed that the sucrose content of water chestnuts (*Trapa* sp.) were found to be almost similar in both of the varieties (green 0.59 and red 0.60%).

Starch is the most important source of carbohydrate in human diet. As water chest nut has been consuming by a large number of people for its rich nutrient contents (Pandit and Quadri, 1986) as well as low price, it is very important to determine the amount of starch contents to the different varieties of water chest nuts. The results presented in Table 2 indicated that both of the varieties contained a significant amount of starch, i.e., 8.7 and 8.2% in green and red varieties respectively. In contrast to these findings, Tulyathan *et al.* (2005) and Alfasane *et al.* (2011) reported a higher amount of starch and protein in the storage organ coteledon of *Trapa bispinosa* Roxib. This discrepancy of results may be due to differences of the methods used, variety of the plant, location, maturity and storage condition etc. It has been reported that aquatic plants differ widely in their chemical composition depending upon species, season, location, growing conditions, maturity and the storage environment (Mazumdar, 1985; Anonymous, 1984; Unni, 1984). Vitamin-C takes part in the formation of tissue collagen. Recent research has established the role of ascorbic acid in the conversion of folic acid to a physiologically active form tetrahydrofolic acid. Vitamin-C also involves in oxidation reduction reaction in cells. The amounts of

vitamin-C present in water chestnuts (*Trapa* sp.) at different varieties are given in Table 2. It may be concluded from the results that the vitamin-C content of water chestnuts (*Trapa* sp.) was higher (1.1 mg/100 g) in green variety compared to red variety (0.9 mg/100 g). These results suggest that water chestnuts (*Trapa* sp.) are a good source of vitamin-C.

β-Carotenes are precursors of vitamin A. Animal cannot synthesize it but can convert it to vitamin A through enzymatic reaction. In plants, it is very necessary for growth and development of soft tissues through its effect upon protein synthesis. Vitamin A also plays a role in the maintenance of normal epithelial structure. Therefore, in the present study, importance is also given to measure the β-Carotene content of different varieties of water chestnuts (*Trapa* sp.) The data obtained from the analysis indicated that β-Carotene content was higher in red variety (92 μg) and lower in green variety (60 μg) (Table 2).

Phenolic compounds enjoy a distribution in the plant kingdom and they are particularly prominent in fruits and vegetables where they are important in determining color and flavor (Lee and Jaworski, 1987). The amount of phenol present in water chestnuts (*Trapa* sp.) at different varieties is given in Table 2. Water chestnuts (*Trapa* sp.) contained low amount of phenol. In green variety the amount of phenol was 0.5 mg (per 100 g) and in red varieties the amount of phenol was 0.6 mg (per 100 g). It may be concluded from the results that the phenol content of water chestnuts (*Trapa* sp.) was slightly higher in red variety. These results are indirectly supported by a report of Malviya *et al*. (2010) who demonstrated that the aqueous extract of *Trapa natants* L. fruit possesses a good amount of phenolic, flavonoids and tannin compounds and has significant antioxidant activity against free radicals.

The crude fiber content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration and quantity. The amounts of crude fiber in two cultivars of the water chestnuts (*Trapa* sp.) were determined to be 2.13±0.03 and 2.27±0.05% for green and red varieties, respectively (Table 2).

Minerals are inorganic elements exist in the body and in food as organic and inorganic combination. In foods mineral elements are present as salt. They combined with organic compound, e.g., iron in hemoglobin. Minerals are required for the teeth and bone formation. Minute amount of mineral elements are constituents of various regulatory compounds such as vitamins, enzymes and hormones. For example, some enzymes require calcium for their activities as lipases and succinate dehydrogenase. Iron requiring enzymes are ferredoxin, catalase, indophenol oxidase, aldehyde oxidase etc. The mineral elements present in the intra and extra cellular fluid maintained water and acid-base balance. They regulate transmission of impulses and contraction of muscles. The deficiencies of minerals cause many disease in human being. As mineral is essential nutrient for human being, therefore this study was also extended to investigate the different types of mineral contents in the two varieties of water chest nut. The amount of potassium, sodium, calcium, phosphorus, sulpher, Iron, copper, manganese and zinc present in Water chestnuts (*Trapa* sp.) are shown in Table 3. The results indicated that the amount of potassium was slightly lower in green variety. Potassium content of water chestnuts (*Trapa* sp.) were 5.22 and 5.32% in green and red varieties, respectively (Table 3). Table 3 also indicated that the sodium content of water chestnuts (*Trapa* sp.) was markedly higher in green variety (0.64%) as compared to red variety (0.59%).

A similar amount of calcium was observed in both of the varieties of water chestnuts i.e., 0.25 and 0.26% in green and red varieties, respectively (Table 3). Calcium is an important nutrient element for human body. It plays an important role in formation of bone and teeth. It plays an important role as second messenger for some hormone action. Calcium acts through calmodulin component of phosphorylase kinase and activates phosphorylase. Ca^{++} activates some enzymes e.g., lipase, ATP-ase, succinate dehydrogenase etc.

From Table 3, it is found that phosphorus content was same in both varieties. In both varieties phosphorus content was 6.77%. Like calcium phosphorus is also essential for bone and teeth formation, as well as acid base regulation. Phosphorus is also used in the form of phosphate (as high energy compounds e.g., ATP, UTP, CTP, creatinine phosphate, GTP etc.) in the synthesis of phospholipids, constituents of cell membranes and nerve tissues etc.

Sulpher content in water chestnuts (*Trapa* sp.) was higher in green variety. Sulpher content of water

Table 3: Mineral contents of two varieties of water chestnuts (*Trapa* sp.)

	Amounts	
Parameters	Green	Red
Potassium (%)	5.22	5.32
Sodium (%)	0.64	0.59
Calcium (%)	0.25	0.26
Phosphorus (%)	6.77	6.77
Sulpher (%)	0.38	0.32
Iron (ppm)	200	200
Copper (ppm)	430	450
Manganese (ppm)	90	110
Zinc (ppm)	600	650

Table 4: Free amino acid content of the two varieties of water chestnuts

	Varieties	
Amino acid	Green	Red
Alanine	+	+
Arginine	+	-
Aspartic acid	-	-
Asparagine	-	+
Cystine	+	-
Valine	-	-
Glutamine	-	+
Glutamic acid	+	+
Histidine	-	-
Hydroxyproline	-	-
Isoleucine	-	-
Leucine	+	+
Lysine	+	+
Methionine	-	-
Phenylalanine	-	-
Proline	+	-
Serine	-	-
Threonine	-	-
Tyrosine	+	+
Tryptophan	+	+

+, -: Signs indicate the presence and absence of amino acid in the indicated variety, respectively

chestnuts (*Trapa* sp.) was 0.38 and 0.32% in green and red varieties, respectively (Table 3).

Iron is important mineral for human being. The primary function of iron is to form hemoglobin and for the formation and maturation of red blood cells. It carries oxygen in the blood in the form of oxyhemoglobin myoglobin is an iron containing chromoprotein. There is some iron requiring enzymes, such as xanthine oxidase, cytochrome C reductase, aconitase, Acyl CoA dehydrogenase, succinate dehydrogenase etc. Iron content of water chestnut (*Trapa* sp.) was same in both varieties. The amount of iron was 200 ppm in both varieties. Copper content of water chestnut (*Trapa* sp.) was 430 and 450 ppm in green and red varieties, respectively indicating that red variety contained a higher amount of Cu.

Manganese and zinc content of water chestnut (*Trapa* sp.) was significantly higher in red variety as compared to green variety. Manganese content was 90 and 110 ppm whereas zinc content was 600 and 650 ppm in green and red varieties, respectively (Table 3).

The free amino acids present in the different varieties of water chestnuts were identified for the first time by two dimensional paper chromatography. As shown in Table 4, nine amino acids were detected in the green variety whereas, eight amino acids were detected in the red variety. The amino acids, glutamic acid, tryptophan, tyrosine, alanine, lysine and leucine were present in both of the varieties. Moreover, cysteine, arginine and proline were detected only in the green variety. Furthermore, the red variety but not the green variety contained free amino acids, glutamine and asparagine.

CONCLUSION

From the results of the present study, it appears that there are very few differences in physicochemical properties and nutrients composition between the two varieties of water chestnuts. However, only a significant difference was observed in the case of the β-carotene, some minerals specially sodium, manganese, zinc, copper and some amino acids contents in the both of the varieties of water chestnuts. The present study has demonstrated that, Water chestnuts (*T. bispinosa* Roxb.) which is consumed by the people of Bangladesh may be an important source of carbohydrate, protein, lipids, vitamins and minerals and thus, suggesting its suitability for incorporation in human diet. Moreover, this biochemical analysis may contribute a lot by supplementing existing knowledge on aquatic plants by providing data particularly on the chemical and mineral composition of fruits of the plant in details.

ACKNOWLEDGMENT

The authors are grateful to Bangladesh Council for Scientific and Industrial Research Institute, Shyampur, Rajshahi, Bangladesh for providing the laboratory facilities in the determination of mineral contents.

REFERENCES

AOAC., 1980. Official Methods of Analysis Association of Official Analytical Chemists. 13th Edn., Association of Official Analytical Chemists, Washington, DC. USA.

Alfasane, M.A., M. Khondker, Z.N.T. Begum, L.A. Banu, M.M. Rahman and U.F. Shahjadee, 2008. Fruit production and biochemical aspects of seeds of *Euryale ferox* Salisb. under *ex-situ* conditions. Bangladesh J. Bot., 37: 179-181.

Alfasane, M.A., M. Khondker and Z.N.T. Begum, 2009. Biochemical composition of the seeds of *Nelumbo nucifera* Gaertn. Dhaka Univ. J. Biol. Sci., 18: 83-85.

Alfasane, M.A., M. Khondker and M.M. Rahman, 2011. Biochemical composition of the fruits of water chestnut (*Tapa bispinosa* Roxb.). Dhaka Univ. J. Biol. Sci., 20: 95-98.

Anonymous, 1984. Making Aquatic Weeds Useful: Some Perspectives for Developing Countries. National Academy of Sciences, Washington D.C., pp: 175.

Bessey, O.A. and C.G. King, 1933. The distribution of Vitamin C in plant and animal tissues and its determination. J. Biol. Chem., 103: 687-698.

Blake, G.R., 1965. Particle Density. In: Methods of Soil Analysis. Part I, Black, C.A. (Ed.). American Society of Agronomy, Madison, Wisconsin, pp: 371-373.

Bligh, E.G. and W.J. Dyer, 1959a. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bray, H.G. and W.V. Thorpe, 1954. Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Anal., 1: 27-52.

Clegg, K.M., 1956. The application of the anthrone reagent to the estimation of starch in cereals. J. Sci. Food Agric., 7: 40-44.

Daniel, P., E. Vajravelu and J.G. Thiyagaraj, 1983. Considerations on *Trapa natans* L. from Peninsular India. J. Econ. Tax. Bot., 4: 595-601.

Hansen, P., 1970. 14C-Studies on apple tree. VI. The influence of the fruits on the photosynthesis of leaves and the relative photosynthetic yield s of fruits and leaves. Physiol. Plant., 23: 805-810.

ICOMR., 1971. A Manual of Laboratory Techniques. Indian Council for Medical Research, National Institute of Nutrition, India, pp: 2-6.

Irfanullah, H.M., 2002. Studies on aquatic vascular plants in Bangladesh: An appraisal. Bangladesh J. Plant Taxonomy, 9: 85-116.

Jayarayamau, J., 1981. Estimation of Reducing Sugars: Laboratory Manual in Biochemistry. Wiley Eastern Ltd., New Delhi.

Khan, M.S. and M. Halim, 1987. Aquatic Angiosperms of Bangladesh. Bangladesh National Herbarium, Dhaka, Bangladesh, Pages: 120.

Kosuge, T., M. Yokoto and K. Suglyara, 1985. Studies on antitumour activities and antitumous principles of Chinese herbs. 1. Antitumour activities of Chinese herb. Yakugaku Zassi, 105: 791-795.

Kowtaluk, H. and A.O. Kopan, 1997. Food for Today. 6th Edn., Glencoe/McGraw-Hill, USA., Pages: 62.

Kumar, L., S.P. Singh and S.S. Pahuja, 1985. Studies on vegetative reproduction rate of water hyacinth and water chestnut. Indian J. Agric. Res., 19: 54-58.

Kusum, B. and V. Chandra, 1980. Water Chestnut (*Trapa*): A supplement to cereals and a conserver of riverine waste land. Biol. Member, 5: 5-12.

Lee, C.Y. and A. Jaworski, 1987. Phenolic compounds in white grapes grown in New York. Am. J. Enol. Vitic., 38: 277-281.

Loomis, W.E. and C.A. Shull, 1937. Methods in Plant Physiology. McGraw Hill, New York.

Lowry, O.H., N.J. Rosebough, A.L. Farr and R.J. Randall, 1951. Proteine measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Majid, F.Z., 1986. Aquatic Weeds: Utility and Development. Agro Botanical Publishers, India, ISBN-13: 9788185031125, Pages: 96.

Malviya, N., S. Jain, A. Jain and R. Gurjar, 2010. Evaluation of *in vitro* antioxidant potential of aqueous extract of *Trapa natans* L. fruits. Acta Pol. Pharm., 67: 391-396.

Mazumdar, B.C., 1985. Water chestnut the aquatic fruit. Wild Crops, 37: 42-44.

Metcalf, R.L., 1957. Methods of Biochemical Analysis. 5th Edn., Interscience Publishers Inc., New York.

Miller, G.L., 1972. Use of Dinitrosalisylic Acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Oser, B.L., 1965. Hawk's Physiological Chemistry. 14th Edn., McGraw Hill Book Company, New Yark.

Pandit, A.K. and M.Y. Quadri, 1986. Nutritive value of some aquatic life forms of Kashmir. Environ. Conver., 13: 260-262.

Ranganna, S., 1979. Manual of Analysis of Fruits and Vegetables Products. Tata McGraw Hill Publ. Co. Ltd., New Delhi, India, Pages: 634.

Tulyathan, V., K. Boondee and T. Mahawanich, 2005. Characteristics of starch from water chestnut (*Trapa bispinosa* Roxb.). J. Food Biochem., 29: 337-348.

Unni, K.S., 1984. Seasonal changes in growth rate and organic matter production of *Trapa bispinosa* Roxb. Trop. Ecol., 25: 125-133.