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# CHANGES IN THE NUTRACEUTICAL POTENTIAL OF CEYLON OLIVE (*ELAEOCARPUS SERRATUS* L.) AT DIFFERENT STAGES OF FRUIT MATURATION

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"Eating healthy" has been a common slogan among educated consumers. This means eating a balanced diet that provides sufficient proteins, carbohydrates, fats, and vitamins. Many edible wild plants contribute significantly to the field of nutraceutical industry, for the production of natural pharmaceuticals and bioactive compounds with disease preventing or medicinal properties. The nutraceutical potential of many plant species remains unexploited. In the present study the oval, pale green, soft, thin-skinned small delicious fruits of *Elaeocarpus serratus* were analysed for their nutritional, antinutritional and antioxidant characteristics at various stages of fruit maturation. The moisture content of fully ripened fruit was high (75.67%) while the total ash content was very low (9mg/g). Considerable amount of crude protein (1.2 mg/ g tissue) and sugar (40.50mg/g) and crude fibre (24mg/g) were observed in mature fruits. The level of total carbohydrate content in mature fruits was very high (103 mg/g). Though, the fruits are good source of essential nutrients, certain secondary metabolites such as polyphenols and tannins with least nutrient value were also found in very little quantities. These compounds are generally catagorised as antinutritional factors. The level of total polyphenolics ranged from 6.1 mg/g to 4.28 mg/g FW at different stages of fruit maturation. 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 acids increases further the antioxidant potential. Appreciable quantities of β-carotene (324μg/g), ascorbic acid (300μg/g) and tocopherol (450μg/g tissue) were observed in mature fruits. Significant variation was noticed in the level of β-carotene; ascorbic acid and tocopherol content at different phases of fruit maturation. The high antioxidant potential strongly correlates with the high β-carotene and tocopherol in addition to total phenolics and tannin content, at various stages of fruit development. The biochemical and analytical data reveal the nutraceutical potential of this underutilized wild fruit, enriched with valuable nutrients and natural antioxidants.

**Keywords:** Aminoacids; Antinutritional factors; Antioxidants; Ascorbate; β-carotene; *Elaeocarpus serratus*; Nutritional; Nutraceutical; Polyphenols; Tannins; Tocopherol; Wild food plants.

## Introduction

Oxidative damage, as a result of normal metabolism or secondary to environmental pollutants, leads to free radical formation which has been considered to play a central role in cancer[1] and atherosclerosis [2,3]. Therefore, antioxidants, which can neutralize free radicals, may be important in the prevention of these diseases. Edible wild plants provide higher amounts of vitamin E and vitamin C than cultivated plants. In addition to the antioxidant vitamins, edible wild plants are rich in phenols and other compounds that increase their antioxidant capacity. A number of dietary antioxidants, such as flavonoids, carotenoids, polyphenols and sulfides, etc., are bioactive and work synergistically as do vitamin C and vitamin E[4,5]. It is therefore important to systematically analyze the total antioxidant capacity of wild plants and promote their commercialization in both developed and developing countries. 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[6].

Ceylon olive (*Elaeocarpus serratus* L.) also known as 'Veralu' is a very important underutilized fruit tree indigenous to Sri Lanka. It belongs to the family Elaeocarpaceae. It is also grown in a number of tropical countries for food, medicine and aesthetic value as an ornamental tree. In India this evergreen tree is widely grown in the forests of Western Ghats and in home gardens for the soft pale green edible fruits. The inhabitants of the forest areas of Western Ghats have been traditionally consuming the fruits of this valuable tree, as a nutraceutical foodstuff especially during famine periods. Leaves of *E. serratus* are used in the treatment of rheumatism by the tribal communities. Leaves have been proved to be antidote of poison. Little is known about the nutraceutical value of this fruit. Hence, the present investigation is an attempt to evaluate the nutraceutical potential of the edible fruits of *E. serratus* during development and ripening.

**Material and Methods**

*Plant material*: Fruits collected at different stages of growth based on the change in colour viz. dark green (unripe), pale green (half ripe) and yellowish green (ripe).

*Chemicals*: The chemicals used were of analytical grade and purchased from Sigma Chemical Co., St. Louis, MO, USA.

*Proximate composition analysis*: The fruits were analyzed for proximate composition by using standard methodologies. Moisture and ash were determined according to AOAC[7]. Carbohydrates and reducing sugar were determined by the method of Mohan and Janardhanan[8]. Crude protein was obtained by multiplying the total nitrogen content by a factor value proposed by Pearson[9]. Amino acid content of fruits were estimated by the method of Sadasivam and Balasubramanian[10].

*Estimation of total phenols*: Total phenol content of fruit tissues was estimated by the method of Mayr *et al*[11]. 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*[12]. 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[13].

*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[14] 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[15]. 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.

*Estimation of in vitro antioxidant activity:* Antioxidant activity was estimated as per assay method of Benzie and Strain[16].

**Result and Discussion**

*Proximate composition*: The quantity of moisture, crude protein and total sugar in fully ripe fruits is shown in Fig.1. Moisture content in mature fruit was very high (75.67%). The value of protein (1.2mg/g tissue FW) is insignificant as compared to legumes and other wild fruits[17,18].Proteins serve as the major structural component of muscle and other tissues in the body. As the excess protein consumption results in formation of toxic substances, it has been deemed to maintain an upper bound not more than twice the recommended daily allowances of proteins. The protein quality, also known as the nutritional value of a food depends on its amino acid content. Amino acid profile of the species is given in the Table 1. It is observed that tyrosine, proline, cysteine and aspartate are the abundant amino acids followed by serine. Lysine and phenylalanine are essential amino acids necessary for the synthesis of new protein for growth and repair.

The value of total sugar content in mature fruits is shown in Fig.1. The total sugar content (40.5 mg/g) can be comparable to some common edible fruits such as mango (8.51g /100g), orange (8.2g /100g) and grapes (13.2g /100g). The level of fibre content (24mg/g) in ripe fruits was appreciable. Remarkably lower level of sugar and high amount of crude fibre in fresh ripened fruits underscores their importance as a valuable low calorie fruit for diabetic patients.

*Antinutritional constituents:* Besides the essential nutrients the fruits contain few secondary metabolites with least nutritional value. The quantity of tannic acid in fresh fruits ranges from 0.538mg/g (green unripe) to 0.3mg/g (ripe) and the total phenol content (Fig.3) varied from 6.1 mg/g (green unripe) to 4.28mg/g (ripe). The tannin content was found to be decreasing as the fruit matures (Fig.2). High tannin content makes young fruits bitter in taste. The antinutritional effect of tannins is not completely known

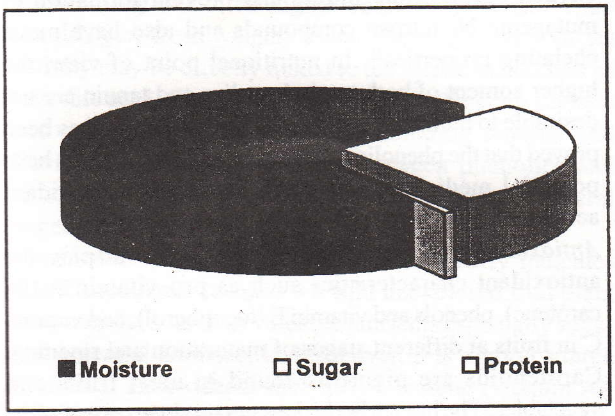


**Fig. 1.** Proximal composition analysis in *Elaeocarpus serratus* fruits.

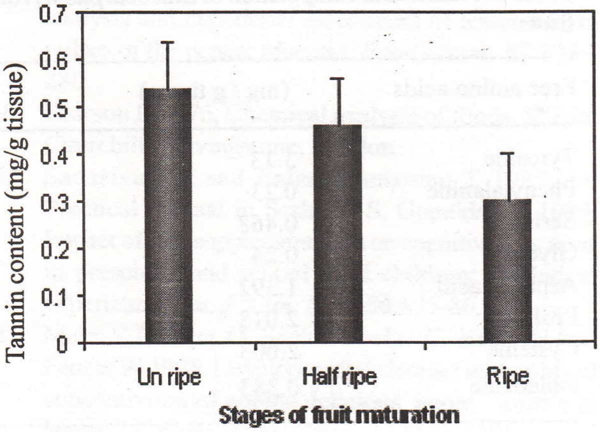


**Fig. 2.** Tannin content in *E. serratus* fruits.

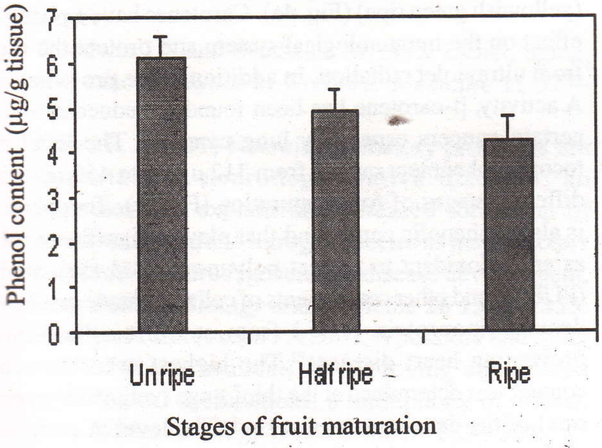


**Fig. 3.** Phenol content in *E. serratus* fruits.

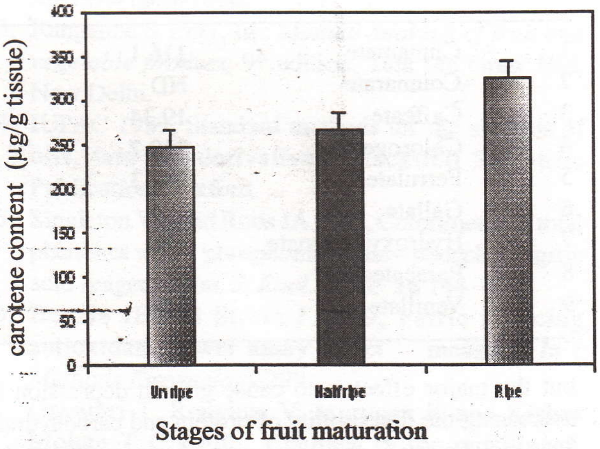


**Fig.4a.** β-carotene content in *E. serratus* fruits.

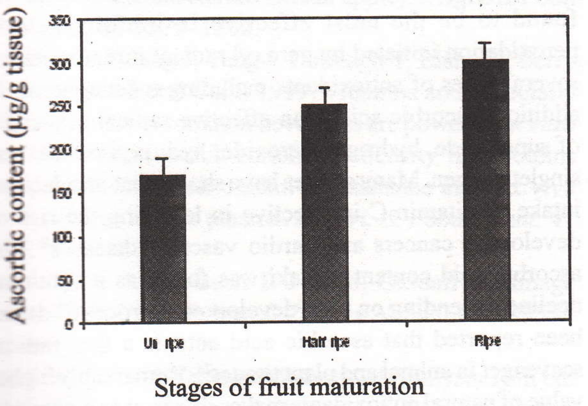


**Fig. 4b.** Total ascorbic acid (vitamin C) in *E. serratus* fruits.

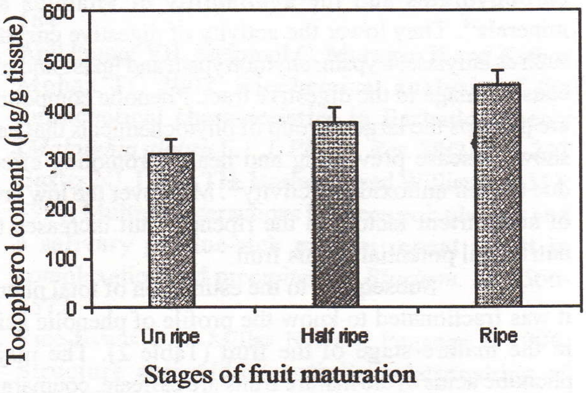


**Fig. 4c.** Tocopherol content in *E. serratus* fruits.

**Table 1.** Aminoacid composition of *Elaeocarpus serratus* fruits.

| Free amino acids | (mg / g tissue) |
|---|---|
| Tyrosine | 3.13 |
| Phenylalanine | 0.23 |
| Serine | 0.468 |
| Glycine | 0.24 |
| Aspartic acid | 1.392 |
| Proline | 2.013 |
| Cysteine | 2.003 |
| Isoleucine | 0.383 |

**Table 2.** Phenolic acids profile of fully ripe fruits of *E. serratus*.

| Sl No | Phenolic acids | (μg / g tissue) |
|---|---|---|
| 1 | Cinnamate | 116.1 |
| 2 | Coumarate | ND |
| 3 | Caffeate | 19.34 |
| 4 | Chlorogenate | 319.2 |
| 5 | Ferrulate | 746.3 |
| 6 | Gallate | 255 |
| 7 | Hydroxy benzoate | 6.4 |
| 8 | Paracatechol | 42 |
| 9 | Vanillate | 0.95 |

but the major effect is to cause growth depression by decreasing the digestibility of protein and carbohydrate. This may be due to the interaction of tannins with either protein or starch to form enzyme resistant substances. Phenolic compounds decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals[19]. They lower the activity of digestive enzymes such as amylase, trypsin, chymotrypsin and lipase and may cause damage to the digestive tract. Phenolic compounds are perhaps the largest group of phytochemicals that have shown disease preventing and health promoting effects due to their antioxidant activity[20]. Moreover the 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 mature stage of the fruit (Table 2). The major phenolic acids in the mature fruits are caffeate, coumarate, chlorogenate 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[21] .In nutritional point of view, the higher content of both total phenolics and tannin are not desirable to human consumption. But recently, it has been proved that the phenolic constituents of various plants have potential medicinal properties, including antioxidant activities[4].

*Antioxidant compounds:* Figs.4a, b and c display the antioxidant characteristics such as pro vitamin A (β-carotene), phenols and vitamin E (tocopherol), and vitamin C in fruits at different stages of maturation and ripening. 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. In the present investigation β-carotene content ranged from 246 μg / g (green-unripe) to 324 μg / g FW (yellowish green ripe) (Fig. 4a). Carotenes have a positive effect on the immunological system and protect the skin from ultraviolet radiation. In addition to the pro -vitamin A activity, β-carotene has been found to reduce risks of certain cancers especially lung cancer[22]. The level of tocopherol content ranged from 312 μg / g to 450μg / g at different stages of fruit maturation (Fig.4c). 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[22].The highest ascorbic acid content was determined at the third stage (yellowish green-ripe) of the developmental period. The level of ascorbic acid ranges from 170μg/g to 300 μg/g at the first and third stages respectively (Fig 4b). 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 α-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[21].The ascorbic acid content of kaki was found as a continual decline depending on their development process[23] .It has been reported that ascorbic acid acts as a free radical scavenger in animal and plant tissues[24]. Remarkably higher value of natural antioxidants makes this fruit as a valuable nutraceutical food.

*In vitro* antioxidant activity (AOX) measured by

FRAP assay exhibited higher value in half ripe (2300 μmol/L/g of fresh weight) 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 flavanoids. Higher correlation between phenolics and antioxidant activity, confirms the earlier results of several vegetables[21].

*Conclusion:* The present investigation clearly indicates that *Elaeocarpus serratus* – a wild unexplored fruit plant is a potential source of valuable nutrients especially sugar and essential aminoacids. Moreover, it is a good source of natural antioxidants such as β-Carotene (vitamin A), ascorbic acid (vitamin C) and tocopherol (vitamin E). Further investigation is warranted both *in vitro* and *in vivo* to know how far these compounds act in the cell systems to reduce the risk of cardiovascular diseases and several other disorders including cancer.

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