

Determination of Nutritive Value and Mineral Elements of some Important Medicinal Plants from Western Part of India

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Abstract

Background: Medicinal plants viz; *Tinospora cordifolia* (Gulvel), *Gymnema sylvestre* (Morasingi), *Tricholepis glaberrima* (Brahmdandi) are very important for treatment of diabetes mellitus and other various diseases. These plants have great importance due to their nutritive value and major source of medicines as they have been found through out human history

Objective: Evaluation of Nutritive value and mineral elements of *T.cordifolia* (Gulvel), *G. sylvestre* (Morasingi), *T. glaberrima* (Brahmdandi).

Methods: The nutrients were analyzed by using different biochemical methods while the mineral elements were analyzed by Flame photometry and by using various titration methods.

Results: *T. cordifolia* (Gulvel), *G. sylvestre* (Morasingi), *T. glaberrima* (Brahmdandi) showed sufficient mineral elements like P, K, Na, Ca, Fe, Zn, N, Mg and low in Cu, Cr with good nutritive value and rich in carbohydrate enough protein but low in fat content

Conclusion: on dry matter basis these medicinal plants shows high nutritive value with maximum percentage of important minerals, which can be used for health care during anemic condition and as food and fodder for livestock.

Keywords: *Tinospora cordifolia*, *Gymnema sylvestre*, *Tricholepis glaberrima*, Mineral elements and Nutritive value

Introduction

Common medicinal plants have been compiled from translations of ancient Indian texts on health and healings [1]. Plants have great importance due to their nutritive value and continue to be a major source of medicines as they have been found through out human history [2] 30 to 40% of today's conventional drugs used in the medicinal & curative properties of various plants are employed in herbal supplements botanicals, nutraceuticals and drug [3]. All human beings require number of complex organic compounds as added [4] caloric requirements to meet the need for their muscular activities, carbohydrates, fats and proteins, while minerals and vitamins from comparatively a smaller part, plant materials form major portion of the diet; their nutritive value is important [5]. Human body comprises chemical compounds such as water, proteins, fatty acids, nucleic acids and carbohydrates, these in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen and phosphorus and may or may not contains minerals such as calcium, iron, magnesium and zinc [6]. The nutritive value of plant plays great role in plant and human being, so material extracted from the natural plant through chemical or biotechnology method [7].

In the present study the shoots of important medicinal plants that are *Tinospora cordifolia*, *Gymnema sylvestre* and *Tricholepis glaberrima* were taken for evaluation of nutritive value and mineral elements from "Sanjivani-Hill, Latur which is located in the state Maharashtra, India having great biodiversity. *T. cordifolia* is an herb commonly called Gulvel, Gulanshe, Guduchi in Hindi, *Tinospora Giloy* in English, commonly found in India and China [8]. Plant possesses bitter, pungent and astringent tastes,

shows post-digestive effect and has hot potency, it has light and dry attributes and alleviates all the three doshas viz, vata, pitta and kapha [9]. It well known for their anti-inflammatory, antacid, antipyretic tonic, neuroendocrine, anti stress, antioxidant and immune modulating effect [10]. *T. cordifolia* increase leucocytes counts and ablate neutropenia, protects against induced infects in mice and rat, cleaning microcirculatory system and other bodily channels [11]. It is effective and unique in its ability to remove exogenous and endogenous toxins (for external and internal sources), its principal constituents are tinosporine, tinosporaside, cordifolide, cordifol and hepatacosanol [12] *G. sylvestre* is a woody climbing plant grows in tropical forest of Central and South India, commonly called Gurmar, Gudmar and Morasingi belongs to the family *Asclepiadaceae* [13] it called as destroyer of madhumeha (glycosuria) and other urinary disorders, on account of its property to abolishing taste of sugar so it is called gurmar, neutralize excess sugar in body in diabetes mellitus [14]. Plant is bitter astringent, acrid, thermogenic, anti-inflammatory, digestive live tonic, diuretic, cardiogenic, antipyretic laxative, and uterine tonic [15] useful in dyspepsia constipation, jaundice, renal and vesicles calculi, cardiopathy and leucoderma [16] it lower the serum cholesterol and triglycerides in animals and prevent weight increase [17] *G. sylvestre* contain *Gymnema*, saponins I-II and gymnemic acid I-IX, which neutralize excess sugar in blood. The major bioactive constituents are group of oleanane type triterpenoid, saponins called gymnemic acid [18, 19].

Tricholepis glaberrima commonly called Brahmdandi. *Bursera serrata*, an herb distributed throughout India grow on bunds of

fields, rocky soil of grasslands and belongs to the family *Asteraceae* [20]. used as folk medicine, treatment of cancer, skin diseases and it acts as best tonic for internal use to get rid from exhaustion [21] *T. glaberrima* used in leucoderma, inflammations promising nervic tonic and possesses unique allelopathic properties [22].

Materials and methods

T. cordifolia, *G. sylvestris* and *T. glaberrima* plants were collected from 'Sanjivani Hill' Latur in the month of August/September 2007. The shoots of all plants were analyzed for nutritive value and mineral elements. The collected plant specimens were identified by a taxonomist Dr. Sirdeshpande B.A.M.S. College, Latur. The plant shoot were washed with deionised water and disinfected with 0.1 % HgCl_2 solution for 5 min and dried in shade [23] to prepare the sample for mineral analysis, the washed and dried materials were ground to fine powder with mortar and pestle and used for dried ashing [24]. For analysis of K, Na, and Ca the powdered plant shoot was taken in pre-cleaned and constantly weighed silica crucible and heated in muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled in desiccator at room temperature. The ash totally free from carbon moistened with Conc. H_2SO_4 and heated on Hot plate till fumes of sulphuric acid get evolved the silica crucible with sulphated ash was again heated at 600°C in muffle furnace till weight of sample was constant (~3-4 hrs) one gram sulphated ash were taken in beaker which dissolved in 100 ml 5 % conc. HCl to obtain solution for determination of K, Na, Ca and Cr through flame photometry (FPM), standard solution of each mineral was prepared and calibration curve drawn for each element using FPM [5].

For determination of protein and Nitrogen using Micro Kjeldahl method, 1 gm of sample of each plant taken in a Pyrex digestion tube and 30 ml of conc. H_2SO_4 carefully added, then 10 gm potassium sulphate and 14 gm copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colorless and clear, allowed to cool, diluted with distilled water and transferred 800 ml Kjeldahl flask, washing the digestion flask, Three or four pieces of granulated zinc and 100 ml of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N sulphuric acids was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using Methyl Red indicator for determination of nitrogen, which in turn give the protein content [25]. For determination of Iron 1 gm of sample in 125 ml deionised water was taken in conical flask pH adjusted to 2-3 by using Congo red paper. 5 drops of Variamine blue indicator was added then content was warmed at 400°C on hot plate and titrated with standard 0.05 M EDTA the initial blue color changes to gray just before the end point and final drop of reagent changes to yellow. Concentration of Iron was calculated by using the formula $1 \text{ mol EDTA} \equiv 1 \text{ mol Iron}$.

For determination of copper the titration cell charged with 1 gm of sample with 10 ml distilled water and 20 ml of acetate buffer (pH 2.2) adding again 120 ml of distilled water. Spectrophotometer adjusted to zero, the solution was filtered, stored and titrated with standard EDTA, absorbance recorded at every 0.50 ml until the value is about 0.20 and subsequently every 0.20 ml titration continued up to 1.0 ml. The end point observed when readings become constant. The absorbance

plotted against volume of titrant added; the intersection of the two straight lines gives concentration of copper in sample which compared with true value. For determination of Chromium 0.50 gm of sample dissolved in 100 ml distilled water, 20 ml of 0.1 M Silver nitrate solution followed by 50 ml of 10 % solution of Ammonium Persulphate. The mixture is then boiled for 10 min. cooled and diluted to 250 ml in graduated flask up to the mark then 50 ml of solution removed and 50 ml 0.1 M Ammonium iron sulphate solution, 200 ml 1 M Sulphuric acid and 0.5 ml of N-Phenylanthranilic acid indicator is added and titrated with standard 0.02 M potassium dichromate solution until the color changes from green to violet red. Ammonium iron sulphate solution standardized against 0.02 M Potassium dichromate, using N-phenylanthranilic acid as indicator. The volume of iron solution calculated which was oxidized by the dichromate originating from the chromium salt and from this the percentage of Chromium was calculated.

For determination of zinc 1 gm of sample in 20 ml deionised water were taken and placed in titration flask and 1 ml pure cyclohexylamine was added, -1.4 V Vs SCE potential applied to deaerate the solution and titrated with standard EDTA using a semi micro burette. From volume of EDTA used concentration of zinc was determined using the relation $1 \text{ ml } 0.01 \text{ M EDTA} \equiv 0.6538 \text{ mg Zn}$.

For determination magnesium 0.1 gm of sample was taken in 25 ml of 5 M hydrochloric acid in 100 ml graduated flask and the volume was made up to mark adding distilled water, standard solution is prepared by using 1 gm Magnesium metal in 50 ml of 5 M HCl and solution made to 100 ml with distilled water then few drop of solochrome black T indicator was added and adjusted to pH to 10.1 the color developed is read at 520

nm and concentration of Mg was calculated Spectrophotometrically by using standard graph [26] For determination of phosphorous 2 gm sample of each plant material taken in 100 ml conical flask two spoons of Darco-G-60 is added followed by 50 ml of 0.5 M NaHCO_3 solution, next flask was corked, and allowed for shaking for 30 min on shaker. the content was filtered and filtrate was collected in flask from which 5 ml filtrate was taken in 25 ml volumetric flask to this 2 drops of 2, 4-paranitrophenol and 5 N H_2SO_4 drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 ml with distilled water and then 4 ml ascorbic acid was added then the mixture was shaken well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbances were compared and concentrations of phosphorous using standard value were calculated [27]. Crude fat were determined by extracting 1 gm of moisture free plant material of each plant with petrol in a soxhlet extractor heating the flask on sand bath for about 1 hr till a drop taken and care was taken that the dripping left no greasy stain on the filter paper should present after boiling with petrol. The residual petrol was filtered using Whatmann No. 40, filter paper and the filtrate was evaporated in a pre weighed clean beaker, increase in weight of beaker gave crude fat percentage from total sample taken.

For determination of crude fibre, 2 gm of moisture and fat free material were treated with 200 ml of 1.25% H_2SO_4 , after filtration and washing, the residue was treated with 1.25% NaOH, filtered, washed with hot distilled water and then 1% HNO_3 and again washed with hot distilled water. The residue was ignited and the ash weighed. Loss in the weight gives the weight of crude fibre [28]. For determination of moisture content plant material is kept in pre weighed watch glass

and dried at 150°C over night in oven. The sample with watch glass is cooled at room temperature in desicator before weighing, the weight loss in sample regarded as moisture content. For determination of ash content, 5 gm of each plant sample weighed and taken in silica crucible and heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3 - 5 hrs at 600 °C temperature. Then the sample was cooled in a desicator and weighed to ensure completion of ashing; it was again heated in muffle furnace for 1 hour, cooled and weighed. This was repeated consequently till the weight of sample became constant (Ash became white or grayish white). The loss in weight of plant sample gives the ash content [29]. The all above procedures were carried out for all plant materials.

Formulae:

Percentage of carbohydrate was given by:

$100 - (\text{Percentage of ash} + \text{percentage Moisture} + \text{percentage fat} + \text{percentage protein}).$

Nutritive value is finally determined by:

$\text{Nutritive value} = 4 \times \text{percentage of protein} + 9 \times \text{percentage of fat} + 4 \times \text{percentage carbohydrate}.$

Results and discussion

Results of the percentage mineral elements in three medicinal plants are given in table 1 and shown in fig 1, while the results of various nutrients summarized in table 2 and shown in fig 2 and nutritive value in table 3 and shown in fig 3. Though the percentage of magnesium higher in *T. glaberrima*, Mg plays important role in formation and function of bones, muscles and prevents high disorders, high blood pressure and depression [30] also Mg plays important role in enzyme activity, deficiency interfere with transmission of nerve and muscle, impulses, causing irritability and nervousness, prevent heart diseases [31]. Chromium was very low in comparison with all other mineral elements in studied plants. Comparatively high in *T. cordifolia* shoot Cr is vital element as it works with insulin to stabilize blood sugar level, help to absorb energy from blood and increase muscle mass reducing fat mass in human body [32]. Deficiency of Cr results in growth failure, cataract, hyperglycemia, neuropathy, atherosclerosis and leads to diabetes in human [33].

Table 1: Percent concentration of various mineral elements

Plants	N	P	K	Na	Ca	Fe	Cu	Zn	Mg	Cr
<i>T. cordifolia</i>	0.45	0.571	0.845	0.33	0.131	0.28	0.031	0.12	6.41	0.006
<i>G. sylvester</i>	0.56	1.075	0.915	0.28	0.124	0.3	0.067	0.23	10.12	0.004
<i>T. glaberrima</i>	0.42	0.175	0.76	0.42	0.145	0.36	0.042	0.09	12.34	0.003

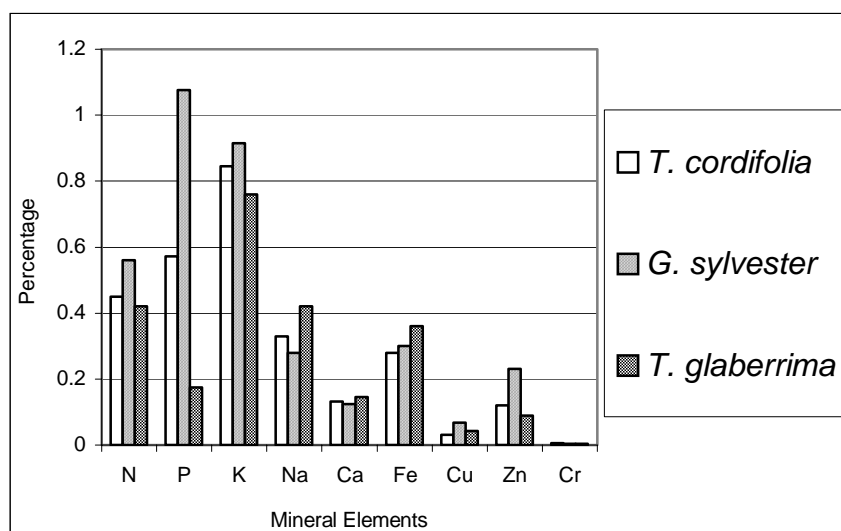


Fig. 1- Percent concentration of various mineral elements

Table 2: Percentage of Ash, Moisture, Fat, Protein, Fibre and Carbohydrate

Sr. No.	Plants	Ash Content	Moisture Content	Crude Fat	Crude Protein	Crude Fibre	Crude Carbohydrate
1	<i>T. cordifolia</i>	12.4	18.34	3.1	4.5	15.9	61.66
2	<i>G. sylvester</i>	4.6	8.02	2.7	4.6	9	80.08
3	<i>T. glaberrima</i>	8.1	20.05	5.5	6.4	13.1	59.5

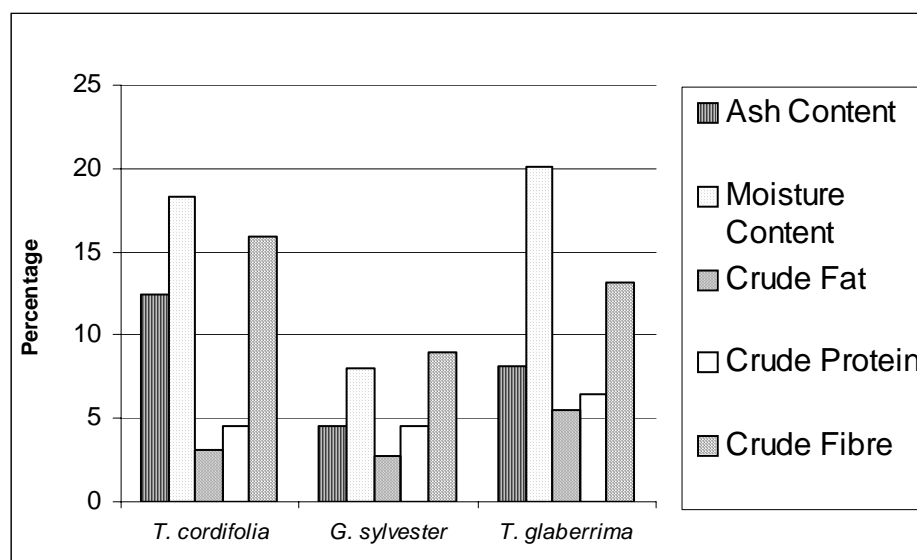


Fig. 2- Percentage of Ash, Moisture, Fat, Protein, Fibre and Carbohydrate

Table 3: Nutritive Value

Sr. No.	Plants	Nutritive Value Cal/100gm
1	<i>T. cordifolia</i>	292.54
2	<i>G. sylvester</i>	363.02
3	<i>T. glaberrima</i>	313.1



Fig. 3- Evaluation of Nutrient Value

Potassium was higher in all studied medicinal plants but they contained less sodium; Na and K. take part in ionic balance of the human body and maintain tissue excitability, carry normal muscle contraction, help in formation of gastric juice in stomach [34], K help in release of chemicals which acts as nerve impulses, regulate heart rhythms, deficiency causes nervous irritability mental disorientation, low blood sugar, insomnia and coma [35]. Iron sufficient in all studied medicinal plants, it make body tendons and ligaments, certain chemicals of brain are controlled by presence or absence of Iron, it is essential for formation of hemoglobin, carry oxygen around the body [36] Iron deficiency causes anemia, weakness, depression, poor

resistance to infection [37]. Calcium is high in *T. glaberrima* but also sufficient in *G. sylvester* and *T. Cordifolia*. Calcium play important role in building and maintaining strong bones and teeth also large part of human blood and extra cellular fluids. It is also necessary for normal functioning of cardiac muscles, blood coagulation, milk clotting and regulation of cell permeability [38]. Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus [39]. Zinc is higher in *G. sylvester* very low in *T. glaberrima* though the percentage of copper is very low in all studied plants. Cu was an important component of many enzyme systems such as cytochrome oxidase, lysyl

oxidase and ceruloplasmin, an iron oxidizing enzyme in blood [40]. Cu deficiency has been associated with cardiac abnormalities in human and animal, cause's anemia and neutropenia [41]. Zinc maintain various reactions of the body which help to construct and maintain DNA, required for growth and repair of body tissues, important element of ligaments and tendons [42]. Zinc deficiency causes clinical consequences, including growth dela, diarrhea, pneumonia, distributed neuropsychological performance and abnormalities of fetal development [43]. Phosphorous is higher in *G. sylvester* shoot and very low in *T. glaberrima*. Phosphorous maintain blood sugar level, normal heart contraction dependent on phosphorous [44] also important for normal cell growth and repair, needed for bone growth, kidney function and cell growth. It plays important role in maintaining body's acid-alkaline balance [45]. Nitrogen was comparatively high

in *G. sylvester*, play important role in digestion of food and growth [46] but in excess it is harmful for living body.

Nutritive value of shoots of *G. sylvester* was high followed by *T. glaberrima* and *T. cordifolia*; on a dry matter (DM basis) these medicinal plants have good nutritive value, which supports their use as food, fodder and good source of various important nutrients for live stock. The crude protein, fat and fibre on DM basis shows variation in there content *T. glaberrima* shoot have comparatively high protein, fibre content also carbohydrate and fat in sufficient amount though *T. cordifolia* with high fibre and sufficient protein and carbohydrate with low fat. *G. sylvester* shoot have good amount of carbohydrate, low fat and sufficient amount of fibre and protein with suitable mineral element and showing high Nutritive value. Seem to be good for younger people, anemic people.

References

1. Swami Brahmananda. Common medicinal plants of India: A complete India to home remedies, Dominant, Delhi. 2000, 8, 327.
2. Balick MJ. Paul and Cox A. Plants that heal; people and culture: The science of ethno botany. Scientific American library, 1996, 73, 25 - 61.
3. Shulz, Volker, Rudolf, Hansel, Mark, and Blumenthal. Medicinal plants, Phytomedicines and Phytotherapy: A physician's guide to herbal medicine. New York, 2001, 4, 1 - 39.
4. William Benton. Encyclopedia Britannica Inc. 1972, 16, 802 - 5.
5. Indrayan AK, Sharma SD. Durgapal N. kumar, and Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valuated plants from Uttaranchal, current science, 2000, 89 (7), 1252-3.
6. Katzmarzyk JL and Waist RR. Circumference and not body mass index explains obesity related health risk. *Am. J. Clin. Nutr.* 2004; 79 (3): 379 - 84.
7. Chapman D. Plant analysis values suggestive of nutrient status of selected crops soil, Sci, Soc of Amer, Madison, 1967; 2: 77 - 92.
8. Pendes VK. Indian drugs. 1981; 10 - 26.
9. Singh SS, Pandey SC, Shrivastava S, Gupta VS and Palio B. Chemistry and medical properties of *T. Cordifolia*. *Indian J. Pharmacol.* 2003; 35: 83 - 99.
10. Thatte VM, Roa SG and Dahanukar SA. *Tinospora cordifolia* induces colony-stimulating

activity in serum, j. post, grad, med, Bombay. 1994, 40, 202 - 3.

11. Thatte VM, SC Chhobria, SM karandikar and SA Dahanukar. Protective effects of Indian medicinal plants against cyclophosphamide neutropenia. *J. Post. Grad, Medicine*. 1987; 33: 185 - 8.

12. An Elementary textbook of Ayurveda; medicine with six thousand year old tradition 1991, 16 - 20.

13. Pankaj oudhia, *Gymnema sylvestre*, Gudmar or Merasingi, R, Br. Society of Parthenium Management India. 2002, 1 - 10.

14. Indian medicinal plants; A compendium of 500 species orient Longman Ltd, Madras, 1995, 3, 107 - 9.

15. Nadkarni KM. Indian material medica, popular prakashan, Bombay, 1993, 1, 596 - 9.

16. Warren RP Warren RM. Weinger MG. Inhibition of the sweet taste by *G. sylvestre*, nature, 1969, 223, 94 - 5.

17. Shigematsu N. Asano R. Shimozaka M and Okazaki M. Effect of administration with the extract of *G. Sylvester* R. Br. Leaves on lipid metabolism in Rats. *Biol. Pharma*. 2001; 24: 707 - 13.

18. Chakravarti KN, Chakravarti DS and Itly MF. Crystalline saponin from *G Sylvester*, Bulletin Scb. Trop med Calcutta. 1996, 4, 126 - 7.

19. Kuzuko Y, Kayoko Shigenoby AA and Kouji M. Structure studies of New anti sweet constituents from *G. sylvestris*, tetrahedron, left, 1989, 30 (9), 1103 - 6.

20. Naik VN. Flora of Marathwada, Amrut Prakashan, 1998, 1, 501 - 2.

21. Pankaj oudhia. A modern Herbal, Society for Parthenium Management, Uttranchal, 2005, 5, 16.

22. Singh V. Wadhwani AM and Johari BM. Dictionary of economic plants of India, Delhi. 1986, 2, 38 - 9.

23. Singh BD. Text book of Biotechnology, kalyani publishers Ludhiana, 2005, 2, 239 - 40.

24. Jonani GK and Sondhi SM. Determination of minerals elements in some Ayurvedic bhasmas used for the cure of various elements. *Phytother. Res*. 2002; 16: 774 - 7.

25. Jayaraman J. Laboratory manual in Biochemistry New age International (p) Ltd., 2005, 24, 75 - 8.

26. Chopra SL and Kanwar JS. In Analytical agricultural chemistry, Kalyani publications, Delhi, 1991, 4, 297 - 8.

27. Mendham JR Denney JD Barnes M and Thomas JK. Vogel's Text book of Quantitative chemical analysis, Pearson Education, Singapore, 2004, 6, 383 - 673.

28. Watanable FS and Olsen SR. Test for ascorbic acid method for determining phosphorus in water and sodium bicarbonate extract of soil. *Proc. soil sci. soc. Am*. 1965; 29: 677 - 8.

29. Sadasivam S and Manickam A. Biochemical methods, New age International, Delhi 1996, 2, 159 - 60.

30. Smith WD and Hammarsten JF. Serum Mg in clinical disorders, *South Mol. J*. 1958; 51: 1116 - 7.

31. Scelig M. Cardiovascular consequences of Mg deficiency and loss; pathogenesis, prevalence and manifestations Ame, Tour, of cardio, 1989, 63, 1101 - 2.

32. Hambridge KM. Chromium nutrition in man. *Am. J. Clin. Nutr*. 1974; 27, 505 - 14.

33. Jamal H, Raza H, Janua KM and Bhatti MK. Effect of minor minerals containing

chromium on human health, pak *J. Sci. Ind. Res.* 1986; 29: 45 - 7.

34. Brody Tom. Nutritional Biochemistry San Diego Academic press, 1998, 11 - 2.

35. Underwood EJ and Suttle NF. The mineral nutrition of Livestock, CABI publishing, New York, 1999, 51 - 101.

36. Alessandra Gaeta and Robert, Hider, C. The crucial role of metal ions in neuro degeneration; the basis for promising therapeutic strategy *Brit J. of Pharmalo.* 2005; 146: 1041 - 59.

37. Weight LM Jalobes P and Noakes TD. Dietary Iron deficiency and sports anemia *Brit, J. of Nutri.* 1992; 68: 253 - 60.

38. Heaney RD. Thinking straight about calcium *The New Eng. J. of med.* 1994; 328 (7): 503 - 5.

39. Hasling C. Sondergard K and Charles Pand Moselkiloe. Calcium metabolism in postmenopausal osteoporotic woman is determined by dietary calcium and coffee intake *Am. ins. of nutria.* 1991; 23: 119 - 26.

40. Mills DF. Symposia from the XII International congress on Nutrition *Prog. Clin.*

Biol. Res. 1981; 77: 165 - 71.

41. Smith JC. Copper nutritive and cardiovascular integrity in: Hemphill DD. Ed proceedings of 21st annual conference on trace substances in Environmental health, Columbia, 1987, 499 - 513.

42. Diaz-Gomez NM. Domenech E. Barroso F. Castells S, Cortabarría C and Jimenz A. The effect of zinc supplementation on linear growth body composition and growth factors in preterm infants, *pediatrics*, 2003, 111 (5), 1002 - 9.

43. Michel Hambidge. Human zinc deficiency *Tour of Nutrition Denver*, 2000, 130, 1344 - 9.

44. Linder and Manria C. Nutritional Biochemistry and metabolism with clinical applications. Appleton and Lange, Norwalk, 1991, 2, 191 - 212.

45. Johns T and Duquette M. Deficiency of phosphorus in man. *Am. J. of clin. Nutr.* 1991; 53: 448 - 56.

46. Cooper J. Structure and Biological activity of nitrogen and oxygen; coordinated nicotinic acid complexes of chromium, *Inorganica chemica, ACTA*, 1984; 91: 1 - 9.