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ANTOXIDANT AND NUTRITIONAL ANALYSIS OF EDIBLE CUCURBITACEAE VEGETABLES OF INDIA

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Abstract: Nutrition related problems are on rise in the under developed and developing countries and require immediate attention. The physical and mental health of a person solely lies on the consumption of nutritious food. Vegetables are rich source of carbohydrates, proteins, vitamins, fats, and minerals and also good antioxidants. *Momardica charantia* (Descourt), *Momardica dioica* (Roxb), *Trichosanthes dioica* (Roxb) and *Coccinia indica* (Wight & Arn) are considered for studying their nutritional composition by using standard analytical procedures due to their availability all over India. The proximate and ultimate analyses such as moisture, ash content, fats, proteins, reduced sugars, dietary fibres, minerals (Calcium, Magnesium, Iron and Phosphorus), vitamins (folic acid and vitamin C) and total phenols have been assessed. Considerable variations have been noticed in their nutritive values, minerals (Calcium, Magnesium, Iron, Phosphorus) and vitamin C, Folic acid compositions. Good concentrations of Pectin (5.04g/100g), Magnesium (48.6mg/100g), and Vitamin C (16.6mg/100g) were observed in *M. charantia* and of fats (2.28g/100g) and Total phenols (2,15x10GAE/l) in *M. dioica*. *T. dioica* had high concentration of minerals such as Iron (218mg/100g), and Calcium (115.7x10mg/100g) and good concentrations of protein (1.17g/100g). High concentrations of Phosphorous (24.11mg/100g) were observed in *C. indica.* Total phenol concentrations (>50.0x10mg GAE/g) and DPPH and FRPA assays indicated the antioxidant potential of these vegetables. These can be considered as alternate natural nutrient supplements and medically useful for common man.

Keywords: Common Vegetable, Nutrition, Antioxidants, DPPH, FRPA, total Phenols

INTRODUCTION

Inadequate nutrition can be one of the causes of the diseases. The development of nutritional products to treat and fight against diseases and malnutrition is necessary. Medical nutrition and pharmaceutical applications of plant origin will be the new research mandate for healthy future. Vegetables are considered to be protective foods and highly beneficial for the maintenance of good health and prevention of diseases[1,2]. They are sources for nutrients such as carbohydrates, proteins, vitamins, fibre and minerals required for human health. Antioxidants (such as polyphenols) are also one of important constituent of vegetables and play significant role in inhibiting the oxidation of organic molecules and defend living systems against oxidative stress. Phenolic antioxidants interrupt the propagation of the free radical auto-oxidation chain by developing a relatively stable free radical incapable of propagating further oxidation processes. The under developed and developing countries are facing grievous problem of malnutrition which can be prevented by accepting vegetables as resources and utilizing them to the maximum. Adequate research and education are needed to identify the vegetables rich in proteins and minerals.

Basic components and ingredients of Indian diet are based on plant foods (raw / cooked or fresh / frozen / dried) and consumed in large quantities. RDA has given the requirements of constituents of the diet with relation to age to maintain healthy life. Education of the available common vegetables as natural food source, and their consumption in diet with respect to their nutritional quality in diet enables a common man to address the malnutrition problem successfully. The nutrient and antioxidant levels of four Cucurbitaceae vegetables *viz., Momardica charantia* (Descourt), *Momardica dioica* (Roxb), *Trichosanthes dioica* (Roxb) and *Coccinia indica* (Wight & Arn) were assessed during the present study. Commercial exploitation of the vegetables can be ensured with this study with respect to their nutritional and medicinal values.

Cucurbitaceae family members have great diversity and considered to be major caterers of common vegetables. Spine gourd (*Momordica dioica*), Bitter gourd (*Momordica charantia*), snake gourd, Pointed guard, ivy gourd (*Coccinia grandis*) and Melothria (*Solena amplexicaulis*) etc. are to state a few. The plants often creepers with membranous, deeply lobed into 3-5 triangular lobes and punctuated leaves; axial, solitary, monoeceous, yellow / white colored flowers and considered as anunuals / perinneals. Cucurbitaceae members have been characterized with insecticidal/ wormicidal control, anti-hypoglycemic, anti-

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inflammatory, anti-lipidemic characteristics. National and international studies on common / wild varieties of selective genera of cucurbitaceae members were conducted on seed composition[3], nutritional levels[4]. Current study was aimed at assessing their capability as nutritional supplements.

MATERIALS AND METHODS

The unripe fruit samples of *Trichosanthes dioica, Coccinia indica, Mimordica dioica* and *M. charantia* were collected, wiped with wet cloth to remove adhering soil, cut into small pieces, mixed and used as samples for analytical purposes. The collected samples were sent to Department of Botany, Mithibai College and Chauhan Institute of Science for taxonomic identification. Samples in fresh and dry conditions were used for proximate and ultimate analysis.

The proximate analysis comprising protein[6,7] percentage moisture, total ash, crude fibers, pectin, crude fat, sugars, starch was assessed[5] . The ash content of the fruits was estimated by igniting the pre-weighed sample in a weighed crucible at 450°C for about 3 hrs in a muffle furnace. The moisture content was determined using oven method. The crude fiber was determined by hydrolyzing the sample with 0.128 ml of H_2SO_4 and 0.223 ml of KOH and fat determination was by Soxhlet extraction method. Minerals such as Calcium, Magnesium, Phosphorous and Iron (Ca^{2+}, Mg^{2+}, P^{4+}, Fe^{2+}) were determined by colorimetry using dry ash sample[7]. Sample solution was prepared from white dry ash of known quantity by using 30% HCl and used for individual mineral determination. The estimations were done in triplicates using Digital Colorimeter EQ-650-A (Equiptronics Company). Fresh samples were considered for estimation of vitamins such as Vitamin C and Folic acid[5]

Solvent extractions of unripe vegetables were considered for the analysis of antioxidant activities and total phenol concentrations analyses. Methanol extracts were prepared in triplicates from fresh vegetables with 20ml of 80% methanol per 0.5 g of the sample, and kept in water bath for 120 min. The supernatant of the extracted material was considered as Methanol extracts of *Trichosanthes dioica, Coccinia indica, Mimordica dioica* and *M. charantia* (TD,CI,MD, and MC respectively) for the determination of total phenols by Folin ciocalteau method[7]; antioxidant properties by DPPH scavenging activity method[8], and FRPA assay by Oyazu method[9].

Qualitative test analysis was performed to determine chemical constituents such as Polyphenols present in the methanol extracts of TSD, CI, MD and MC. The concentration of total polyphenolic compounds in the extracts was determined by Folin-Ciocalteau micro-method. 40µl of extract was mixed with 3160µl of distilled water and 200µl of Folin-Ciocalteau's phenol reagent. After 30 s to 8 min, 600µl of 20% of Sodium carbonate solution was added. The absorbance of samples was measured at 765 nm against blank sample using a Digital Colorimeter EQ-650-A (Equiptronics Company). Estimation was carried out in triplicates and calculated from the calibration curve obtained with Gallic acid as a standard. Final results were expressed as Gallic acid equivalents (x10 mg GAE/L).

DPPH scavenging activity had been evaluated based on the procedure of Nikhat,[8] during the current study. 3.0 ml of methanol extracts of CI, MD, MC and TSD in different concentrations (1–16µg/ml) were added to 1 ml of 0.1 mM DPPH in methanol. A blank was prepared without adding extract. 1 ml of 1% Ascorbic acid in 1 to 16µg/ml concentrations was used as standard. Thirty minutes later, the absorbance was measured at 517 nm using a Digital Colorimeter EQ-650-A (Equiptronics Company). Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{control} - A_{test} / A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Ferrous Reducing Power of methanol extracts of MC, MD, CI and TSD were estimated using the protocol given by Oyaizu[9]. Different concentrations (1-16µg/ml) of methanol extracts of vegetables were prepared. 1ml of each methanol extract solution was mixed with 2.5ml of 0.2M phosphate buffer (pH 6.8) and 1%Potassium ferri cyanide (2.5ml). The mixture was incubated at 50°C for 20min. 2.5 ml of 10% Trichloroacetic acid (TCA) was added to this mixture, and centrifuged at 3000 rpm for 10 min. The 2.5 ml of upper layer of the solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm.

$$\text{Percentage scavenging} = (A_{control} - A_{sample} / A_{control}) 100,$$

Where $A_{control}$ - the absorbance of solution without extract and A_{sample} - the Absorbance with different dilutions of extract. 1% Ascorbic acid (1-16 µg/ml) was used as reference standard. The antioxidant activity of the fruit extract and standard Ascorbic acid was expressed as EC_{50}. The EC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the ferrous reduction power by 50%.

Total phenol concentration and Antioxidant activities of MC, MD, CI and TSD methanol extracts were obtained using DPPH and FRPA assays. Data are presented as the mean ± SEM of each parameter. Three readings were considered for each chemical analysis, each extract and each time. The assessments were done in triplicates. Scatter plot, Pearson correlation and ANOVA were estimated using SYSTAT, the results were considered to be statistically significant (p, 0.05)

RESULTS

The proximate analysis revealed remarkable differences in nutrition composition among the vegetables (Fig.1). Water content present in vegetables as free/ bound form due to physical and chemical forces is estimated as moisture content. The given samples were dried at 55°C for four days in the oven and the percentage weight lost by foods due to evaporation considered as moisture content. The moisture content was high in *M. charantia* (89±0.11%) and low in *T. dioica* (42.9%±0.11). The previous studies of *M.dioica*[10,11]; *T. dioica* and *M. charantia* [12] showed a marginal variation of 90-92% moisture.

Pectin has nutrition value as an important soluble fiber which can controls lipid concentrations in human body. The Pectin values showed a remarkable variation between 1.34±0.23 (*T. dioica*) to 5.04±3.4 (*M. charantia*) g/100g.

The crude Fat residue obtained by Soxhlet's extraction varied from a low of 0.77±0.01 (*M. charantia*) to 2.28±0.01 (*M. dioica*) g/100g. Reducing Sugar content of vegetables was estimated by Shaffer and Somogyii micro method using iodometric titration of cuprous oxide and Dextrose as standard. Reducing sugars in the plant material were estimated by use of Copper reagent of the type. The reducing sugars varied between 0.76±0.11 (*T. dioica*) to 1.66±0.09 (*M. dioica*) and starch between 0.68 ±0.11 (*T. dioica*) to 1.44±0.09 (M. dioica) g/100g. Protein was extracted and estimated by Biuret method from the vegetable sample[6]. The protein content ranged between a low of 0.97±0.01 (*M. charantia*) to a high of 1.17±0.005 (*C. indica*) g/100g. The protein values of the earlier studies varied by *M. dioica* 3g/100g[10,11];2.0g/100g in *T. dioica* and 1.6g/100g in *M. charantia*[12] which could be due to different methodologies considered for the estimation.

Ash content of the fruits is the measure of mineral content of the food[13]. The percentage yield of white ash varied between 0.54±0.010 (*T. dioica*) to 1.66±0.01 g% (*M. dioica*). The earlier studies of *M. dioica* [10,11] the ash content showed variation of 9.1g/100g.

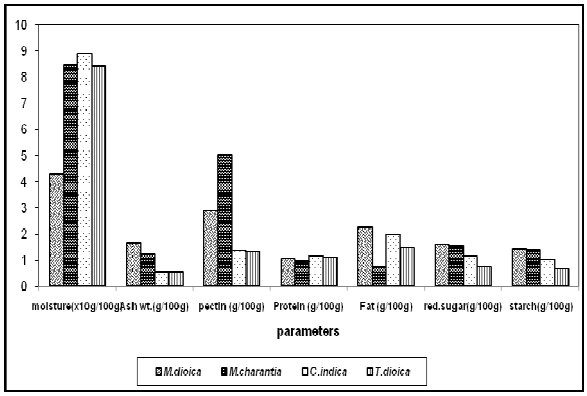

Fig.1: Proximate analysis of four selected edible vegetables of India

Table.1: Summary of Nutritional Value of Selected Edible Vegetables of India

Parameters (mean± se)	*M. dioica*	*M. charantia*	*C. indica*	*T. dioica*
Moisture (g/100g)	84.23±0.76	89±0.11	84.33±0.16	42.9±0.11
Ash wt. (g/100g)	1.66±0.01	1.25±0.83	0.55±0.001	0.54±0.01
Pectin (g/100g)	2.9±0.11	5.04±3.4	1.38±0.01	1.34±0.23
Protein (g/100g)	1.07±0.001	0.97±0.01	1.17±0.005	1.11±0.05
Fat (g/100g)	2.28±0.01	0.77±0.01	2±0.56	1.5±0.56
Red sugar (g/100g)	1.6±0.09	1.54±0.01	1. 16±0.01	0.76±0.11
Starch (g/100g)	1.44±0.09	1.39±0.01	1.04±0.01	0.68±0.11
Calcium (10xmg/100g)	74.06±14.4	62±11.3	94.66±6.25	115.7±4.05
Magnesium (mg/100g)	35.97±5.54	48.67±0.66	41.74±0.25	30.92±0.08
Iron (mg/100g)	129.73±17.28	177.3±12.39	188±3.9	218.93±0.02
Phosphorous (mg/100g)	21.94±6.3	17.33±2.6	24.11±1.38	22±1.17
Vitamin C (mg/100g)	39.4±5.2	16.67±11.4	4.67±0.65	9.33±4.56
Folic acid (mg/100g)	0.28 ±0.06	0.88±1.33	0.23±0.10	1.76±0.01
Total phenol (GAEx10mg /L)	215±12.00	73±12.9	75±16.95	58.3±3.16
DPPH assay (mcg/ml) (Ascorbic acid 1.6mcg/ml)	6.0	4	4	2.4
FRPA (mcg/ml) (Ascorbic acid 3.0 mcg/ml)	3.2	5.6	7.0	10.5

values are expressed as mean±std. error (p=0.05)

The ultimate analysis indicated that the selected vegetables as good source of micro elements. Minerals such as Calcium, Magnesium, Iron and Phosphorous concentrations were estimated by standard methods[7]. Studies indicated variations in the composition of Calcium (x10mg), high in 115.7±4.05 (*T. dioica*) and low 62±11.3 in (*M. charantia*) /100g; Magnesium, 30.92±0.08 (*T. dioica*) to 48.67±0.66 (*M. charantia*) (mg/100g); Iron, (mg/100g) 129.73±17.28 (*M. dioica*) to 218.93±0.02 (*T. dioica*) and Phosphorous 17.33±2.6 (*M. charantia*) to 24.11±1.38 (*C.indica*) mg/100g respectively(Table-1). The previous studies of *M.dioica*[10,11] indicated 7.39mg /100g Calcium and 5.04mg/100g of Iron.

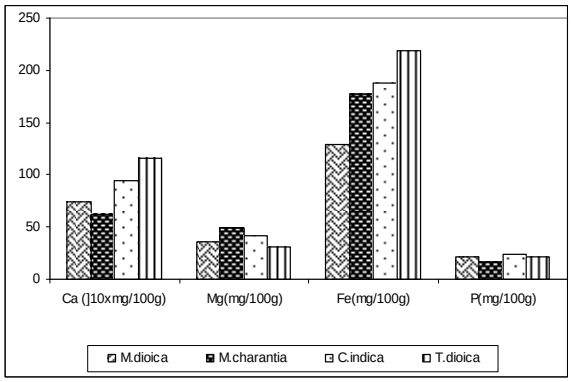

Fig.2: Ultimate analysis of four selected edible vegetables of India

Vitamins such as Folic acid and Ascorbic acid in the four cucurbitaceous members were assessed Folic acid was extracted from the vegetable samples using mild alkaline buffer, oxidized with permanganate to produce diazotized compound which will be read at 550nm. The present study indicated remarkable variations (Fig.3) in the concentrations of Folic acid 1.76±0.01 (*T. dioica*) to 0.23±0.10 (*M. charantia*) mg/100g. Viramin C varied from a high concentration of 39.4±5.2 (*M. dioica*) and a lowest of 4.67±0.65 (*C. indica*) mg/100g of vegetable. The Vitamin C concentrations were remarkably low, 9.33mg/100g as compared to 29mg/100g of *T.dioica*[12] and 16.67mg/100g in *M. charantia,* as compared to the previous studies (88mg/100g) conducted by Rahman[12] showed a marginal variation of 90-92% moisture.

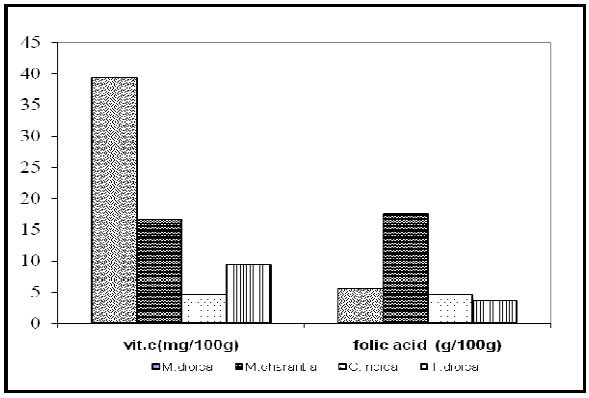

Fig. 3: Vitamin C and Folic acid composition of selected edible vegetables of India

Phenolic compounds are natural products of plant origin with great importance as potential antioxidants. Their consumption may significantly contribute to human health[14], to prevent oxidation of fats and oils, antioxidants and important constituents in foods and cosmetics[15]. The methanol extracts were assessed for total phenolic components by Folin-Ciocalteau micro-method with Gallic acid as standard. The total phenols were expressed as Gallic acid equivalents (x10 mg GAE/L) which varied from 58.3±3.16 (*T. dioica*) to 215±12.00 (*M. dioica*). Antioxidant assays such as Ferrous reducing power assay (Fig.4) showed variation in EC_{50} values from 3.2 (*M. dioica*) to 10.5 µg/ml (*T. dioica*) against standard Ascorbic acid of 3.0 µg/ml. IC_{50} value of DPPH assays varied between 2.4 (*T. dioica*) to 6.0 (*M. dioica*) µg/ml against the standard Ascorbic acid 1.6 µg/ml (Fig.5). Folic acid and phenols concentrations were not considered in the previous studies. DPPH scavenging activity was comparatively higher in *T. dioica* and close to that of the standard Ascorbic acid. The ferrous reduction power was similar to that of standard Ascorbic acid as in the case of *M. dioica* (3.2µg/ml).

Pearson correlation was performed using SYSTAT statistical package. The results indicated perfect correlation between *T. dioica, M. charantia and C. indica* (r=0.9) represented as one group, whereas *M. dioica* had lower correlation values with the others (r = < 0.75) (p=0.05) (Fig.6). The scatter plot indicated a close association between three vegetables *T. dioica, M. charantia and C. indica which* was represented as one group as they occur as common vegetables in all regions and seasons of India. *M. dioica* has occupied different position which can be attributed to rich nutrients such as fats, vitamin C and total phenols and its availability in high altitudes.

A correlation between the DPPH scavenging activity, Ferrous reducing power assay and total Phenol concentration was estimated. The methanolic extracts of three cucurbitacean vegetables showed positive correlation between DPPH scavenging activity and phenols (0.91, p=0.05). The results indicated that DPPH and Phenol have significant positive correlation which could be due to the secondary metabolites i.e. phenolic compounds, which are known as hydrophilic antioxidants[16].

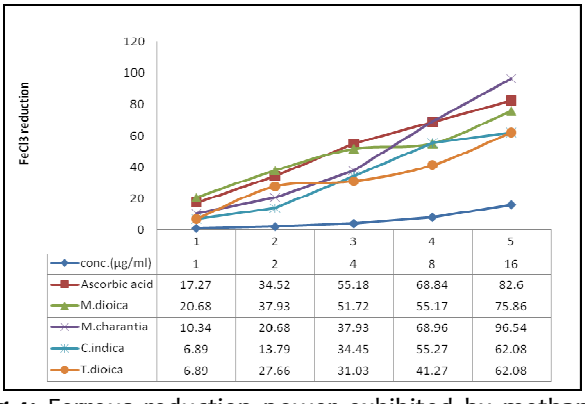

Fig.4: Ferrous reduction power exhibited by methanol extracts of selected edible vegetables of India

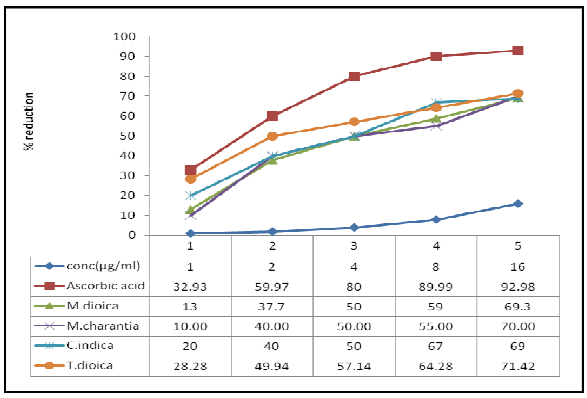

Fig.5: DPPH scavenging activity exhibited by methanol extracts of selected edible vegetable of India

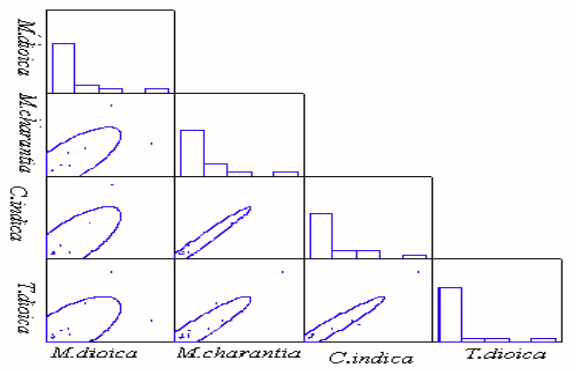

Fig.6: Scatter plot representation of interrelationship between the selected edible vegetables of India

DISCUSSION AND CONCLUSION

Research on nutritional, genetic and medicinal aspects was carried out in different *cucurbitaceous* members. The fruits, leaves, flowers and roots of the cucurbitaceae members are consumed as food. They have medicinal importance as diuretics, alexiterics, laxatives, hepato protectives, antivenumous, anti-hypertension, anti-diabetic, astringent, antihelminthic, aphrodisiac, antiseptic characteristics and capable of curing pimples or acne[17,18,19]. The cucurbitaceae family generally known as gourd family has very important role as vegetables with nutritional value. 90% of the common vegetables are consumed in the main course of food all over India. The current study indicated *M. dioica, T. indica* as the protein rich vegetables as the other two for medicinal rich components. According to the recommended dietary allowances (RDA)[20], the nutritional importance of the cucurbitacean fruits could be good supplement for nutrients such as Fibre, Calcium, Phosphorous, vitamin C, lipid, Protein and Carbohydrates (Table-2).

Table.2: balanced diet required for a man with moderate work (based on new RDA)

Nutrient	Vegetarian diet	Required as per RDA
Protein(G)	81.4	60
Total Fats (G)	60	30+30
Calcium(mg)	886.3	600
Iron(mg)	20.6	17.0
Ascorbic acid(mg)	192	40
Folic acid (mcg)	83.4	200
These values are derived from Nutritive value of Indian foods(1989)		

The study of cucurbitacean members indicated rich composition of Pectin, reducing sugars, starch, Magnesium, and vitamins like Folic acid (*M. charantia*); fats, vitamin C along with Ferrous reduction capacity (*M. dioica*); high concentration of minerals (Iron and Calcium) and Total phenol components (*T. dioica*), and protein and Phosphorous (*C. indica*). The stastical analyses indicated closed association among the domesticated common vegetables and distance from that of the wild vegetable *M. dioica* which requires domestication. These common, easily available and affordable vegetables can be a source of nutrient replenishments and also to maintain good health and to combat against several diseases. An awareness to consume these vegetables can prevent malnutrition in under developed and developing countries.

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