Research Article

Effect of Some Antinutritional Factors on the Bioavailability of Minerals along with the Study of Chemical Constituents & Antioxidant Property in *Typhonium trilobatum & Spinacia oleracea*

Swati Banerjee[1], Kamala Adak[1], Dr. Mohini Mohan Adak[2], Dr. Sumana Ghosh[3], Amitava Chatterjee[4*]

[1]Dept. of Home Science, University of Calcutta, India;
[2]Department of Chemistry, Asutosh College, University of Calcutta, India
[3]Department of Chemistry, HMM College for Women, Kolkata, India
[4]Department of Health & F.W., Govt. of West Bengal, India

Abstract

The bioavailability of nutrients like Fe and Ca in food materials are often minimized owing to the presence of certain antinutritional factors like oxalate, phytate etc. resulting severe deficiencies leading to anaemia, osteoporosis. Thus, the authors searched for cheap, easily available vegetables having less antinutritional components like oxalate, phytate to combat anaemia & osteoporosis amongst the vulnerable section of the society and studied *Typhonium trilobatum (Tt),* commonly known as Bengal arum/Ghatkanchu and *Spinacia oleracea (So)*(spinach) - leafy vegetables having similar components and analysed the chemical constituents w.r.t. proximate constituents, minerals, vitamin C, phytochemicals, antinutritionals & antioxidant activity. Investigation revealed *So* leaves to be rich in minerals like Na, K and Ca but Fe in comparison to *Tt* leaves. As the Fe (58.40±0.50 mg%) and Vit C (108.08±1.70 mg%) contents of *Tt* are very higher than *So* (Fe:1.91±0.56 & Vit C:27.01±1.50 mg%) and the two antinutritional factors, oxalate (*Tt*:7.19±0.58 & *So*:658.07±0.45 mg%) and phytate (*Tt*:0.35±0.01 & *So*:B.D.L.µg%) contents are fairly lower, so it can be said that the former is a better bioavailable source of iron and calcium than spinach, as binding actions of oxalates & phytates are appreciably lesser. Study further depicted *Tt* leaves to be higher in crude fibre and protein; equivalent content of carbohydrates; lower contents of lipids and total ash in comparison with *So* leaves.

The polyphenols, total hydrolysable tannins & flavonoids contents (21.19±0.07, 22.17±0.15 & 11.25±0.06 mg/gm) of *Tt* are comparable with *So* leaves (18.70±0.21, 20.45±0.33 & 14.81±0.12 mg/gm). Antioxidant activity of *Tt* leaves w.r.t. DPPH free radicals is higher than *So* (*Tt*:432.90±1.90 & *So*:455.13±2.05 µg/ml) and equivalent w.r.t. the $ABTS^{+}$ radicals (*Tt*:155.31±0.90 & *So:*150.20±2.08 µg/ml).

Keywords: bioavailability, Fe, Ca, oxalate, phytate, phytochemicals, antioxidant activity

***Correspondence**
Amitava Chatterjee,
Email: amitavachatterjee.phl@rediffmail.com

Introduction

Vegetables are the fresh and edible portions of herbaceous plants. They are important food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which can be successfully utilized to build up and repair the body. Vegetables are valuable in maintaining alkaline reserve of the body. They are valued mainly for their high carbohydrate, vitamin such as β-carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as iron, calcium and phosphorous content [1]. In nature, there are many underutilized leafy vegetables, which are available seasonally, and practically no information is available on the

nutrient content and antinutritional factors of such vegetables. They have remained underutilized due to lack of awareness and popularization of technologies for utilization[2]. Looking into the prevalence of high level of micronutrient malnutrition among the vulnerable sections in the developing countries and the increasing prevalence of chronic degenerative diseases globally, the need for exploration of underutilized foods is significant to overcome the nutritional disorders. The diet and food based approach in combating micronutrient malnutrition is essential for its role in increasing the availability and consumption of micronutrient rich foods [3]. Increasing the utilization of GLV in our diet, known to be rich sources of micronutrients as well as dietary fibre can be a food-based approach for ensuring the intake of these nutrients. It is essential that the locally available GLV, which are inexpensive and easy to cook, be used in the diets to eradicate micronutrient malnutrition and also to prevent the degenerative diseases [4]. On the other hand, increasing research on underutilized vegetables in different regions showed that most of these wild greens have great nutritional values and antioxidant properties, which are comparable to those commercially cultivated vegetables [5,6]. **One of such less focused leafy vegetable is *Typhonium trilobatum*, which is the subject of our study.**

Typhonium trilobatum belongs to the Araceae family and it is small to moderate sized perennial herb, commonly known as Bengal arum, Ghatkanchu or Ghatkol in Bangladesh. It is widely grown in India, Bangladesh, China, Thailand, Vietnam, Malaysia, and Srilanka [7]. It has been valued in Ayurveda and Unani system of medicine for possessing variety of therapeutic properties. The rhizome of *Typhonium trilobatum* has been used for the treatment of vomiting, cough, asthma, excessive expectoration, pyrogenic sore throat, headache, gastric ulcer, abscess, snake bite [8, 9], diarrhoea and dysentery [10], stimulant and menstrual troubles [11]. Leaves are cooked as vegetables and given to the patient suffering from piles and rheumatism [12, 13]. The use of this plant as traditional medicine confirms that it may possess some important biological activities. Though *Typhonium trilobatum* has been used for variety of therapeutic purposes since the ancient times, no such scientific data are available on its nutritional or other important biological activity. So the aim of the present study is to evaluate the nutrient contents and focus on this lesser known medicinal leafy vegetable.

Experimental

Collection and identification of plant materials

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) stable radical free radical were purchased from Sigma Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.); ascorbic acid, trichloroacetic acid (TCA), ferric chloride, Anhydrous Sodium Carbonate, Potassium acetate , Potassium permanganate, Aluminum chloride, Butylated Hydroxytoluene, Methanol , Tannic acid, Dichlorophenol-Indophenol dye, Sulphuric acid, Perchloric acid, Nitric acid, Oxalic aid were supplied by E. Merck India Pvt. Ltd. (Kolkata, India). 2,4,6-Tripyridyl-S-Triazine(TPTZ), 2,2 -azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) cation(ABTS +) free radical, Potassium persulfate, Folin Ciocalteu's phenol reagent, gallic acid and Quercetin were purchased from SRL (Sisco Research Laboratory, Mumbai, India).

Plant Material

The samples were collected from three different district of West Bengal as well as from three different local markets of Kolkata to obtain the mean value and to exclude the percentage of error as well as possible. The plant materials were identified and authenticated by Botany Department of Calcutta University.

The leaves were separated from roots, washed under running water, followed by double glass distilled water. They were drained completely and used for analysis.

Proximate analysis

Proximate composition includes moisture, total ash, crude fibre, fat, protein, carbohydrate and minerals. Moisture was determined by oven dehydration method at 105°C up to the constant weight and expressed in percentage. Protein was determined by estimating the nitrogen content of the plant material using kjeldahl method. For extraction of total

sugar Anthrone-suphuric acid reagent test was applied [14]. For extraction of total lipid, Chloroform:Methanol mixture (2:1) test was applied [14]. Crude fiber was determined by Acid digestion - Alkali digestion and by using fibertech. Ash content was determined in muffle furnace at 550°C for 6 hours. The samples were used in triplicate for all these determinations, according with AOAC14.

Vitamins Assay
Vitamin C was determined in fresh vegetable samples by Dichlorophenol-Indophenol dye reduction method [14].

Mineral Assay
For minerals analysis viz K, Ca, and Na, Fe the samples were digested by using $HClO_4$/ HNO_3 method [15]. The digested samples were used for selected minerals analysis, using Atomic Absorption Spectrometer (Perkin Elmer model 2380) and Flame Photometer (Jenway PFP7).

Oxalate assay
Total oxalate was analysed by extraction with hydrochloric acid and soluble oxalate with water followed by precipitation with calcium oxalate from deprotinized extract and subsequent titration with potassium permanganate [16].

Phytate assay
Phytic acid was extracted and determined according to the precipitate analysis method of Thompson and Erdman 1982 [17] with some modification.

Phytochemicals and *in vitro* antioxidant assay:

Preparation of plant leaf extract:
After washing the samples were allowed to dry in shades for 3-4 days. After complete drying of this plant leaves, they were ground into smaller pieces. Each plant sample (10g) was mixed with 200ml 80:20 methanol water by maceration for 24 hour. Then the solution was filtered and the filtrates were dried using rotary evaporator under reduced pressure. The extract yields of sample and spinach were 20% and 18.8% respectively. Stock solution (1mg/ml) of each plant extract was prepared by using 80% methanol as solvent.

Determination of Total Polyphenol Content (TPC):
Total polyphenol content of the flower extracts was determined by the method described by Matthaus 2002 [18]with some modification. Briefly, 0.2 mL of different concentrations of the extracts were taken, to which 1mL of Folin-ciocalteu reagent (diluted to 10-folds) and 0.8 mL of 2% Na_2CO_3 were added, and the volume was made up to 10 mL with methanol:water (6:4). After 30 min the absorbance was read at 740 nm wavelength against a standard calibration curve. The results were expressed as Gallic acid equivalents per gram of extract.

Determination of Flavonoids Content (TFC):
Total flavonoid content was measured by the aluminum chloride colorimetric assay [19]. An aliquot of extracts (50 and 100 μg/mL) or the standard solution of quercetin (20 to 100 μg) was added to a 10 mL volumetric flask containing 4 mL of distilled H2O. 0.3 mL 5% $NaNO_2$ was added. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At 6th min, 2 mL 1 M NaOH was added and the total volume was made up to 10 mL with water. The solution was mixed well, and the absorbance was measured against prepared reagent blank at 510 nm.

Determination of Total Hydrolysable Tannin Content (HTC):
Content of tannins was determined by Folin-Denis reagent [20] based on colorimetric estimation of tannins (measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannins in alkaline medium 0.5gm of dry sample was added to 75ml of distilled water, heated gently at first and then boiled for 30 minutes. Then it was centrifuged at 2000 rpm for 20 minutes. The supernatant liquid was collected and made up to the volume of

100ml (volumetric flask) by distilled water. After that 0.1ml of this solution was taken in a test tube and 7.5ml of water, 0.5ml of Folin-Denis reagent and 1ml of 35% Na2CO3 were added to it. The volume was made up to 10ml (volumetric flask) by using distilled water and shaken well. The test tubes were incubated for 30 minutes at room temperature and the absorbance of the solution was measured at 700nm wavelength against a standard calibration curve. Each experiment was carried out in triplicate and results averaged and expressed as $\pm$SD in µg/100g sample. Tannic acid was used as a standard and the calibration curve was prepared in the range of 20µg-100µg using 20, 40, 60, 80 and 100 µg as the standard concentrations at a wavelength of 700nm (λ_{max} of tannic acid).

***In vitro* Antioxidant activity**

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay:

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, BHT (Butylated hydroxy toluene). Different concentrations of the plant extracts were used to scavenge DPPH. The antioxidant activity of each sample was expressed in terms of IC_{50}, and was calculated from the graph after plotting inhibition percentage against extract concentration. DPPH assay was carried out after making some modifications in the standard protocol [21]. 3 ml of 0.1 mM DPPH solution was mixed with 1 ml of various concentrations (10 to 300 µg/ml) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. BHT was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{control}-A_{test})/A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control (BHT) and A_{test} is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

IC_{50} value

Inhibition Concentration (IC_{50}) parameter was used [22] for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

Determination of $ABTS^+$ scavenging activity:

For ABTS assay, the procedure followed was the method of Dimitrina and Roberta [23,24] with some modifications. ABTS was dissolved in water to make a concentration of 7 mmol/L. $ABTS^+$ was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the test of samples, the $ABTS^+$ stock solution was diluted with 80% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 4.85 mL of diluted $ABTS^+$ to 0.15 mL of stock samples solution (final concentration 1mg/mL), the absorbance reading was taken 6 min after the initial mixing. BHT (0.1 mg/mL) and Quercetin (0.05 mg/mL) were used as positive controls. The activities of the samples were evaluated by comparison with a control (containing 4.85 mL of ABTS solution and 0.15 mL of 80% Methanol). Each sample was measured in triplicate and averaged. This activity is given as percentage $ABTS^+$ scavenging that is calculated by the following formula:

$$ABTS^+ \text{ scavenging activity(\%)} = Ac–As/Ac \times 100$$

Where, A_C is the absorbance value of the control and A_S is the absorbance value of the added samples test solution. The antioxidant capacity of test compounds was expressed as EC_{50}, the concentration necessary for 50% reduction of $ABTS^+$.

Results and Discussion

Table 1 A comparison of Nutrient contents of *Typhonium trilobatum* and *Spinacia oleracea* leaves on fresh weight basis

Experimentation	Parameters	*Typhonium trilobatum*	*Spinacia oleracea*
Proximate composition	Moisture %	88.84±0.43	92.01±0.35
	Carbohydrate gm%	2.94±0.20	3.02±0.19
	Lipid gm%	0.66±0.03	0.86±0.04
	Total Ash gm%	1.30±0.20	1.62±0.02
	Protein gm%	2.81±0.60	1.96±0.42
	Crude Fibre gm%	2.44±0.25	0.59±0.05
	Vitamin C mg%	108.08±1.70	27.01±1.50
Minerals	Sodium mg%	0.03±0.01	58.53±0.02
	Potassium mg%	5.23±0.02	206.04±0.01
	Calcium mg%	22.59±0.06	72.05±0.04
	Iron mg%	58.40±0.50	1.91±0.56
Antinutritionals	Phytate µg%	0.35±0.01	B.D.L
	Oxalate mg%	7.19±0.58	658.07±0.45

Values are presented as mean ± SD, n = 3. Values are significantly different at P < 0.05.
B.D.L. – Below Detectable Limit

Table 2 A comparison of phytochemical contents in T*yphonium trilobatum* and *Spinacia oleracea* leaves

Phytochemicals	*Typhonium trilobatum* mg /gm extract	*Spinacia oleracea* mg /gm extract
Total polyphenols	21.19±0.07	18.70±0.21
Total flavonoids	11.25±0.06	14.81±0.12
Total hydrolysable tannins	22.17 ±0.15	20.45±0.33

Values are presented as mean ± SD, n = 3. Values are significantly different at P < 0.01.

Table 3 A comparison of antioxidant activity in *Typhonium trilobatum* and *Spinacia oleracea* leaves and standard BHT

Variety	DPPH radical scavenging activity IC_{50} µg/ml	$ABTS^{+}$ radical scavenging activity EC_{50} µg/ml
Typhonium trilobatum	432.90±1.90 µg/ml	155.31±0.90 µg/ml
Spinacia oleracea	455.13±2.05 µg/ml	150.20±2.08 µg/ml
Standard BHT	28.19±1.80 µg/ml	75.01±0.50 µg/ml

Values are presented as mean ± SD, n = 3. Values are significantly different at P < 0.01.

Nutrient contents:

Proximate composition:

The nutrient contents of the leaves of two plants were analyzed on fresh weight basis and expressed as gm/mg/µg per 100 gm sample. The results of proximate composition of *Typhonium trilobatum* leaves **(Table 1)** showed high moisture content (88.84±0.43gm%) which was lower than *Spinacia oleracea* (92.01±0.35) gm%. Although, the leaves of *Typhonium trilobatum* depicted lower values w.r.t. total ash and lipid contents (1.30±0.20 & 0.66±0.03gm%) than the leaves of *Spinacia oleracea* (1.62±0.02 & 0.86±0.04gm%), carbohydrate contents of the two plants leaves were comparable (2.94±0.20 & 3.02±0.19gm% for *Typhonium trilobatum* and *Spinacia oleracea* respectively). On the contrary, higher values have been observed w.r.t. protein (2.81±0.60gm%) as well as crude fibre (2.44±0.25gm%) contents of *Typhonium trilobatum* leaves than its counterpart (1.96±0.42gm% protein & 0.59±0.05gm% crude fibre). Quantitative estimation of the leaves of two plants revealed a remarkable 75% higher value of vitamin C content in the leaves of *Typhonium trilobatum* (108.08±1.70mg%) in comparison with spinach (27.01±1.50mg%), thus, establishing itself as a potent source of vitamin C amongst the leafy vegetables.
Thus, *Typhonium trilobatum* leaves resulted in higher contents w.r.t. vitamin C, crude fibre and protein; equivalent content of carbohydrates; lower contents of lipids and total ash when compared with the most commonly used *Spinacia oleracea* leaves.

Minerals:

The experiment revealed that although, sodium, potassium and calcium contents of leaves (0.03±0.01, 5.23±0.02, 22.59±0.06) mg% respectively are quite low in comparison with spinach leaves (58.53±0.02, 206.04±0.01, 72.05±0.04) mg% respectively **(Table1)**, iron content is fairly richer than its counterpart (58.40±0.50 and 1.91±0.56mg% respectively for *Typhonium trilobatum* and *Spinacia oleracea*).
Thus, *Spinacia oleracea* leaves may be described as an excellent source w.r.t. the minerals like Na, K and Ca but Fe in comparison to *Typhonium trilobatum* leaves, whereby, the later may be considered as a potent source of Fe nutrition.

Phytate and Oxalate: Effect of presence w.r.t. bio-availability of minerals:

Phytates: Bioavailability of Fe & Ca:

Seeds — such as nuts, edible seeds, beans/legumes and grains — store phosphorus as phytic acid. When phytic acid is bound to a mineral in the seed, it's known as phytate. It's often considered an anti-nutrient because it binds minerals

in the digestive tract, making them less available to our bodies. When we eat the plant, phytates are hydrolyzed during digestion to myo-inositol-1,2,3,4,5,6-hexkisphosphate (IP6) and lower inositol polyphosphates including IP1 through IP5 (these are phytate degradation products).

Phytic acid can bind minerals like iron, zinc, and manganese in the gut before they are absorbed and influence digestive enzymes. Phytates also reduce the digestibility of starches, proteins, and fats. Vegan eaters often consume more iron than omnivores. Yet, they also consume more anti-nutrients, including phytates, and these reduce the amount of iron available to their bodies. Consuming 5-10 mg of phytic acid can reduce iron absorption by 50%. This is why vegetarian eaters should eat more iron than omnivores (33 mg for veg eaters vs. 18 mg for omnivores). Not only that, but it seems to bind heavy metals (e.g., cadmium, lead in particular), helping to prevent their accumulation in the body[25].

The interference of phytic acid with the utilization of calcium of cereals has also been recognized for some time, and it has found practical application in England where 14 oz. (370 g.) of calcium carbonate are added to every 280 lb. (129 kg.) of 85% flour to neutralize the effect of phytic acid in wheat [26].

Phytates and Vitamin C:

Vitamin C appears strong enough to overcome phytic acid. In one study, adding 50 mg of vitamin C counteracted the phytic acid load of a meal. In another study, 80 mg of ascorbic acid (vitamin C) counteracted 25 mg of phytic acid[27].

Oxalates: Bioavailability of Ca:

The availability of calcium in relation to the oxalic acid content of the diet is a problem of some importance to tropical nutrition.

With respect to the availability of the calcium of spinach, it is generally agreed that the presence of oxalates in the food impairs the absorption of calcium. Confirmatory findings on dog, man and rat have been reported by McClugage & Mendel [28], Fincke & Garrison [29], and Fairbanks & Mitchell [30]. Fairbanks & Mitchell [30] state that the addition of sodium oxalate to diets of skimmed milk powder depresses the calcium utilization by 24-38 %.

It is obvious from these facts that calcium determinations on foodstuffs are of little significance from the nutritional point of view, unless taken in conjunction with the oxalic or phytic acid content or both. Thus Fairbanks & Mitchell [30] found that the calcium of spinach was hardly used by the rat. Closely associated with this problem is the incidence of oxaluria following the ingestion of oxalate-rich foods. Aykroyd [31] states that the daily calcium intake of the adult Indian is less than 0.2 g., compared with 0-8 g. recommended by the National Research Council. Consumption of oxalate-rich foods would further aggravate the already existing deficiency in calcium. Thus, it would be better to avoid oxalate-rich foods than to take measures to neutralize the effect of oxalic acid, especially when other sources of green vegetables are available.

The result of our study showed that *Typhonium trilobatum* contain negligible amount of phytate (0.35±0.01µg%) and the oxalate content of *Spinacia oleracea* (658.07±0.45mg%) is more than 90% higher than that in the former (7.19±0.58mg%). This trifle amount of phytates might not interfere with the bioavailability of rich iron that is present in the *Typhonium trilobatum* leaves (58.40±0.50mg%), which is appreciably higher (~30%) than the Fe content in spinach (1.91±0.56mg%).

Though, spinach is rich source of many nutrients, specially, minerals and phytochemicals but its high oxalic acid content (658.07±0.45mg%) may interfere with absorption of minerals specially calcium and iron. It is known from literature that oxalic acid present in spinach, combines with calcium to form an insoluble complex of calcium oxalate

which cannot be absorbed. **Approximately 55% of the oxalic acid in spinach is in the form of free and soluble oxalic acid rather than insoluble form, calcium oxalate. Free oxalic acid in such foods as spinach can reduce absorption of calcium and iron in other foods** [32]. Though, calcium content of *Typhonium trilobatum* is low (22.59±0.06mg%) in comparison with spinach but its low oxalate (7.19±0.58mg%) do not hamper appreciably towards calcium or iron absorption. Whereas, in case of spinach though the calcium content is high (72.05±0.04mg%), but its extremely rich oxalate content (658.07±0.45mg%) would make the maximum amount of calcium and iron present in spinach and even the calcium present in other foods unavailable for absorption.

Moreover, Ascorbic acid in sufficient amount can partly counteract this inhibition. As the iron (58.40±0.50mg%) and ascorbic acid (108.08±1.70mg%) contents of *Typhonium trilobatum* are very higher than spinach (1.91±0.56mg% Fe; 27.01±1.50mg% Vit C) and the two antinutritional factors, oxalate and phytate contents are fairly lower, so it can be said that the former is a better source of iron and calcium than spinach. So, in that point of view *Typhonium trilobatum*, (a cheap less focused easily available leafy vegetable) is quite good source of nutrients in comparison with spinach because the bioavailability of nutrients specially iron, calcium present is better than the most commonly used, familiar and quite expensive leafy vegetable, spinach.

Phytochemicals:

As plants have versatile chemical compound and their therapeutic value is due to the phytochemicals it is necessary to assay the phytochemical content for determination of antioxidant activity. Polyphenols and flavonoids are known to be the contributors of antioxidant capacity of fruits and vegetables [33]. The total polyphenol (Gallic acid was used as standard), flavonoids (Quercetin was used as standard), and tannin (Tannic acid was used as standard) contents were shown in **Table 2**. The polyphenol and total hydrolysable tannin contents (21.19±0.07 and 22.17±0.15mg/gm) of *Typhonium trilobatum* are higher than the spinach (18.70±0.21 and 20.45±0.33mg/gm). Though, the flavonoid content (11.25±0.06mg/gm) of *Typhonium trilobatum* is not as much as spinach (14.81±0.12mg/gm) but, it is quite satisfactory when compared with other less focused medicinal herbs.

Statistical Analysis:

All determinations were performed in triplicate, with the results reported as the means of those determinations with the standard deviations. Results were analyzed for significant differences using Independent sample t test by SPSS version 17.

DPPH Radical Scavenging Activity

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determine by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity [13,14]. **Table 3** shows DPPH radical scavenging activity of *Typhonium trilobatum*, *Spinacia oleracea* and BHT (which was used as standard), and it was calculated in the form of IC_{50} value. Activity of *Typhonium trilobatum* methanolic leaf extract was found at a concentration of 432.90±1.90µg/ml which is greater than *Spinacia oleracea* leaf extract activity (455.13±2.05µg/ml). So, the sample is able to scavenge DPPH free radical at lower concentration than spinach which depicts its better free radical scavenging activity than spinach.

ABTS Radical Scavenging Activity

ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by addition of potassium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. **Table 3** shows ABTS radical scavenging activity of standard BHT, the leaf extracts of *Typhonium trilobatum and Spinacia oleracea*. Though the ability of spinach to scavenge ABTS radical cation is little bit higher than the Typhonium leaf extract in term of EC_{50} value but the difference between two EC_{50} values (155.31±0.90 and 150.20±2.08µg/ml respectively) is negligible. So, it can be said that the activity of both samples are more or less similar w.r.t. the ABTS radicals.

Conclusion

Typhonium trilobatum leaves resulted in higher contents w.r.t. vitamin C, crude fibre and protein; equivalent content of carbohydrates; lower contents of lipids and total ash when compared with the most commonly used *Spinacia oleracea* leaves. *Spinacia oleracea* leaves may be described as an excellent source w.r.t. the minerals like Na, K and Ca but Fe in comparison to *Typhonium trilobatum* leaves, whereby, the later may be considered as a potent source of Fe nutrition. As the iron (58.40±0.50mg%) and ascorbic acid (108.08±1.70mg%) contents of *Typhonium trilobatum* are very higher than spinach (1.91±0.56mg% Fe; 27.01±1.50mg% Vit C) and the two antinutritional factors, oxalate and phytate contents are fairly lower, so it can be said that the former is a better source of iron and calcium than spinach. So, in that point of view *Typhonium trilobatum*, (a cheap less focused easily available leafy vegetable) is quite good source of nutrients in comparison with spinach because the bioavailability of nutrients specially iron, calcium present is better than the most commonly used, familiar and quite expensive leafy vegetable, spinach. The polyphenol and total hydrolysable tannin contents (**21.19**±0.07 and **22.17**±0.15mg/gm) of *Typhonium trilobatum* are higher than the spinach (18.70±0.21 and 20.45±0.33mg/gm). Though, the flavonoid content (**11.25**±0.06mg/gm) of *Typhonium trilobatum* is not as much as spinach (14.81±0.12mg/gm) but, it is quite satisfactory when compared with other less focused medicinal herbs. *Typhonium trilobatum* is able to scavenge DPPH free radical at lower concentration than spinach which depicts its better free radical scavenging activity than spinach. Activity of both samples are more or less similar w.r.t. the ABTS radicals.

Acknowledgments

Research conducted with contribution from: Dept. of Home Science, University of Calcutta, West Bengal, India, for funding and infrastructural support and WBPHL, Dept. of Health & F.W. Govt. of West Bengal, India for infrastructural and technical support.

References

[1] Robinson D.S., *Food biochemistry and nutritional value*, Longman scientific and technical publisher, New York. USA, **1990**.

[2] Borneo R, León E.A., Aguirre A, Ribotta P, Cantero J.J., Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their in vitro testing in model food system. *Food Chem*., **2008**, 112, 664-670.

[3] FAO, Preventing micronutrient malnutrition: *a guide to food based dietary approach*, Washington, FAO & ILSI., **1997**.

[4] Gupta A S, Lakshmia J, Manjunathb M N, Prakash J, Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. *Swiss Society of Food Science and Technology*, 38, **2005**, 339–345.

[5] Maisuthisakul P, Suttajit M, Pongsawatmanit, Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry*., **2007**, 100(4), 1409-1418.

[6] Glew R, Vanderjagt D, Chuang L.T., Huang Y. S., Millson M, Glew R, Nutrient Content of Four Edible Wild Plants from West Africa. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum).*, **2005**, 60(4), 187-193.
[7] World Health Organization, *Medicinal plants in Viet Nam*. WHO Regional Publications, WHO Regional Office for the Western Pacific, Manila., **1990**, 3.
[8] Haldar K M, Ghosh P, Chandra G, Evaluation of target specific larvicidal activity of the leaf extract of Typhonium trilobatum against Culex quinquefasciatus Say. *Asian Pac J Trop Biomed*., **2011**, 199-203.
[9] Kandhasamy M, Arunachalam K D, Efficacy of Typhonium trilobatum (L.) Schott tuber extracts on pathogenic bacteria. *Elect J Nat Subs.***, 2008,** 3, 1-7.
[10] Shanmugam S, Kalaiselvan P M, Selvalumar S K, Rajendran K, Ethnomedicinal plants used to cure diarrhea and dysentery in Sivagangai district of Tamil Nadu,India. *Int J Res Ayur Pharm.*, **2011,** 2, 991-994.
[11] Biswas A, Bari M, Roy M, Vadra S K, Inherited folk pharmaceuticals knowledge of tribal people in the Chittagong Hill tracks, Bangladesh. *Indian J Trad Know*., **2010**, 9, 77-89.
[12] Das H B, Mojumder K, Datta B K, Ray D, Ethnobotanical uses of some plants by Tripuri and Reang tribes of Tripura. *Nat Prod Rad.*, **2009**, 8**,** 172-180.
[13] Rahmatullah M, Rahman L, Rehana F, Kalpana M A, Khatun M A, Jahan R, A scientific evaluation of medicinal plants used in the folk medicinal system of five villages in Narsinghdi District, Bangladesh. *Am-Eurasian J Sustain Agric.*, **2010,** 4, 55-64.
[14] Sawhney S K, Singh R, *Introductory practical Biochemistry*. Narosa Publishing House Pvt. Ltd. Delhi., **2006**, 190.
[15] Steekel J E, Flannery R L, Automated determination of P, K, Ca, Na, Mg in wet digestion solution of plant tissues. *In technicon symposia, Automation in analytical chemistry.*, **1965**,116-122.
[16] Baker C J L, *The determination of oxalates in fresh plant material*, Analyst., **1952**, 77: 340–344.
[17] Thompson D, Erdman J W, Phytic acid determination in soy beans. *Journal of Food Science*., **1982**, 47, 513–517.
[18] Matthaus B, Antioxidant activity of extracts obtained from residues of different oilseeds. *J Agric Food Chem*., **2002**, 50, 3444–52.
[19] Ebrahinzadeh M A, Pourmorad F, Bekhradnia A R, Iron chelating activity screening, phenol and flavanoid content of some medicinal plants from Iran. *Afr J Biotechnol.*, **2008**, 32, 43–9.
[20] Parimala M, Shoba F G, Phytochemical Analysis and Invitro Antioxidant Activity of Hydroalcoholic Seed Extract of Nymphaea nouchali Burm. *Asian Pacific Journal of Tropical Biomedicines.*, **2013**, 3, 887-895.
[21] Hsu C Y, Chan Y P, Chang J, Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol Res.*, **2007**, 40, 13-21.
[22] Brand-Williams W, Cuvelier M E, Berset C, Use of a free radical method to evaluate antioxidant activity, Lebensmittel- Wissenschaftund-Technologie. *Food Sci Technol.*, **1995**, 28, 25- 30.
[23] Zheleva-Dimitrova D, Nedialkov P, Kitanov G, Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. *Pharmacogn mag*., **2010**, 6, 74–80.
[24] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*., **1999**, 26, 1231–7.
[25] Hunt J R & Roughead Z K. Nonheme-iron absorption, faecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. *Am J Clin Nutr.*, **1999**, 69, 944-952.
[26] Peters R A, The metabolism and functioning of vitamin like compound. *Nature*, Lond., **1942**, 150, 125.
[27] Siegenberg D, Ascorbic acid prevents the dose dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am J Clin Nutr.*, **1991**, 53, 537-541.
[28] McClugage H B, Mendel L B, Experiments on the utilization of nitrogen, calcium, and magnesium in diets containing carrots and spinach. *J. biol. Chem*., **1918**, 35, 353.
[29] Fincke M L, Garrison, The availability of the calcium of spinach and kale. *J. Home Econ. Proc*., **1936**, 28, 572.
[30] Fairbanks B W, Mitchell H H, The availability of calcium in spinach, in skim milk powder and in calcium oxalate. *J. Nutrit.*, **1938**, 16, 79.

[31] Aykroyd W R, *The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets*,. Health. Calcutta, Government of India Press., **1941.**
[32] Srilakshmi B, *Nutrition science*, New Age International Publishers, New Delhi., **2012**, 4.
[33] Teixeira S, Siquet C, Alves C, Boal I, Marques M P, Borges F, Structure-property studies on the antioxidant activity of flavonoids present in diet. *Free Rad Biol Med.*, **2005**, 39, 1099

Publication History
Received 31st Mar 2015
Revised 18th Apr 2015
Accepted 11th May 2015
Online 30th May 2015