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Screening of nutritional factors from some wild vegetables in Poladpur

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Abstract

Nutritional factors like proteins, carotenoids, polyphenols, total flavonoids, reducing sugar, total sugar, starch, ascorbic acid analysed from six wild vegetables. These factors were analysed by different methods. It was observed that leaves of *Clerodendrum serratum* contain higher protein, carotenoids, total polyphenols, total flavonoids and ascorbic acid as compared to other studied wild plants. Leaves of *Cassia obtusifolia* contain high amount of starch.

Keywords: wild vegetables, protein, carotenoids, total polyphenols, total flavonoids, ascorbic acid

Introduction

Poladpur is a Taluka in Raigad District of Maharashtra State, India. It belongs to Konkan region. Wild vegetables are economic source for adivashi people in Poladpur and adjoining areas during rainy season. These are known to be important sources of protective foods (Nnamami *et al.* 2009; Sheele, *et al.*, 2004) [19, 22]. Vegetables have also been reported to be good sources of, carbohydrates, minerals as well as vitamins (Adenipenkun, and Oyetunji 2010) [1]. The nutritional value of a food depends upon its nutritional contents. The purpose of this study therefore is to evaluate the levels of nutritional factors of some common wild vegetables in Poladpur region of Raigad district.

Every year during rainy season various vegetables growing wild are eaten by people on the basis of traditional knowledge. Their nutraceutical ability is not yet understood in term of modern scientific perspectives. Attempts have been made to identify and analyse unconventional wild vegetables from markets in Poladpur city for their nutritional value. Some of the wild vegetables like *Cleorodendrum serratum* (L.) Moon, *Chlorophytum borivillianum* Sant. and Fern, *Holarrhena antidyenterica* Wall. Ex D.C., *Colocasia esculenta* (L.) Schott and *Cassia obtusifolia* (L.) which are utilized by people are analyzed and discussed here.

Material and Methods

Some wild vegetables collected locally from nearby villages and some collected from local market. Leaves of *Cleorodendrum*, *Chlorophytum*, *Colocasia* and *Cassia* separated from plants. Pods and flowers of *Holarrhena* were used as vegetable also separated from plant. Samples were washed with tap water and then with distilled water to remove dirt and dried at 50°C in hot air oven. Samples were powdered with grinding machine and stored in airtight container.

Soluble Protein: Soluble protein was determined by the method of Lowry *et al.* (1951) [13]. 0.2 g of sample was extracted with 10ml of the 0.1M Phosphate buffers using mortar and pestle. Sample was filtered through Whatman No. 1 filter paper. Filtrate was used for protein estimation. 0.2ml of the sample extract was taken into test tube. The volume was made to 1ml in the all the test tubes with distilled water. A tube with 1ml of water serves as the blank. 5ml of reagent C (50 ml Reagent A (2% sodium carbonate in 0.1 N sodium hydroxide and 1ml of Reagent B (0.5 % copper sulphate in 1% potassium sodium tartrate) added to each test tube and mixed properly. After 10 min. 0.5 ml Folin-ciocalteau reagent added and incubated at room temperature in dark for 30 min. blue colour developed is measured at 660nm on Digital Dual Beam Spectro Photo Meter EQ-824. A standard curve of bovine serum albumin (0.1mg.ml⁻¹) was prepared and the soluble protein was calculated.

Carotenoids

The carotenoid content was determined from the 80% acetone extract by recording the absorbance at 470 nm on Digital Dual Beam Spectro Photo Meter EQ-824 using 80% acetone

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as blank. The carotenoids were calculated by using the following formula (Kirk and Allen, 1965)

$$\text{Total Carotenoids (mg.100-1g)} = \frac{A_{470} \times \text{volume of extract} \times 10 \times 100}{2500 \times \text{weight of plant material (g)}}$$

Total polyphenols

The method of Folin and Denis (1915) [4] was adopted for determination of the total polyphenol content in sample. Five hundred milligrams of dry powdered material was homogenized in 30 ml of 80 % acetone and filtered through Whatman No.1 filter paper. The residue was washed 2-3 times with 80 % acetone and the final volume of filtrate was made 50 ml with 80 % acetone. 2 ml of plant extract were taken in 50 ml capacity Nessler's tubes and to each Nessler's tube 10 ml of fresh 20 % Na₂CO₃ and 2 ml of Folin Denis reagent were added. The final volume of reaction mixture was made 50 ml with distilled water. Similar way set of standards were prepared using standard tannic acid (0.1 mg/ml) in place of taking plant extract along with above reagents. After 20 minutes absorbance was read at 660 nm against reagent blank

Total flavonoids

Total flavonoids were estimated according to the method of Luximon-Ramma *et al.* hundred milligrams of samples were extracted in 80 % acetone in cold mortar and pestle. The homogenate was filtered through Buchner's funnel using Whatman No. 1 filter paper. Final volume of the filtrate was made 35 ml with 80 % acetone. The reaction mixture contained 1.5 ml of the plant extract and 1.5ml, 2% Methanolic Aluminum Chloride (2 g Aluminium chloride dissolved in 100 ml pure methanol). Blank was prepared with distilled water in place of sample. The absorbance of the reaction mixture was measured at 400 nm on Digital Dual Beam Spectro Photo Meter EQ-824.

Carbohydrates

Carbohydrates were estimated according to the method described by Nelson (1944) with some modifications. 0.2 g oven dried plant material was homogenized in mortar with pestle and extracted with 80% alcohol. It was filtered through Bucher's funnel using Whatman No.1 filter paper. The residue on filter paper was washed with 80% alcohol repeatedly. All the washings and filtrate were mixed together. This filtrate was used for estimation of total (soluble sugars) and reducing sugars while the residue was saved for estimation of starch.

1. Reducing sugars

The filtrate was condensed on the water bath to about 2-3 ml and to it, were added Lead acetate and Potassium oxalate (1 g each) to decolorize the extract. It was mixed together with the help of glass rod with the addition of some water. It was again filtered and washed with distilled water 2-3 times, collecting the washings in the same filtrate. The final volume of filtrate was made to 50 ml with distilled water. This filtrate was used for estimation of reducing sugars (A). Reducing sugars were estimated following the method described for estimation of starch given below.

2. Total sugars (soluble sugars)

Estimation of total sugars was carried out according to Dey (1990) method. For this 10 ml extract of reducing sugars was taken into a test tube and heated in water bath at 60°C for an hour. The contents were cooled and from this extract 2 ml

were taken in a test tube into which 1 ml. 5% phenol and 5 ml concentrated sulphuric acid were added carefully. This was mixed thoroughly with glass rod. The contents were cooled and absorbance was read at 485 nm on Digital Dual Beam Spectro Photo Meter EQ-824. The amount of soluble sugars was estimated using standard glucose (0.1 mg ml⁻¹). The values are expressed in g 100g⁻¹.

3. Starch

The residue on the filter paper saved for starch estimation was transferred to a conical flask with 50 ml of distilled water and 3-5 ml of conc. HCl. This was hydrolyzed, neutralized with Na₂CO₃ and filtered. This filtrate contains reducing sugars produced as a result of hydrolysis of starch. The sugars so available were estimated to determine the starch present in the tissue (C). The volume of the filtrate was also noted down. The requisite quantity, (2ml filtrate A and 0.1 ml of filtrate C) were taken separately in test tubes. 1 ml of alkaline copper tartrate reagent- (4 g CuSO₄.5H₂O, 24 g anhydrous Na₂CO₃, 16 g Na-K- tartrate and 180 g anhydrous Na₂SO₄ were dissolved in distilled water and volume was made to 1000 ml) was added to each test tube. All the test tubes containing the reaction mixtures were subjected to boiling water bath for about 10 min and then cooled to room temperature. 1ml of Arsenomolybdate reagent (25 g ammonium molybdate in 450 ml distilled water and to this were added 21 ml of conc. H₂SO₄. This was mixed with solution containing 3 g sodium arsenate dissolved in 25 ml distilled water. The mixture of the solutions was placed in an incubator at 37 °C for 48 hours) was added to each test tube and shaken vigorously. The volume of the reaction mixture in each test tube was made 10 ml with distilled water. A blank was prepared in the same way but without sugar solution. After 15 minutes, the absorbance was read at 560 nm on Digital Dual Beam Spectro Photo Meter EQ-824. A standard curve of glucose (0.1mg/ml-1) was prepared and the sugar content was calculated.

Ascorbic Acid

Ascorbic acid content was determined according to Sadasivam and Manickam procedure with slight modification. 0.5g dry powdered samples were extracted with 4% oxalic acid and filtered through Whatman No. 1 filter paper. The final volume made up to known volume. 5ml of this sample pipette out. 10ml of 4% oxalic acid were added and titrated against dye (42mg sodium bicarbonate into a small volume of distilled water. DCPIP (dissolve 52mg of 2, 6, dichloro indophenol in it and make up to 200ml with distilled water). Prepare a standard graph by using 0.1mg/ml ascorbic acid in 4% Oxalic acid.

Results and Discussions

1. Soluble proteins

Proteins have high nutritional value. High protein value increases nutritional value of plants. Protein content of wild vegetables showed in Table 1. Results indicate that leaves of *Cleorodendrum serratum* (10.88 g 100g⁻¹), pods (11.63 g 100g⁻¹) and flowers (9.25g 100g⁻¹) of *Holarrhena antidysenterica* and leaves of *Cassia obtusifolia* (9.5 g 100g⁻¹) have high protein content. Protein content from leaves of *Delonix elata* has 7.1 g 100g⁻¹ by Gupta *et al.* (2005) [5]. Protein content also reported from lesser known wild leafy vegetables such as *Momordica balsamina* (11.29%), *M. oleifera* (20.72%), *Lesianthera Africana* (13.10 – 14.90%), *S. americanum* (11.33%) and *Leptadenia hastata* (19.10%) (Isong and Idiong, 1997; Sena *et al.*, 1998; Lockett *et al.*,

2000; Hassan and Umar, 2006)^[9, 21, 12]. Protein content value of *Cleorodendrum serratum* is in the range of *Momordica balsamina*. Umar *et al.* (2011)^[24] reported 35.11% protein from spiny *Amarathus viridis*. Results indicate that *Cleorodendrum serratum*, *Holarrhena antidysenterica* and *Cassia obtusifolia* acts as source of protein.

2. Carotenoids

Carotenoids are organic pigments that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria, where they play a critical role in the photosynthetic process. They are split into two classes, xanthophylls and carotenes. β -carotene is a precursor for vitamin A. Carotenoids are efficient free-radical scavengers, and they enhance the vertebrate immune system. Table 1 represents carotenoid contents of wild vegetables. A carotenoid content of present study was found to be in range of 12.60 to 142.80 mg 100g⁻¹ dry wt. *Cleorodendrum serratum*, *Colocasia esculenta* and *Cassia obtusifolia* had higher carotenoid content. Total carotene content of underutilized green leafy vegetables recorded by Gupta *et al.* (2005)^[5] ranged between 10 and 35 mg/100 g fresh wt. in all with exceptionally high amount in *Cocculus hirsutus* (67 mg/100 g) and *Delonix elata* (60 mg/100 g fresh wt.). Nambiar and Seshadri (1998)^[17] have reported total carotene content of 16 common leafy vegetables to be less than 20 mg/100 g. carotenoid content of present study found higher except flowers of *Holarrhena antidysenterica* indicating rich source of Carotenoids.

3. Total polyphenol content

Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol units or building blocks per molecule. It is the product of secondary metabolism. Various roles have been attributed to phenolic compounds in plants. These are considered to be protective in function against different types of disease in plants (Wallace and Mansell, 1975; Lunderstadt, 1976)^[25, 14]. Phenolic compounds have an antioxidant activity (Zhu *et al.* 2004)^[26]. They play a role in inhibiting the oxidization of low-density proteins (Frankel *et al.*, 1993).

Polyphenol content of present study showed in Table 3. Higher polyphenol content recorded in *Holarrhena antidysenterica* flowers (1.32 g100g⁻¹ of dry wt.), *Cassia obtusifolia* (0.92 g100g⁻¹ of dry wt.), *Cleorodendrum serratum* (0.89 g100g⁻¹ of dry wt.) and *Holarrhena antidysenterica* pods

(0.86 g100g⁻¹ of dry wt.). Mane (1993)^[16] recorded high concentration of the polyphenols in leaves (1.97g100g⁻¹ dry wt.), stem (0.95 g100g⁻¹ of dry wt.) and root (0.19g100g⁻¹ of dry wt) of *Jatropha curcas*. Thombre (1987)^[23] has recorded 0.27 and 0.47% polyphenols (fresh wt.) in *Portulaca quadrifida* and *Setaria italica* and its polyphenols respectively. Polyphenol content of present study found higher than these the earlier records.

4. Total flavonoids

Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). An attempt has made to know antioxidative potential of wild vegetables of plants of Poladpur by knowing flavonoids absorbance (depicted in Table 1). Among studied wild vegetables *Cleorodendrum serratum*, *Colocasia esculenta* and *Cassia obtusifolia* showed maximum absorbance. It indicates that these vegetables had higher flavonoid content. According to Hanasaki *et al.* flavonoids can either directly scavenge superoxides, or can scavenge the highly reactive oxygen derived radical called peroxynitrite. Higher flavonoids content indicates great nutritive potential. Flavonoid content is now regarded as valuable antioxidant supplied by the plants to human beings.

5. Ascorbic acid

Ascorbic acid known as vitamin C is an anti-scorbutic. Generally it is present in all fresh vegetables and fruits. It is water soluble heat-labile vitamin. It is natural antihistamine which prevents histamine release and increases the detoxification of histamine (Johnston *et al.*, 1992)^[10]. Vitamin C is also useful in lowering serum uric acid level resulting in lower incidence of gout (Choi *et al.*, 2009)^[2] and an oxidized version that can cross blood brain barrier may reduce neurological deficits and mortality following stroke (Huang *et al.*, 2001)^[8].

Table 1 depicts ascorbic acid contents in wild vegetables of Poladpur. *Cleorodendrum serratum* and pods of *Holarrhena species* showed higher ascorbic acid content. Gupta *et al.* (2005)^[5] recorded higher ascorbic acid content from *Delonix elata* (295mg 100⁻¹ g fresh wt.). Umar *et al.* (2011)^[24] recorded ascorbic acid from *Amaranthus viridis* (21.45). The result of this analysis is a point out that the vegetables *Cleorodendrum serratum*, *Lactuca virosa* and pods of *Holarrhena species* could be an important dietary source of ascorbic acid.

Table 1: Levels of nutritional factors in wild vegetables of Poladpur

Sr. No.	Name of plant	Protein g 100g ⁻¹ dry wt	Carotenoids mg100 g ⁻¹ dry wt.	Polyhenols g100 g ⁻¹ dry wt	Total Flavonoid O.D. at 400nm	Reducing sugar g 100g ⁻¹	Total sugar g 100g ⁻¹	Starch g 100g ⁻¹	Ascorbic acid mg100g ⁻¹
1	<i>Cleorodendrum serratum</i> (Leaves)	10.88	142.80	0.89	0.59	0.48	1.78	7.05	118.83
2	Pods of <i>Holarrhena antidysenterica</i> (Pods)	11.63	32.20	0.86	0.08	1.57	2.58	7.05	26.09
3	<i>Holarrhena antidysenterica</i> (flowers)	9.25	12.60	1.32	0.05	5.09	12.89	3.00	18.60
4	<i>Chlorophytum borivilianum</i> (Leaves)	3.5	75.60	0.17	0.09	0.61	2.39	12.01	19.77
5	<i>Colocasia esculenta</i> (Leaves)	4.75	128.80	0.43	0.21	2.18	6.58	3.00	13.95
6	<i>Cassia obtusifolia</i> (Leaves)	9.5	92.40	0.92	0.16	2.18	4.67	21.01	18.60

Summary and Conclusions

Wild vegetables are rich in nutrients such as proteins, vitamins, and mineral nutrients. *Cleorodendrum serratum*, *Holarrhena antidysenterica* and *Cassia obtusifolia* had high protein content. Results indicate that these wild vegetables act as source of protein. *Cleorodendrum serratum*, *Colocasia*

esculenta and *Cassia obtusifolia* had higher carotenoid content. High carotenoids content value represents these vegetables are edible as these have antioxidant potential. Higher polyphenol content recorded in *Holarrhena antidysenterica* flowers, *Cassia obtusifolia*, *Cleorodendrum serratum* and *Holarrhena antidysenterica* pods indicating

higher antioxidant activity. Higher starch, total sugar and non reducing sugar recorded in *Cassia obtusifolia*. Higher total sugar found in *Colocasia esculenta*. Other vegetables had less carbohydrate level. *Cleorodendrum serratum* and pods of *Holarrhena species* showed higher ascorbic acid content indicating important source of ascorbic acid.

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