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BIOCHEMICAL ANALYSIS OF THE NUTRACEUTICAL CHARACTERISTICS IN BARBADOS CHERRY (*MALPIGHIA GLABRA* L.)

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Many edible wild plants contribute significantly to the nutraceutical industry, which has a wide scope, encompassing natural pharmaceuticals and bioactive compounds with health promoting, disease preventing or medicinal properties. The regular consumption of fruits and vegetables has been strongly linked to a reduction in the risk of cardiovascular disease, cancer, diabetes, and age- related disorders caused by free radicals and reactive oxygen species. In the present study the three lobed soft, juicy, thin-skinned small delicious fruits of Barbados cherry were analysed for their proximate composition including mineral constituents, antinutritional and antioxidant characteristics at various stages of fruit maturation. The proximate analysis of ripe fruits revealed moisture (85%), total ash content (8.3mg/g), crude fiber (12mg/g), total sugar (2.135mg/g), crude protein (0.84mg/g), and fat content (0.14mg/g FW). Mature fruits were found to be rich source of essential minerals. In addition to the major nutrients fruits contained some antinutritional characteristics such as tannins, (17.9μg/g tissue), phytic acid (4.3μg and 2.5μg/g) and total phenol content (18.1mg /g FW). The value of antinutritional factors was below the standard value of World Health Organization (WHO). Appreciable quantities of β-carotene (0.1μg/g), ascorbic acid (3411.75mg/g) and tocopherol (8.9μg/g tissue) were observed in mature fruits. Considerable variation was noticed in the level of β-carotene; ascorbic acid and tocopherol content at different phases of fruit maturation. The high antioxidant potential (2913 μmol) strongly correlates with the high ascorbic acid and total phenol content during the stages of fruit development. Subsequently, the fruits were further analysed for fractionating the polyphenolics by RP-HPLC. A positive correlation was noticed between phenolic acids and total phenol content suggesting their role as precursors of many of the secondary metabolites of the plant. The presence of the phenolic acids such as caffeic, coumaric, chlorogenic, ferulic, gallic and vanillic acid increases further the antioxidant potential. The biochemical and analytical data clearly suggest that the Barbados cherry have enormous potential to meet the nutritional and antioxidant need in the diets of human populations as an excellent nutraceutical food.

Keywords Antinutritional factors; Antioxidants; Ascorbate; β-Carotene; Minerals; Nutraceutical; Phenolic acids; Phenols; Tocopherol; Wild food plants.

Introduction

Nutrition plays a critical role in wellness, not only by providing essential nutrients, but also by promoting good health and preventing diseases[1]. These properties in food have given rise to the nutraceutical industry. The term nutraceutical simply means 'a foodstuff that provides health benefits' which is derived from the words nutritive and pharmaceutical. Many edible wild plants contribute significantly to the nutraceutical industry, which has a wide scope, encompassing natural pharmaceuticals and bioactive compounds with health promoting, disease preventing or medicinal properties. The regular consumption of fruits and vegetables has been strongly linked to a reduction in the risk of cardiovascular disease, cancer, diabetes, and age- related disorders caused by free radicals and reactive oxygen species[2]. With increasing interest in finding new alternative food source, the wild or unutilized plants receives more attention which offer a good scope to meet the increasing demands for vegetable protein, carbohydrates, omega-6 and omega-3 poly unsaturated fatty acids (PUFAs) and natural antioxidants such as provitamin A (β-carotene), vitamin C (ascorbic acid), vitamin E (α- tocopherol), polyphenols, flavonoides etc. The major problem encountered with the exploitation

of the potential of many wild plants was the occurrence of some antinutritional factors like tannins, protease inhibitors, saponins, lectins, phytic acid, goitrogens, allergens, antivitamins and several phenolic derivatives in higher or lower levels along with nutritional components.

Barbados cherry, (*Malpighia glabra* L.) also known as acerola cherry, grown in sub tropical countries, is well known for its high concentration of natural vitamin C (ascorbic acid) content. The three lobed soft, juicy, thin-skinned fruits are light to deep crimson colored with pleasant flavor when ripe. The over ripe fruits bruise easily and, hence, has a very short shelf life, which in turn makes transportation and storage difficult. In India this exotic wild plant is cultivated in home gardens as an ornamental. Ripe cherries possess high nutraceutical value as it is an excellent source of natural antioxidants mainly ascorbic acid, phenolic compounds such as phenolic acids, flavonoids, their glycosides and anthocyanins. Recent studies have shown the ability of barbados cherry extract to inhibit chemically induced lung tumorigenesis in mice and NO production in mouse macrophage like cells[3]. Hexane extract of acerola fruits exhibited tumor specific cytotoxic effects, which could be used in application for cancer therapy.Little is known about the nutritional, antinutritional and antioxidant status of Barbados cherry. So the present investigation is an attempt to understand the fruit as a whole with special reference to the nutritive, antinutritive and antioxidant characteristics.

Materials and Methods

Plant material: Fruits were collected from the garden of the Department. Fruits collected at different stages of growth based on the change in colour viz. dark green (unripe), pale green (half ripe), yellow red (pre ripe), crimson (ripe) and cherry red (post ripe).

Chemicals: All biochemicals used were obtained from Sigma Chemical Co., St. Louis, MO, USA and other chemicals were of analytical grade.

Proximate composition analysis: The fruits were analyzed for proximate composition by using standard methodologies. Moisture and ash were determined according to AOAC[4].Crude fibre content was determined by the method described by Pearson[5]. Carbohydrates and reducing sugar were determined by the method of Mohan and Janardhanan[6]. Crude protein was obtained by multiplying the total nitrogen content by a factor value proposed by Pearson[5].

Estimation of total phenols: Total phenol content of fruit tissues was estimated by the method of Mayr *et al*[7]. The total phenols/g tissue was calculated from the standard graph.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) of phenols: Phenolic components of extracts were separated using HPLC following the method of Beta *et al*[8]. Standard phenolic acids such as gallic, vanillic, p-hydroxybenzoic, ferulic, chlorogenic sinapic, para coumarate and cinnamic acids were injected into the column separately. By comparing the retention time of the standard phenolic acids, various phenolic acids in the sample were identified. Height of the peaks was taken as a parameter for quantification.

Estimation of ascorbate: Ascorbate was extracted and quantified as per the methodology of Ranganna[9].

Estimation of β carotene and tocopherol: The carotene and tocopherol content of fruit samples was estimated by extracting the tissues by soxhlet method using the solvent Hexane[10] and the absorbance was recorded at 429 nm and 292 nm, respectively.

Antinutritional factor analysis: A quantitative analysis of tannins was carried out spectrophotometrically using Folin- Dennis reagent[11]. Extraction was done with methanol / water. Tannic acid was used to prepare the standard graph. Total phenol contents of fruit tissues were estimated by the method described earlier. The total phenols /g tissue was calculated from the standard graph. Phytic acid content was determined by the method of Ravindran and Ravindran[12].

Estimation of in vitro antioxidant activity: Antioxidant activity was estimated as per assay method of Benzie and Strain[13]. 100 µl of ethanolic extract was added to 3 ml of FRAP reagent (10 mM 2,4,6- tripyridyl S – triazine (TPTZ) in 40 mM HCl and 20 mM ferric chloride in 300 mM sodium acetate buffer, pH 3.6 in the ratio of 1:1: 10 (v/v) and mixed thoroughly and absorbance noted after 4 min, at 593 nm against water blank. Calibration was against a standard curve (50- 100 µM) ferrous ion produced by the addition of freshly prepared ammonium ferrous sulphate. Values were obtained from three replications and expressed as µmol FRAP g^{-1} fresh weight.

Results and Discussion

The proximate analysis of mature fruits reveals moisture (85%), total ash content (8.3mg/g), crude fiber (12mg/g). The results of the proximal composition of fruits at various stages of development i.e. dark green (unripe) to cherry red (post ripe) indicates appreciable variation in crude protein content, total sugar, crude fat and moisture content. The crude protein values ranged between 1.73 mg/g and 0.84 mg /g fresh weight (FW). Unripened fruits showed the highest value (1.73 mg/ g) with regard to protein content. The total sugar content (reducing and non

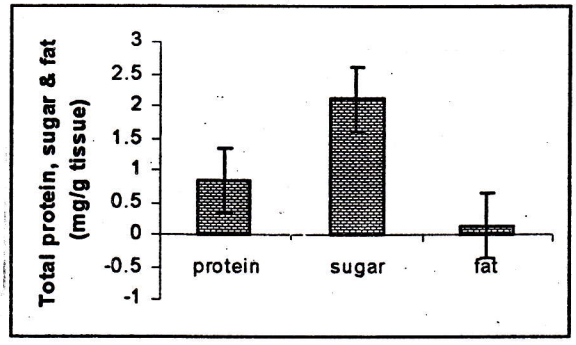


Fig. 1. Nutrient composition in mature fruits of Barbados cherry.

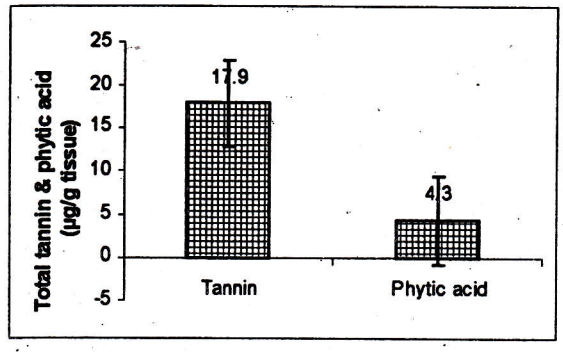


Fig. 2. Antiutrient content in mature fruits of Barbados cherry.

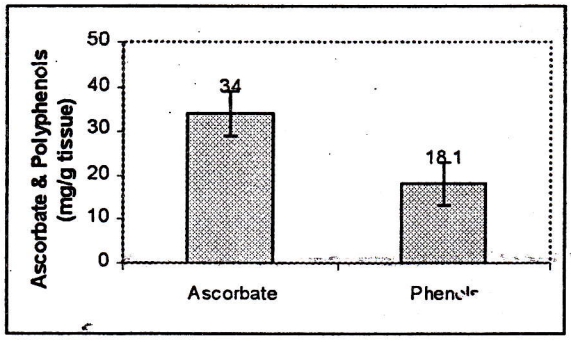


Fig. 3a. Ascorbate and polyphenols in mature fruits of Barbados cherry.

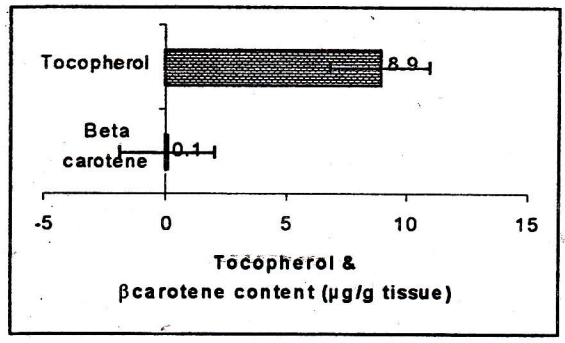


Fig. 3b. β-Carotene and tocopherol in mature fruits of Barbados cherry.

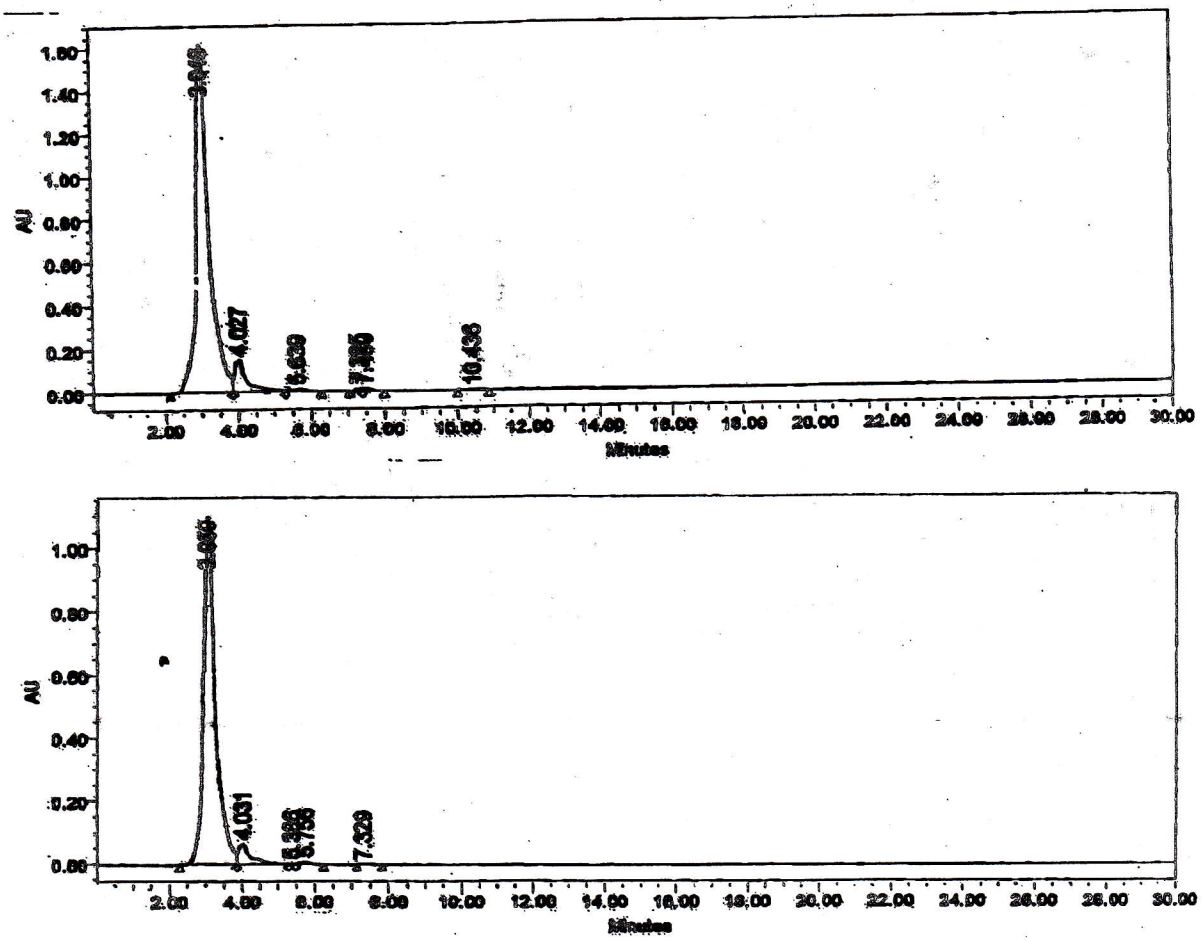
Table 1. Amino acid profile in Barbados cherry.

Aminoacids	mg/g tissue
Lysine	0.11
Arginine	0.5
Histidine	0.08
Glutamate	5.7
Aspartate	1.5
Tyrosine	0.59
Leucine	0.25
Tryptophan	1.0
Serine	0.9
Glycine	0.4
Alanine	2.0
Cysteine	1.8
Methionine	0.05
Valine	1.42
Isoleucine	1.5
Phenylalanine	0.4
Threonine	0.24
Proline	0.42

Table 2. Phenolic acid in young and mature cherry fruit.

Phenolic acids	Green stage	Fully ripen
Caffeate	50.2	5.37
Coumarate	48379.7	2165.2
Chlorogenate	927.8	433
Ferulate	9478	6189.8
Gallate	742	546
Paracatechol	ND	ND
Vanillate	3.2	ND
Cinnamate	301	32.23
Hydroxy benzoate	16.7	1.8

reducing) varied from 6.287 mg / g FW in young green unripe fruits to 2.135 mg / g in mature cherry red fruits. Interestingly, a gradual decrease in total sugar content was observed at different stages of fruit maturation, which may be correlated to the biosynthesis of ascorbic acid (vitamin C) where D- glucose was found to be the substrate. Fully mature fruits exhibited lower level of crude fat (0.14mg/g), fiber (12mg/g) and ash content (8.3mg/g) that is in agreement with most of cherries (Fig. 1). A progressive



Figs. 4 a & b. Chromatogram showing the profile of phenolic acids in Barbados cherry (young and fully ripe state).

increase in fat content was observed at various stages of fruit maturation. The data on amino acid profile is given in Table 2. It is observed that valine and glutamate are the most abundant aminoacids and others in substantial amounts. The fruits contain appreciable levels of nutritionally valuable minerals such as calcium (0.322mg/g), phosphorous (0.358mg/g) and iron (0.012mg/g). High Ca/P ratio helps to increase the absorption of Ca in the small intestine[14].

Antinutrients: Fig. 2 represents the values of antinutritive constituents such as total free phenolics, tannins and phytic acid in fruit at maturation. Antinutritional factors refer to naturally occurring compounds that act to reduce nutrient utilization and food intake. The presence of antinutritional factors adversely affects the nutritional qualities of many wild and cultivated fruits. A preliminary evaluation of some of these factors was made in *Malpighia*. A slight increase in phytic acid content (2.5 µg to 4.3 µg / g) was noticed at different stages of fruit ripening. A very important mineral storage compound in many seeds is phytate, a mixed cation salt of phytic acid (*myo*-inositol hexaphosphoric acid). This compound is often considered to be an antinutritional substance in human diets, but it may have a positive nutritional role as an anti-oxidant and an anti-cancer agent[15]. The quantity of tannin in fresh fruits ranged between 9.65µg (unripe) and 17.9µg / g (post ripe) and the total phenolic content ranged from 30.8 mg / g to 18.1 mg / g (Fig. 3a). Phenolic compounds decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals. They lower the activity of digestive enzymes such as amylase, trypsin, chymotrypsin and lipase and may cause damage to the digestive tract[16]. Remarkably low level of antinutrient factors in the ripened fruit increases the nutritional potential of this fruit. Subsequent to the estimation of total phenol it was fractionated to know the profile of phenolic acids in the

young and mature stage of the fruit (Fig. 4a & b) (Table 2). The major phenolic acids in the mature fruits are cinnamate, caffeate, coumarate, chlorogenate,ferullate, hydroxy benzoate and gallate. These phenolic acids are effective antioxidants because they scavenge reactive oxygen species, trap nitrate and prevent formation of mutagenic N- nitroso compounds and also have metal chelating properties[19].

Antioxidants: Fig. 3 a and b displays the antioxidant characteristics such as vitamin C and phenol, pro vitamin A (β-carotene) and vitamin E (tocopherol) in fruits. Carotenoids are pigments found in most fruits and vegetables. The human body does not produce carotenoids; therefore, they need to be supplied through diet. β-Carotene is the most important and frequently studied among all carotenoids. No significant change in total carotenoids was observed at different stages of fruit maturation. In the present investigation β-carotene content ranged from 0.349 μg / g (green-unripe) to 0.1 μg / g FW (cherry red- post ripe) (Fig. 3b). Carotenes have a positive effect on the immunological system and protect the skin from ultraviolet radiation[17]. In addition to the pro -vitamin A activity, β-carotene has been found to reduce risks of certain cancers especially lung cancer[17]. The level of tocopherol content ranged from 20.62 μg / g to 8.9μg / g and ascorbic acid from 3106.94mg / 100g to 3411.75 mg / 100g at young and fully mature stages, respectively. Phenolic compounds are perhaps the largest group of phytochemicals that have shown disease preventing and health promoting effects due to their antioxidant activity. Tocopherol is also a phenolic compound that plays a significant role as an antioxidant to protect polyunsaturated fatty acids (PUFAs) and other components of cell membrane and low-density lipoprotein (LDL) from oxidation, therefore preventing heart diseases[17]. Ascorbic acid is perhaps the most important antioxidant in extra cellular fluids. It was found to be the most effective in inhibiting lipid peroxidation initiated by peroxyl radical initiator among several types of antioxidants including a-tocopherol. In addition, ascorbic acid is an effective radical scavenger of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. Many studies have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers and cardio vascular diseases[18]. Remarkably higher value of antioxidants especially ascorbic acid makes this fruit as a valuable nutraceutical food.

In vitro antioxidant activity (AOX) measured by FRAP assay exhibited higher value in post ripe (2913 μmol) fruits. The higher antioxidant activity may be directly linked to the increased amount of ascorbic acid, total phenolics, rich phenolic acids content and other natural antioxidants including tannins and phytic acid. Higher correlation between phenolics and antioxidant activity, confirms the earlier results of several vegetables[19].

The present study indicates that Barbados cherry (acerola) can be considered as an excellent source of valuable minerals and natural antioxidants. In North America, acerola is used for its high content of vitamin C. Dried acerola fruit extracts can now be found in tablet form and as an ingredient in many over-the-counter multivitamin products in the United States as a natural form of vitamin C. Acerola has not been the subject of much clinical research since it is mainly consumed as a food, rather than used as an herbal remedy. Recent research in cosmetology indicates that vitamin C is a powerful antioxidant and free radical scavenger for the skin, and acerola extracts are now appearing in skin care products that fight cellular aging. In addition to its vitamin content, acerola contains mineral salts that have shown to aid in the remineralization of tired and stressed skin, and its mucilage and proteins have skin-hydrating properties and promote capillary conditioning. Further research work is warranted on *in vitro* and *in vivo* experiments related to biological evaluation and health promoting aspects one needed to reduce the risk of cardiovascular diseases, cancer, diabetes, and age- related disorders caused by free radicals and reactive oxygen species.

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