Estimation of Minerals in *Carica papaya* L. Leaf found in Northern India by using ICP-OES Technique

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Abstract - The aim of this Research Paper is to determine the minerals by using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method in *Carica papaya* L. leaf (papaya leaf) found in Northern India. Three types of samples; green, yellow and brown leaves of *Carica papaya* L. collected from plane and hill regions, more particularly, the healthy papaya leaves were collected from National Capital Region (NCR) as plane & from Shimla (Himachal Pradesh), height about 2205 m or 7234 ft from the sea level was chosen for the collection from hill region, both the regions are the parts of Northern India. All the three types of leaves collected from plane as well as hills were analysed for the estimation of mineral constituents. The collected leaves were dried & finely powdered after removal of pedicle. All the six samples were extracted and digested with 2.0 per cent w/v solution of nitric acid in deionised water separately and analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The appreciable results were obtained and the highest value of Ca in green leaves collected from hill region; Cr in green leaves from both, plane & hill region; Mg, Fe, Zn, Mn & Cu in the brown leaves collected from plane region, K in the brown leaves collected from hill region and Na in yellow leaves from hills were obtained. Thus, brown papaya leaves from plane & hill both regions of Northern India having a good source of essential minerals while yellow papaya leaves were found a nice source of sodium and green leaves of calcium. The most significant feature of this research is the estimation of nine minerals in *Carica papaya* L. leaf which are highest in number estimated so far & the instrument used is most advance, sophisticated & sensitive also i.e. ICP-OES which is capable to quantify the multi elemental determination having a wide working range which can quantify the metals or minerals in traces up to ppb level. Therefore, papaya leaves due to its potent therapeutic properties can be formulated in herbal formulations as a medicine/dietary supplement and may be marketed as a nutraceutical product and as well as drug in curing the several diseases like Dengue fever, malaria etc.

Index Terms-ICP-OES, *Carica papaya* L. leaves (CP), Nitric acid, Deionised water, Minerals/nutrients, Phytochemical, Therapeutic Properties & Dietary Supplement etc.

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1-INTRODUCTION

Carica papaya Linnaeus (pawpaw or papaya) found in different parts of the world including India. It's a herbaceous succulent plant that possess a supporting stem. Papaya belongs to family Caricaceae and it is commonly found in tropical areas. The papaya is a large perennial herb with a rapid growth rate. The plants are male hermaphrodite or female. The male trees are uncommon, but sometimes when homeowners collect their own seeds. The hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped succulent fruits. These plants are self pollinated. Several books, journals & literature like Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) & United States Pharmacopoeia (USP) were surveyed but none any literature about Carica papaya L. leaf was found. The stem, leaves, seeds, flowers and the fruit of papaya plant have medicinal importance and are commonly used in traditional folk therapy to fight against infection, fever, beri-beri, malaria, aid in digestive health. An infusion of dried leaves is purgative and may cause abortion. The flowers are used in jaundice[1]. Papaya mostly cultivated in Maharashtra, Bengal, Bihar, Haryana, Punjab, Delhi, Uttar Pradesh, Uttarakhand, Himachal Pradesh and Andhra Pradesh in India.

In Indian System of Medicine (ISM), *Carica papaya* (Papeeta) is an important medicinal plant and its fruit juice, leaves and roots have been used in various ailments and as health tonic. The plant is traditionally used for the treatment of gastric ulcers, dental caries, to expel intestinal worms, as heart tonic, for severe jaundice, for inflammation, haemorrhoids etc. Reports indicates that the pharmacological activities of *C. papaya* include antihypertensive, ulcer protective, antimicrobial, antioxidant, anti-fertility, antifungal & anthelmintic[2, 3, 4, 5 & 6]. *Carica papaya* contains alkaloid (carpine and carposide), citric acid, Vitamin-C, Nicotinic acid, papain or papayotin, papaya oil.

It was concluded that the acute toxicity study of CP leaf extract at 2000 mg/kg body weight (BW) administered orally to Sprague Dawley rats did not cause any death or acute adverse

effect on the clinical observation and mortality to the treatment rats. However, from the blood investigation, it showed that CP leaf extract consumption may cause dehydration as demonstrated by increased in HGB, HCT and RBC as well as total protein level. Other parameters except triglyceride were normal. This finding will be monitored in the sub acute toxicity study[7].

Since CP leaf extract can mediate a Th1 type shift in human immune system, our results suggest that the CP leaf extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders, and may also serve as immune adjuvant for vaccine therapy[8].

Carica papaya L. fruit juice and leaf extracts are known to have many beneficial medical properties. Recent reports have claimed possible beneficial effects of CP leaf juice in treating patients with dengue viral infections. The extracts of both the leaves and fruit are known to contain several proteins and alkaloids with important pharmaceutical, medical, and industrial applications. Interestingly, *C. papaya* L. fruit juice and leaves extracts have demonstrated anti-cancer. Carica papaya L. leaf tea or extract plays an important role as a tumour-destroying agent[9].

The results of a critical study showed that the CP leaves may be used as a complementary drug in dengue fever which accelerates the increased in platelet counts, and shortened the hospitalisation period[10].

In a recent study, the powder from papaya leaves has substances responsible for the release and/or production of thrombocytes (platelets). The study was initiated and led by Dr S. Kathiresan of AIMST University. Dr Kathiresan said the leaves of papaya & fruit were high in complex vitamins that might help bone marrow to rapidly increase blood platelet production. Dengue fever continues to be a major health threat to Malaysia & India. The main effect of dengue virus is on platelet production. Normally, a platelet in our body lasts for about 5 to 10 days and the body replenishes them when required. This virus destroys the body's capacity to produce new platelets during the period, when the virus is effective. The platelet count for a normal person varies from 150,000 to 250,000 per micro litre (μl) of blood. On becoming infected, a patient's platelet count starts falling. A platelet count below 100,000 per 1 μl s alarming and an immediate medical attention is required. A platelet count below 50,000 can be fatal. A fall in the platelet count prevents formation of clots and this leads to haemorrhaging, which results in both internal and external bleeding. Once such bleeding starts, the situation is almost irreversible. Dr Kathiresan noted that interest in the papaya began with a simple experiment with papaya leaves which were ground and administered to mice. The platelet counts before, and 72 hours after dosing, revealed they were significantly higher[11].

The objective of this study is to compare the mineral contents of different types of CP leaves (green, yellow and brown collected from the plains as well as from hills) as a basis to advising the traditional medicine practitioners, herb users, herb sellers, health institutions and farmers on the health and economic importance of CP leaves.

The minerals mainly estimated/obtained in papaya leaf are calcium, magnesium, potassium, iron, manganese and sodium so far. The presence and the quantity of minerals/nutrients also depend upon the soil, climatic conditions like temperature, humidity etc. hence it varies according to the conditions where plant grow. So, taking into consideration, the material for the research was collected from the plain as well as from the hill area of the Northern India just to identify the responsible factors

which affect the quantity & the kinds of the minerals present in papaya leaves.

Fresh as well as dried green papaya leaf is an antibacterial and cures Dengue fever. The green, yellow and brown dried papaya leaf is the best as an iron tonic and blood purifier. Although, the brown leaves contain maximum content of iron and it may be a very herbal drug for the patients of anaemia. The tea, prepared with the green papaya leaf, promotes digestion and aids in ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart.

The potent antioxidant activity of papaya leaves is attributed to the array of phenolic compounds. *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf, bark and twig tissues which possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides[10]. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle ingredient papain, chymopapain etc. are found.

2-MATERIALS AND METHODS

Nitric acid, Methanol, water bath, conical flask, measuring cylinder and Whatman Filter paper No 4 etc. Chemicals/reagents were purchased from E. Merck Ltd. Mumbai, India which were used for the successive digestion/extraction during the experiment. CP leaves were collected from plane as well as from hills of Northern India, Shimla, Himachal Pradesh in July/August 2012. The plant was identified and authenticated from Raw Materials Herbarium and Museum at National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

3-MINERALS ANALYSIS

Minerals were determined by digesting with 2 per cent nitric acid in ultrapure water obtained by Milli Q system (Millipore, France) was used for sample digestion & further dilution of the samples. After the complete digestion, filtered by ashless Whatman Filter paper No. 4 with the pore size 20-25 μm by using the Inductively Coupled Plasma Emission Optical Spectrophotometer (ICP-OES), Make- Perkin Elmer, Model-Optima-2100-DV for the estimation of minerals like Calcium, Magnesium, Manganese, Iron, chromium, copper, zinc, potassium and sodium.

4-RESULTS OBTAINED

(The results of Mineral analysis of the leaves given in the Table -1)

In the CP leaves, the highest value in (mg/kg) of Ca, 3480 in green leaves collected from hill region; Cr, 7.50 in green leaves of plain & hill region; Mg, 5928; Fe, 558; Zn, 33.4; Mn, 22.88; Cu, 2.16 in the brown leaves collected from plain region, K, 8754.50 in the brown leaves collected from hill region and Na, 1912.60 in yellow leaves from hill region were obtained. Thus brown papaya leaves from both plain & a hill region of Northern India is a good source of essential minerals while yellow CP leaf shown a good source of sodium and green leaves of calcium.

5-DISCUSSION

The appreciable amounts of essential minerals (9 nos.) were obtained in all the three types (Six samples) of CP leaves obtained, which are as below:

1- Yellow papaya leaves collected from Plain contain least amount of Calcium (Ca), while green, brown and yellow leaves from hills have almost equal and an appreciable amount of calcium.
2- Brown leaves collected from plain as well as hills, contain maximum amount of Magnesium (Mg), green and yellow have in a decreasing order, but all the papaya leaves contain an appreciable amount of Magnesium.
3- Brown leaves, both collected from plain as well as hills contain maximum amount of Manganese (Mn), Green and yellow leaves have in decreasing order but all the leaves contain an appreciable amount of Manganese.
4- Green, yellow & brown leaves collected from plain as well as hills contain appreciable amount of Sodium (Na) but the leaves collected from hill region contain slightly higher amount of sodium than plain.
5- Brown leaves collected from plain & hill area contain maximum amount of Potassium (K) although green and yellow leaves of both regions also contain good quantity of Potassium.
6- Brown leaves collected from both the regions contain maximum amount of Iron (Fe) while green and yellow leaves collected from plain and hill area also contain an appreciable amount of Iron (Fe).
7- Brown leaves collected from both the regions contain maximum amount of Zinc (Zn) while green leaves collected from plain and hill area also contain an appreciable amount of Zinc and the yellow leaves collected from both regions contain slightly lower quantity of zinc but all the 06 samples contain an appreciable amount of Zinc (Zn).
8- Brown leaves collected from plain & hill area contain maximum amount of Copper (Cu) although green and yellow leaves of both regions also contain good and almost equal quantities of copper, all the 06 samples of papaya leaves contain a good amount of Copper (Cu).
9- Green leaves collected from both regions contain maximum amount of Chromium (Cr), yellow and brown leaves collected

from plane area contain slightly lower quantity while from hills, contain lowest quantity among all the 06 sample of papaya leaves. Although, all samples contain good quantity of Chromium (Cr).

6-CONCLUSION

The study on CP leaves done herewith has shown very rich minerals composition in green, yellow and brown leaves, which were collected from the plain as well as hill area of Northern India and CP leaves, can be seen as a potential source of useful drug, food & dietary supplement. The brown leaf can be used as anti-anaemic agent. It possesses immune-stimulants with anti-oxidant property. In the recent case study report, CP leaves extract controls dengue infection, has opens new gateway and raised eyebrows of many scientists. On the other hand, the presence of important anti-oxidants & vitamins may suppress various free radicals species induced during the viral infection. Particularly, the mineral composition may balance the mineral deficiency promoted by the virus and strengthen the immune cells against the dengue virus. The usefulness of these nine minerals obtained, like Ca, Mg, Mn, Na, K, Fe, Zn, Cu and Cr, which are found in rich quantity in CP leaves. The minerals are the elements that originate in the Earth and cannot be made by living organisms. Plants obtain minerals from the soil, and most of the minerals in our diets come directly from plants or indirectly from animal sources. Minerals may also be present in the water we drink, but this varies with environmental, climatic or geographic conditions. Minerals from the plant sources may also vary from place to place, because soil mineral content varies geographically. Hence, the leaves of CP were collected from northern India and in the months of July & August to find out the quantity and the kinds of minerals present in this region and whether these parameters differs from the reported results so far. Ca is responsible for coagulation of blood, the proper functioning of the heart, nervous system and normal contraction of the muscles. The normal range for total calcium in the blood is about 9 to 10.5 mg/dL and range for free calcium is about 4.5 to 5.6 mg/dL. Calcium is also found stored inside cells in special calcium – containing structures. Calcium should take up to 500 mg at one time, the higher doses cannot be readily absorbed in human body. The physiological functions of calcium are so vital to survival that the body will demineralise bone to maintain normal blood calcium level when calcium intake is inadequate. Thus, adequate dietary calcium is a critical factor in maintaining a healthy skeleton[12, 13]. Magnesium plays important roles in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, 6% to 7% is found in other cells, and less than 1% is found outside the cells[14]. Magnesium is involved in more than 300 essential metabolic reactions[15], some of which are instant energy production; the metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required by the adenosine tri-phosphate (ATP), a synthesising protein in mitochondria of the cell. Mg assists in the assimilation of phosphorous, the lack of the magnesium can be responsible for tetanous, tuberculosis, diabetes, cancer and all nerve related diseases. Calcium and magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types. Such effects on cell migration may be important in wound healing[16]. Manganese (Mn) plays an important role in a number of physiologic processes as a constituent of multiple enzymes and an activator of other enzymes. A number of manganese-activated enzymes play important roles in the metabolism of carbohydrates, amino acids and cholesterol[17]. Mn deficiency symptoms are ataxia, fainting, hearing loss, weak tendons and ligaments, can be a cause of diabetes. Medical studies indicate that manganese deficiency impairs glucose metabolism and reduced insulin production. The deficiency has been linked to myasthenia gravis. Manganese activates several enzyme systems and supports the utilization of vitamin C, E, choline, and other B-vitamins. Inadequate choline utilization reduces the acetylcholine synthesis, causing conditions such as myasthenia gravis (loss of muscle strength). Symptoms of increased manganese levels are; psychiatric illnesses, mental confusion, impaired memory, loss of appetite, mask-like facial expression and monotonous voice, spastic gait, neurological problems, impaired Thiamine (B1) metabolism, iron deficiency & increased demand for vitamin C and copper. It can also cause kidney failure, hallucinations, as well as diseases of the central nervous system. High hair manganese levels indicate problems with calcium and/or iron metabolism[18]. Na, i.e. Salt of sodium called as sodium chloride is essential for life. The tight regulation of the body's sodium and chloride concentrations is so important that multiple mechanisms work in concert to control them. Although scientists agree that a minimal amount of salt is required for survival, the health implications of excess salt intake represent an area of continued investigation among scientists, clinicians, and public health experts[19]. K (Potassium) is an essential dietary mineral and electrolyte. The term electrolyte refers to a substance that dissociates into ions (charged particles) in solution, making it capable of conducting electricity. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells [20]. Iron has the longest and best described history among all the micronutrients. It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes [21, 22]. Haeme is an iron-containing compound found in a number of biologically important molecules. Haemoglobin and myoglobin are haeme-containing proteins that are involved in the transport and storage of oxygen. Haemoglobin is the primary protein found

in red blood cells and represents about two thirds of the body's iron. The vital role of haemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen as needed during its circulation through the tissues. Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles [23, 24]. Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important role in growth and development, the immune response, neurological function, and reproduction. On the cellular level, the function of zinc can be divided into three categories: (1) catalytic, (2) structural, and (3) regulatory[25]. Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes[26]. Copper (Cu) is an essential trace element for humans and animals. In the body, copper shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms, though the majority of the body's copper is in the Cu^{2+} form. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions and in scavenging free radicals[27]. Although, Hippocrates is said to have prescribed copper compounds to treat diseases as early as 400 B.C.[28]. Scientists are still uncovering new information regarding the functions of copper in the human body. A biologically active form of chromium (Cr) participates in glucose metabolism by enhancing the effects of insulin. Insulin is secreted by specialized cells in the pancreas in response to increased blood glucose levels, such as after a meal. Insulin binds to insulin receptors on the surface of cells, which activates the receptors and stimulates glucose uptake by cells. Through its interaction with insulin receptors, insulin provides cells with glucose for energy and prevents blood glucose levels from becoming elevated. In addition to its effects on carbohydrate (glucose) metabolism, insulin also influences the metabolism of fat and protein. A decreased response to insulin or decreased insulin sensitivity may result in impaired glucose tolerance or type 2 diabetes, also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM). Type 2 diabetes is characterized by elevated blood glucose levels and insulin resistance [29]. The precise structure of the biologically active form of chromium is not known. Recent research suggests that a Low-Molecular-Weight Chromium, binding substance (LMWCr) may enhance the response of the insulin receptor to insulin. The following is a proposed model for the effect of chromium on insulin action.

Carica Papaya L. leaves can be formulated in herbal formulations for various regimens of diseases. The CP leaves having an appreciable amount of minerals/nutrients and vitamins which play a vital role in curing the Dengue Haemorrhoid Fever (DHF). More research/investigation is required in this regard.

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S.No	Name of Mineral Obtained	Green Leaves (collected from)		Yellow Leaves (collected from)		Brown Leaves (collected from)	
		Plain	Hills	Plain	Hills	Plain	Hills
1.	Calcium (Ca)	3386.00	3480.00	3012.00	3140.00	3260.00	3120.00
2.	Magnesium (Mg)	4390.00	4686.00	3392.00	3268.00	5928.00	5648.00
3.	Manganese (Mn)	18.60	19.82	14.60	14.08	22.88	21.80
4.	Sodium (Na)	1512.50	1747.50	1627.50	1912.60	1820.00	1895.00
5.	Potassium (K)	6762.50	8512.60	7015.00	8502.60	8645.60	8754.50
6.	Iron (Fe)	320.00	324.00	332.00	358.00	558.00	528.00
7.	Zinc (Zn)	21.40	22.20	14.82	15.68	33.40	32.00
8.	Copper (Cu)	0.68	0.70	0.68	0.68	2.16	1.68
9.	Chromium (Cr)	7.50	7.50	7.00	4.80	6.26	5.00

8-REFERENCES

1- Review on Indian Medicinal Plants: Vol.-5, Indian Council of Medical Research (ICMR), New Delhi, 46, CARICA Linn. (Caricaceae), page 500-519.

2- Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS, Idowu TO (2006) Afr. J. Biotech., 5(20): 1947-1949. Antisickling agent in an extract of unripe pawpaw (Carica papaya): Is it real?

3- M Indrann et al/West Indian med. j. vol. 57 no.4 Mona Sept., 2008. Protective effect of Carica papaya L leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats.

4- Ogunyemi CM, Elujoba AA, Durosinmi MA (2008). J. Nat. Prod., 1: 56-66. Antisickling properties of *Carica papaya* Linn.

5- N O A Imaga et al/ African Journal of Biochemistry Research Vol.3 (4), pp 102-106 April, 2009. Antisickling property of Carica papaya leaf extract.

6- Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kehinde MO, Bamiro SB (2009).. Afr. J. Biochem. Res. 3(4): 102-106. Antisickling Property of Carica papaya leaf extract.

7- Journal of Medicinal Plants Research Vol. 5(xx), pp. 1867-1872, 18 May, 2011, Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats S. Z. Halim1 *, N. R. Abdullah1,2, A. Afzan1, B. A. Abdul Rashid1, I. Jantan3 and Z. Ismail.1

8- J Ethnopharmacol.2010 Feb 17;127(3):760-7. doi: 10.1016/j.jep.2009.11.024. Epub 2009 Dec 2. Aqueous extract of Carica papaya leaves exhibits anti-tumor activity andimmune-modulatory effects. Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C. Division of Clinical Immunology, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

9- Rahmat A, Rosli R, Wan Nor IW, Endrini S, Sani HA. Antiproliferative activity of pure lycopene compared to both extracted lycopene and juices from watermelon (*Citrullus vulgaris*) and papaya (*Carica papaya*) on human breast and liver cancer cell lines. J Med Sci 2002; 2:55-8.

10- The effect of *Carica papaya* L. leaves extract capsules on platelets count andhematocrit in dengue fever patient FennyYUNITA[1], Endang HANANI[1], Jusuf KRISTIANTO[2]. ([1]Faculty of Pharmacy, University of Indonesia, Depok, 16424, Indonesia & [2]Faculty of Public Health, University of Indonesia, Depok, 16424, Indonesia.)

11-The six-month study, titled Thrombocyte counts in mice after the administration of papaya leaf suspension, published in October 2009, was funded by AIMST University and Universiti Sains Malaysia. Dr Kathiresan was assisted in the study by Dr Surash Rama-nathan, Dr Sharif M. Mansor and Dr Mas Rosemal M.H. Haris of Universiti Sains Malaysia, Penang, and Walther H. Wernsdorfer of Medical University of Vienna, Austria, Bernama.

12-Weaver CM, Heaney RP. Calcium. In: Shils M, Olson JA, Shike M, Ross AC, eds. Modern Nutrition in Health and Disease. 9th ed. Baltimore: Lippincott Williams & Wilkins; 1999:141-155.

13- Heaney RP. Calcium, dairy products and osteoporosis. J Am Coll Nutr. 2000;19(2 Suppl):83S-99S. Pub Med.

14- Shils ME. Magnesium. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential minerals. New York: Marcel Dekker, Inc; 1997:117-152.

15- Food and Nutrition Board, Institute of Medicine. Magnesium. Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington D.C.: National Academy Press; 1997:190-249. National Academy Press.

16- Rude RK, Shils ME. Magnesium. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins; 2006:223-247.

17- Spencer H, Norris C, Williams D. Inhibitory effects of zinc on magnesium balance and magnesium absorption in man. J Am Coll Nutr. 1994;13(5):479-484. Pub Med. 1-Keen CL, Zidenberg-Cherr S. Manganese. In: Ziegler EE, Filer LJ, eds.

18- Present Knowledge in Nutrition. 7th ed. Washington D.C.: ILSI Press; 1996:334-343.

19- Blaurock-Busch, E. Wichtige Nahrstoffe fur Gesunde Haut und Haare, Kosmetik Internat. 3/87.

20- Taubes G. The (political) science of salt. Science. 1998;281(5379):898-901, 903-897. Pub Med.

21- Peterson LN. Potassium in nutrition. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential minerals. New York: Marcel Dekker, Inc; 1997:153-183.

22- Wood RJ, Ronnenberg AG. Iron. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ,eds. Modern Nutrition in Health and Disease. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2006:248-270.

23- Beard JL, Dawson HD. Iron. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential minerals. New York: Marcel Dekker, Inc; 1997:275-334.

24- Yip R, Dallman PR. Iron. In: Ziegler EE, Filer LJ, eds. Present Knowledge in Nutrition. 7th ed. Washington D.C.: ILSI Press; 1996:277-292.

25- Brody T. Nutritional Biochemistry. 2nd ed. San Diego: Academic Press; 1999.

26 - Cousins RJ. Zinc. In: Bowman BA, Russell RM, eds. Present Knowledge in Nutrition. 9th ed, Vol. 1. Washington, D.C.: ILSI Press; 2006:445-457.

27- Food and Nutrition Board, Institute of Medicine. Zinc. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C.: National Academy Press; 2001:442-501. National Academy Press.

28-Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. Am J Clin Nutr. 1996;63(5):797S-811S. Pub Med.

29- Turnlund JR. Copper. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. Modern Nutrition in Health and Disease. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2006:286-299.