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Determination of mineral content and heavy metal content of some traditionally important aquatic plants of tripura, India using atomic absorption spectroscopy

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Mineral and heavy metal concentration of six aquatic plants possessing health promoting effects and used in indigenous medicine (as medicinal food) were determined using Atomic Absorption Spectroscopy. Different elemental constituents at trace levels of plant play an effective role in the medicines prepared. A total of 11 elements Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb and Zn have been measured. Their concentrations have been found to vary in different samples. Result of the present study provides vital data on the availability of some essential minerals, which can be useful to provide dietary information for designing value –added foods and for food bio fortification. Apart from this, data on the contaminated levels of heavy metals highlights the necessity on the quality and safety concerns about their use. Medicinal properties of these plant samples and their elemental distribution also correlated.

Key Words: Elemental analysis, Medicinal plants, Atomic Absorption Spectroscopy

Introduction

All human beings required sufficient food for growth, development and to lead an active and healthy life and it depends upon the quality and quantity of foodstuff he or she is able to include in his regular diet. The quality of food depends upon the presence of relative concentration of various nutrients such as proteins, fat, carbohydrate, vitamins and mineral. Plant materials form a major portion of their diet: their nutritive value is important. Nutrition plays a critical role in wellness, by not only providing essential nutrients, but also promoting good health and preventing diseases (Willet, 1994).

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Traditional wild vegetables contain a number of organic photochemical have been linked to the promotion of good health. In many tropical countries, rural people traditionally harvest wide range of leafy vegetables, roots, tubers, fruits from wild because of its taste, cultural uses, as food supplements or to tide over food shortage (Mahapatra *et al*., 2012). Among alternatives available to meet the food demands cultivable and wild vegetables are cheep source of food for the marginal communities (Hussain *et al*., 2009a). Wild plants have been recognized to have potential to meet household food and income security (Guinand and Dechassa, 2000; Kebu and Fassil, 2006). To apprehend the situation, interests have been centralized on the exploitation, quantification and utilization of food plants, especially the vegetables (Dini *et al*., 2005).

Vegetable being the rich source of carbohydrates, fats and proteins, which form the major portion of the human diet, are the cheaper source of energy. Various scientists (Sreedevi and Chaturvedi, 1993; Mathews *et al*., 1999; Kalita *et al*., 2007) have recorded the importance of these biochemical's. Besides these biochemical's, the moisture, fiber, and ash contents and the energy values of individual vegetable and plant species have also been regarded important to the human health. The wild plant species have antibacterial, hepatoprotective and anticarcinogenic properties, and therefore having medicinal values (Bianco *et al*., 1998). Trace element and heavy metals have certain risks (Schumacher *et al*., 1991) thus it is important to determine the levels of these compounds in common, popular, and widely used herbal plants. In general, information on edibility and therapeutic properties of wild plants is scanty but data on their nutritional composition and mineral content is negligible (FAO, 1984; Aloskar *et al*., 1992).

Aquatic plants grow profusely in lakes and waterways all over the world and have in recent decades their negative effects magnifies by humans intensive use of natural water bodies. Eradication of this water plants are has proved almost impossible and even reasonable control is difficult. Several authors (Banarjee and Matai, 1990; Boyd, 1968) have emphasized the potential of aquatic plants as food and feed. Large growths of aquatic plants in lakes and waterways of tropical countries, although a menace, represent a natural resource of green leaves (NAS, 1983). With increasing interest in finding new alternatives food sources, the wild or unutilized plants receive more attention that offers a good scope to meet the increasing demand for vegetable protein, carbohydrate, natural antioxidants, polyphenols etc. These plants could be useful components of the diet, especially for rural families since the plants are abundant and collection for food would be a relatively easy task.

Materials and methods

Sample preparation

Certain traditional plants (6 species) used by local tribal community of state Tripura collected from wild condition. The sample collected were air dried at room temperature and ground into fine powder in a mortar and pestle to avoid chromium contamination from stainless steel utensils. The powder placed temporarily in airtight polythene pouches until further analysis. The identity of plant materials confirmed with assistance from local practitioners and botanists.

Apparatus

The most commonly used techniques for qualitative and quantitative determination of minerals in food samples are atomic absorption spectrometry (AAS) (Dean and Ma, 2008; Dolan and Capar, 2002; Zafar *et al*., 2010). In the present study, the minerals and heavy metal content of the powdered samples were determined through AAS Perkin Elmer 3110 at Sophisticated Analytical Instrumental Facility Centre, North Eastern Hill University, Shillong following AOAC (1990) method.

Chemical analysis

The minerals [calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), and zinc (Zn)] contents of the powdered samples was determined. Briefly, a known amount of the sample was digested with a mixture of concentrated nitric acid, sulphuric acid, and perchloric acid (10:0.5:2, v/v), and the analysis was conducted using the All the glassware was cleaned by soaking overnight in a 10% nitric acid solution and then rinsing three times with deionised water. The Total Carbon and Hydrogen content was determined using CHNO Perkin Elmer 2400 Series II Analyzer.

Results and discussions

Table 1 show the wild edible traditional hydrophytes and marsh plants of Tripura and their part used along with their therapeutic uses. The different biochemical analysis of the different wild edible aquatic plants presented in (Tab 2). The mineral contents in these six vegetables showed that potassium was the most abundant secondary macro element present, ranging from 9.19 ppm (*Monochoria vaginalis*) to 1.72 ppm (*Euryale ferox* seed) K is important in for its diuretic nature and Na plays an important role in the transport of metabolites. The ration of K/Na in any food is an important factor in prevention of hypertension arteriosclerosis, with K depresses and Na enhances blood pressure (Saupi *et al*., 2009).The ration of K/Na is significant in *Neptunia prostrata* (27), *Monochoria vaginalis* (7.67), *Euryale ferox* aril (7.19) and compared with leafy vegetables (Cabbage 17.5, beet 3.9). In the present study K/Na, also show positive relation. The Mg content of the studied plant taxa varied from 3.028 ppm (*Monochoria vaginalis*) *to* 1.037 ppm (*Euryale ferox* seed). In humans, Mg is required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium. It is required in many enzyme – catalysed reactions, especially those in which nucleotide participate where the reactive species is the magnesium salt, e.g., $MgATP^{2-}$.Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system. The range of Fe in the studied plant varies from 0.15 ppm to 11.88 ppm. The permissible limit set by FAO/WHO (1984) in edible plants was 20 ppm. Fe is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in human body (Kaya and Incekara, 2000) and normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats (Adeyeye and Otokiti, 1999).The occurrence of anaemia in Fe deficiency may probably be related to its role in facilitating iron absorption and in the incorporation of iron into haemoglobin (FAO/WHO 1984). *Neptunia prostrata* exhibit higher concentrations of Ca that is 34.6 ppm than the other parts and *Euryale ferox* possess minimum concentration of Ca (0.204 ppm). Ca is important because of its role in bones, teeth, muscles system and heart functions (Brody, 1994). The lowest concentration of Cu that is 0.0011 ppm (*Nelumbo nucifera*) and maximum concentration estimated 0.138 ppm (*Monochoria hastata*). The permissible limit set by FAO/WHO (1984) in edible plants was 3.00 ppm. After comparison, metal limit in the studied medicinal plants with those proposed by FAO/WHO (1984) it is found that all plants accumulate Cu below this limit. In the present study, only *Monochoria vaginalis* found to contain Cr (0.001 ppm). Chronic exposure to Cr may result in liver, kidney and lung damage (Zayed and Terry, 2003).The absence of Cr in most of the studied species indicate their food safety value. Among the investigated edible plants *Euryale ferox* seed exhibits higher concentration of Pb 0.17 ppm while *Monochoria vaginalis* contains minimum amount of Pb 0.021 ppm. Most of the studied taxa contain Pd below the permissible level se by FAO/WHO (0.43 ppm). However, for medicinal plants limit was 10 ppm set by China, Malaysia, Thailand and WHO. Pb causes both acute and chronic poisoning, and poses adverse effects on kidney, liver, vascular and immune system (Heyes, 1997). The range of Mn varied between 3.386 ppm in *Monochoria vaginalis* to 0.016 ppm in *Nelumbo nucifera*. Except *Monochoria vaginalis* all other investigated taxa accumulate Mn below the permissible limit set by FAO/WHO (1984) limit in edible plants. However, for medicinal plants the WHO (2005) limits yet not been established. Cd causes both acute and chronic poisoning; adverse effect on kidney, liver, vascular and immune system (Heyes, 1997).In the studied plant Cd concentration ranged from 0.111 ppm in *Euryale ferox* aril to 0.026 ppm in *Euryale ferox* seed. The permissible limit set by FAO/WHO (1984) in edible plant was 0.21 ppm. However, for medicinal plants the permissible limit for Cd set by WHO is, China and Thailand was 0.3 ppm. The low concentration of Cd in all the investigated taxa also indicates their safety. Among the studied taxa, *Neptunia prostrata* contain the highest amount of Zn (4.87 ppm). The positive impact of zinc supplementation on the growth of some stunted children, and on the prevalence of selected childhood diseases such as diarrhoea, suggests that zinc deficiency is likely to be a significant public health problem, especially in developing countries (Osendarp *et al*., 2003; Sian *et al*., 2002; Hussain *et al*., 2009 a, c). According to FAO's food balance data about 20% of the world's population could be at risk of zinc deficiency. A correlation analysis was performed to investigate the relationships between the element concentrations in traditionally used aquatic samples. The data were subjected to statistical analysis, and Tab. 3 reports the correlation matrix (r) between major- and minor-elements for 11 variables. Some of the variable show positive correlation while some others show negative correlation. Fe and Cr, Zn and Ca show highly significant positive correlation.

Table 1. Wild edible traditional hydrophytes and marsh plants of Tripura

Botanical Name (& code name)	Family	Part Used	Therapeutic uses
Euryle ferox Salisbury ; (EUFa)	Nymphaeaceae	Aril	Gonorrhoea
Euryle ferox Salisbury ; (EUFs)	Nymphaeaceae	Seed	Skin disorder
Monochoria hastata (L.) Solm ; (MOH)	Pontederiaceae	Young Shoot	Decoction is used in nausea
Monochoria vaginalis (Burm.f.) Presler ; (MOV)	Pontederiaceae	Young shoot	Gastritis and Asthma
Nelumbo nucifera Gaertner ; (NEN)	Nymphaeaceae	Petiole	Digestive disorder
Neptunia prostrata (Lamarck) Baillon ; (NEP)	Mimosaceae	Shoot	Used in renal disorder.
Nymphaea rubra Roxburgh *ex* Andrews ; (NYR)	Nymphaeaceae	Petiole	Used to increase haemoglobin level

Table 2. Concentration of mineral contents (ppm) of selected wild edible traditional hydrophytes and marsh plants of Tripura

Mineral Content (ppm)	Plant Code Name						
	EUFa	**EUFs**	**MOH**	**MOV**	**NEN**	**NEP**	**NYR**
Cu	0.100	0.058	0.138	0.052	0.0011	0.016	0.043
Fe	0.30	0.058	1.68	11.88	1.57	0.505	0.43
Cr	ND	ND	ND	0.001	ND	ND	ND
Pb	0.060	0.17	0.031	0.021	0.10	0.07	0.10
Mn	0.114	0.028	1.512	3.386	0.016	1.912	0.022
Cd	0.111	0.026	0.060	0.063	0.042	0.028	0.035
Ca	0.279	0.279	2.752	0.786	0.639	34.6	1.504
Mg	2.149	2.149	2.552	3.028	1.554	2.17	1.758
Na	1.558	1.558	3.760	1.198	2.140	0.2	2.124
K	8.33	8.33	8.01	9.19	4.96	5.4	4.67
Zn	0.268	0.254	0.330	0.269	0.095	4.87	0.246

Table 3. Correlation coefficient between major- and minor- element concentrations of selected wild edible traditional hydrophytes and marsh plants of Tripura

	Cu	**Fe**	**Cr**	**Pb**	**Mn**	**Cd**	**Ca**	**Mg**	**Na**	**K**	**Zn**
Cu	1.0000										
Fe	-.0383	1.0000									
Cr	-.0587	9890***	1.0000								
Pb	-.3786	-.5675	-.5057	1.0000							
Mn	.0582	.8144*	.7980*	-.7112	1.0000						
Cd	.5786	.1619	.1610	-.5398	.0336	1.0000					
Ca	-.3595	-.1931	-.1751	-.1086	.3208	-.3651	1.0000				
Mg	.4746	.7461	.7536	-.6174	.8523**	.2865	-.0078	1.0000			
Na	.6259	-.1418	-.2404	-.1465	-.2432	.1575	-.5965	-.0311	1.0000		
K	.6361	.4854	.5138	-.2742	.4070	.5165	-.3759	.7991*	.0913	1.0000	
Zn	-.3614	-.1859	-.1601	-.0928	.3207	-.3462	.9981***	.0063	-.6360	-.3442	1.0000

(***$p<0.0001$;** $p< 0.01$; * $p<0.05$)

Conclusion

Aquatic plants have economic and environmental uses depending on their natural characteristic. Some of them are consume in human diet while other species have medicinal value and still other species are good resource of mineral and vitamin (Vasu *et al*., 2009). Data obtained from wild edible plants show that they have a very high nutritional potential. Therefore, it is of utmost importance to promote the use of those non-conventional plants as foodstuff. Rahman *et al*. (2007) revealed that because of rich content of carbohydrates, proteins in aquatic plants they can be utilize as food, and feed (Dewanji, 1993) indicated that leaf protein extracted from unwanted aquatic plants could be used for food and feed purposes. The elements Fe^{+2}, K^{+}, Mg^{+2}, Na^{+}, Ca^{+2}, , Mn^{+2}, Zn^{+2} and Cu^{+3} have been classified as essential elements, Cr^{+3} are possibly essential while Cd^{+2}, Pb^{+4} are non essential elements for the human body. The researchers are trying to link the contents of the trace elements and medicinal values of the plants. Most of the studied species contains the trace below the permissible limit (FAO/WHO 1984). The study revealed that investigated edible plants are good source of Na, K, Ca, Mg and Fe. However, in some cases they carry very high content of toxic metal whose main reason is the industrial pollution and irrigation by polluted water waste (Rehman *et al*., 2007, Sial *et al*., 2006)The mineral and heavy metal concentration reported herein might not be on par with some of the earlier reports on medicinal plants.

The differences observed might be due to different growth conditions, genetic factors, geographical variations in the level of soil fertility, efficiency of mineral uptake, and the analytical procedure employed (Ozcan and Akgul, 1998).Though much is known about the functional role of a number of elements, the best foreseeable benefit for human health, by mineral nutrition, lies in obtaining the correct amount of supplementation in the right form at the right time. This is for the first time that such an exhaustive work on elemental content has been carried out on the edible medicinal aquatic plants of Tripura. The data obtained in the present work will be useful in synthesis of new herbal drugs with various combinations of plants, which can be used in the treatment of different diseases at global level generally and in Tripura, India particularly.

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References

Adeyeye, E.I., Otokiti, M.K.O. (1999). Proximate composition and some nutritionally valuable minerals of two varieties of Capsicum annum (Bell and Cherry peppers). Discovery and Innovation 11:75-81.

Aloskar, L.V., Kakkar, K.K., Chakra, O.J. (1992). Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Part-I (A-K), 1965- 1981, NISC, CSIR, New Delhi, pp: 265-266.

A.O.A.C. (Association of Official Agriculture Chemists). (1965). Official methods of analysis Washington, D. C.

A.O.A.C. (Association of Official Agriculture Chemists) (1990). Official methods of analysis. Washington, D. C.

Banarjee, A, Matai, A. (1990). Composition of Indian aquatic plants in relation to utilization as animal forage. J. Aquat. Plant Manag 28: 69-73.

Bondi, A., Alumot, E. (1987). Anti-nutritive factors in animal feedstuffs and their effects on livestock. Progr Food Nutr Sci11:115-151.

Boyd, C.E. (1968). Fresh -water plants: A potential source of protein. Eco Bot 22: 359-368,

Brody, T. (1994). Nutritional Biochemistry. San Diego, Academic Press.

Cosgrove, D.J. (1980). Inositol hexakinase phosphatase p 26 -43.In: Cosgrove DJ.(ed.). Inositol phosphatase Theirchemistry, biochemistry and physiology. Amsterdam, Elsevier Scientific Publishing Company.

Dean , J. R. and R. Ma. (2008). Atomic absorption, atomic emission, and inductively coupled plasma spectroscopies in food analysis, 319-346 pp. In : Hanbook of Food Analysis Instruments, Otles S. (Eds.). CRC Press, Taylor and Francis Group.

Dewanji, A. (1993). Amino acid composition of leaf proteins extracted from some aquatic weeds. J.Agri. Food Chem 41:1232- 1236.

Dini, I., Tenore, G.C., Dini, A. (2005). Nutritional and ant nutritional composition of Kancolla seeds: an interesting and underexploited andine food plant. Food Chem 92:125-132.

Dolan, S. P., and S. G., Capar. (2002). Multi-elements analysis of food by microwave digestion and inductively coupled plasma atomic emission spectroscopy. J Food Comp. Anal. 15(5):593-615.

F.A.O./ W.H.O. Contaminants (1984). In: Codex Alimentarius (1st ed, XVII), FAO/WHO, Codex Alimentarius Commission. Rome

Gerloff, G.C., Krombholz, P.H. (1966). Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plant. Limnol Oceano11:529 -539.

Guinand, Y., Dechassa, L. (2000). Indigenous Food Plants in Southern Ethiopia: Reflections on the Role of 'Famine Foods' at the Time of Drought. United Nations Emergencies Unit for Ethiopia (UNEUE), Addis Ababa.

Heyes, R.B. (1997). The Carcinogenicity of metals in humans. Cancer Causes Control 8:371-385.

Hodge, J.E., Hofreiter, B.J. (1962). Determination of reducing sugars and carbohydrates p. 380-394. In: Whistler, R. L., Wolform, M. L. (ed.), Methods in Carbohydrate Chemistry. Academic Press, New York.

Hussain, J., Khan, A.L., Rehman, N., Hamayun, M., Shah, T., Nisar, M., Bano, T., Shinwari ,Z.K., Lee, I.J. (2009). Proximate and nutrient analysis of selected vegetable species: A case study of Karak Region Pakistan. Afr. J. Biotech 8(12):2725-2729.

Hussain, J., Khan, A.L., Rehman, N., Zainullah, S.T., Hussain, F., Shinwari, Z.K. (2009). Proximate and nutrient analysis of selected medicinal plant species of Pakistan. Pak J. Nutr. 8(1):620 – 624.

Hutchinson, G.E. (1975). A treatise on Limnology p264-407. In: Hutchinson GE (ed.) Limnolo Bot. New York.

Kalita, P., Mukhopadhyay, P.K., Mukherjee, A.K. (2007). Evaluation of the nutritional quality of four unexplored aquatic weeds from northeast India for the formulation of cost-effective fish feeds. Food Chem103:204-209.

Kaya, I., Incekara, N. (2000). Contents of some wild plants species consumed as food in Aegean region. J. Turk. Weed Scie 3:56-64.

Kebu, B., Fassil, K. (2006). Ethnobotanical study of wild edible plants in Derashe and Kucha Districts. South Ethiopia. J. Ethnobiol. Ethnomed.2: 53.

Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J. (2003). Tannins in nutrient dynamics of forest ecosystems-a review. Plant Soil 25(6):41-66.

Kumar, V.B., Jeejamol, C., Murugan, K., Mohan, K.T.C. (2007). Biochemical analysis of the nutraceutical characteristics in Barbados cherry (*Malpighia glabra* L.). J. Phytol Res 20(2): 237 – 242.

Lahiry, N.L., Satterlee, L.D., Hsu, H.W., Wallace, G.W. (1977). Characterization of chlorogenic acid binding fractions in leaf protein concentrates. J. Food. Scie 42:83 – 85.
Lindhorst, T.K., Thisbe, K. (2003). Essentials of Carbohydrate Chemistry and Biochemistry. p 112-125 New Delhi, Wiley Eastern Ltd.
Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with follin - phenol reagent. J. Biol. Chem.193: 265-275.
Luo, X.D., Basile, M.J., Kennelly, E.J. (2002). Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). J. Agri. Food Chem. 50:1379–1382.
Mahapatra, A.K., Satarupa, M. , Basak, U.C., Panda, P.C. (2012). Nutrient analysis of some selected edible fruits of deciduous forests of India : an explorative study towards non-conventional bio-nutrient.4(1): 15-21.
Mandal, B., Ghosh, B., Majumder, S., Maity, C.R. (1984). Nutritional evaluation of protein from three non-traditional seeds with or without amino acids supplementation in Albino rats. Proce Ind. Nat. Scie. Acad. 50(1):48 -56.
Mathews, C.E., Van-Holde, K.E., Ahern, K.G. (1999). Biochemistry. Benjamin Cummings.
N.A.S. (National Academy of Sciences). (1984). Making aquatic weeds useful: some perspectives for developing countries. Washington, DC.
Osendarp, S. J., West, C.E., Black, R.E. (2003). The need for maternal zinc supplementation in developing countries: an unresolved issue. J.Nutr.133:817- 827.
Ozcan, M., Akgul, A. (1998). Influence of species, harvest date and size on composition of capers (*Capparis* spp.) flower buds. Food Nahrung 42:102–105.
Pierpoint, W.S. (1983). Reactions of phenolic compounds with proteins and their relevance to the production of leaf protein .p 55-60. In Telek L, Graham HD (ed) Leaf Protein Concentrates.AVI Publishing Co. Westport, CT.
Polisini, J.M., Boyd, C.E. (1972). Relationship between cell-wall fractions, nitrogen and standing crop in aquatic macrophytes. Ecol 53:484-488.
Rahman, A.H., Rafiul, I., Naderuzzaman, A.K.M., Hossain, M.D., Rowshatul, A. (2007). Studies on the aquatic angiosperms of the Rajshahi University campus. Res. J. Agri. Biol. Scie.3:474-480.
Rehman, W., Zeb ,A., Noor ,N., Nawaz, M. (2008). Heavy metal pollution assessment in various industries of Pakistan. J. Env. Geol. 55:353-358.
Sadasivam, S., Manickam, A. (1992). Biochemical Methods. New Age International Publishing Ltd., New Delhi.
Saupi, N., Zakira, M.H., Bujang, J.S. (2009). Analytic chemical composition and mineral content of yellow velvet leaf (Limnocharis *flava* L. Buchenau) edible parts. J. Appl. Scie.9:2969 -2974.
Schumacher, M., Bosque, M.A., Domingo, J.L., Corbella, J. (1991). Dietary intake of lead and cadmium from food in Tarragona Province, Spain. Bull. Env. Cont. Toxicol.46:320-328.
Sial, R.A., Chaudhary, M. F., Abbas, S.T., Latif, M.I., Khan, A.G. (2006). Quality of effluents from Hattar Industrial Estate. J. Zhejiang Univ.Scie. B.7(12): 974-980.
Sian, L., Krebs, N.F., Westcott, J.E. (2002). Zinc homeostasis during lactation in a population with a low zinc intake. The Ame. J. Clin.Nutr.75:99-103.
Sreedevi ,A., Chaturvedi, D. (1993). Effect of vegetable fibre on post *prandial glycemia*. Plant Food Human Nutr.44:71-78.
Stake, E. (1967). Higher vegetation and nitrogen in the rivulet in central Sweden, Schweiz. J. Hydrol 29:107 -124.
Swain, J., Hillis, W.E. (1995). The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. J. Scie. Food Agri. 10:63 -68.

Thompson, L.U., Zhang, L. (1991). Phytic acid and minerals: effect on early markers of risk for mammary and colon carcinogenesis. Carcinogenesis12:2041-2045.

Vadivel, V., Janardhanan, T. (2000). Nutritional and anti nutritional a composition of velvet bean: an underutilized food legume in south India. Internat. J. Food Scie. Nutr.51:279-287.

Vasu, K., Jakku, V.G., Suryam, A., Charya, S.M. (2009). Bimolecular and photochemical analysis of three aquatic angiosperm .Afr. J. Microbiol. Res.3(8):418 -421.

Vijayvergia, R., Kumar, J. (2007).Quantification of Primary Metabolites of *Nerium indicum* Mill. Asian J. Exp. Scie. 21(1):123-128,.

W.H.O. (World Health Organization) (2005). Quality Control Methods for Medicinal Plant Materials. Geneva.

Willet, W.C. (1994). Diet and health: what should we eat? Science 254:532 -535.

Zafar, M., Khan, A.M., Ahmad, M., Jan, G., Sultana, S., Ullah, K., Marwat, K.S., Ahmad, F., Jabeen, A., Nazir, A., Abbasi, M.A., Rehman, A.U., Ullah, Z. (2010). Elemental analysis of some medicinal plants used in traditional medicine by Atomic Absorption Spectrophotometer. J. Med. Plant. Res 4(19):1987-1990.

Zayed, A.M., Terry, N. (2003). Chromium in the environment: factors affecting biological remediation Plant. Plant Soil 249:139-156.

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