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PHYTOCHEMICALS MINERALS AND FREE RADICAL SCAVENGING ACTIVITY, FROM INDIGENOUS THERAPEUTIC HERBAL BONNAYA BRACHIATA LINK & OTTO

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ABSTRACT

Phytochemicals, mineral constituents and antioxidants from the crude extract *Bonnaya brachiata* Link & Otto an indigenous herbal has been explored to unveil trustworthy therapeutic chemicals. The bioactive organic compounds and minerals viz. antioxidant 36µg/ml, saponin, 30 mg/g, flavonoid 20.905 mg/g ,alkaloid 30 mg/g, phenol 17.86 mg/g. tannin 9.42 and minerals mg/g, Potassium 10.4 mg/g, calcium6.48 mg/g, magnesium 4.90 mg/g, iron 2.5 mg/g, sulfur 1.812mg/g, zinc 0.12mg/g, copper 0.06 mg/g, manganese 0.10mg/g, phosphorous 0.02 mg/g etc. reflect the an indispensible sources of precious natural resources of therapeutic chemicals and unique chemodiversity, the only means for survivability of species forever on

earth. The bioactive components not only add to therapeutic chemicals against dreadful diseases, but also intensify to supplements to the nutrients of food consequently contemplate the multifaceted practicabilities of therapeutic herbals for future human race.

KEYWORDS: Free radical scavenging activity, phytochemical constituents and minerals, indigenous herbal *Bonnaya brachiata*.

INTRODUCTION

Phytochemical have long been approved and blessed by many properties including antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic. ^[1] *Bonnaya brachiata* Link & Otto (Local name Keehom- Man, Manipuri) a wonder crude herbal drug plant, traditionally used in Manipur, India since immemorial time in herbal health care system against asthma, dysentery and as general tonic to stimulate muscles, movements and nerves of different parts of the body; to increase cognition, the intellectual functions, the ways of knowing and thinking, the process of perceiving imaging, remembering, reasoning and judging etc. The herbal plant is tasteless and all aerial parts posses a potent of general health tonic and stimulating effect in general. ^[2, 3] Other medicinal utilities includes diaphoretic in the form of decoction, leaves in treatment of spruce, juice in coagulation of blood and checking of bleeding.

In recent years, there has been a growing interest in use of fruit & vegetables under so-called functional foods because they can provide human physiological benefits additional to nutritional and energetic, as, for instance, antihypertensive, antioxidant or anti-inflammatory ^[4]. Between the different compounds with functional properties, antioxidants are the most widely studied. ^[5, 6] These compounds can play an important role in food technology because of their usefulness against lipid peroxidation. Usually, food production, process and storage can generate important losses of endogenous antioxidants that limit their own protection against lipid oxidation. Moreover, the important role of antioxidants in human health has been demonstrated, thus increasing the interest in such products and their demand by consumers. ^[7] Vegetal kingdom have become the main source of antioxidant compounds. Among them the main families are phenolic compounds, carotenoids and tocopherols,

MATERIAL AND METHODS

The test plant *Bonnaya brachiata* collected from Canchipur, Imphal East district Manipur on August - September 2010, were identified by Botanical Survey of India (BSI) Shillong and the voucher specimen was deposited at HRDRI Canchipur. The plant materials were cleaned, rinsed with deionized water and allowed to evaporate at room temperature and grind into uniform powdered in a grinder with utmost care so as not to contaminate with dust.

Mineral element determination

The major elements of calcium, phosphorous, potassium, magnesium and trace elements comprising Iron, Zinc, copper, manganese and sulphur were determined chemically from the plant materials following the methods of analysis described by the standard method. ^[8, 9, 10] The minerals and chemicals determined were patented by using different methods viz. potassium by the flame photometer method, phosphorous by the vanado – molybdate yellow method and sulphur by spectrophotometer; calcium, iron, zinc, magnesium, copper and manganese by atomic absorption.

Determination of free radial

Free radical scavenging of plant extracts was established by the use of DPPH radicals.^[11]

Determination of percentage of alkaloid and Saponin

The alkaloid and saponin percentage were determined following the method of Hamid,^[12] Obodani & Ochuko.^[13]

Estimation of total flavonoid content

Total flavonoid content was estimated following standard method of Chang.^[14]

Total phenol determination

Total phenol was determined following approved method of Malick.^[15]

Tannin determination

Tannin was determined following method of Sadasiyam, and Manickan.^[16]

RESULT AND DISCUSSION

The present investigation on phytochemical constituents of *Bonnaya brachiata* yields a number of organic compounds including antioxidant and allocate in different strengths viz. saponin 30 mg/g, flavonoid 20.9 mg/g, alkaloid 30 mg/g, phenol 17.86 mg/g, tannin 9.42 mg/g and antioxidant 36 µg/ml. The accorded data of phytochemical constituents of *Bonnaya brachiata* are shown in table 1. and graphically in fig. 2 and Fig. 3.

The test herbal was estimated with various minerals in different concentrations viz Potassium account 10.4 mg/g in top followed by calcium6.48 mg/g, magnesium 4.90 mg/g, iron 2.5 mg/g, sulfur 1.812mg/g, zinc 0.12mg/g, copper 0.06 mg/g, manganese 0.10mg/g, phosphorous 0.02 mg/g, and cobalt nil. The accorded data were tabulated in table 2 and graphically represented in Fig.3.

Analysis on Table-1 revealed that the fully matured plant of *Bonnaya brachiata* were full of resourceful resources of therapeutic organic compounds viz antioxidant, phenol, saponin, flavonoid, alkaloid and tannin that available wild in nature. The result of these bioactive compounds freely presence in the plant have empathetically emphasize the medicinal potentials of the test herbal.

Table 1: The phytochemical constituents of flavonoid, saponin, alkaloid, phenol, tannin in mg/g and antioxidant in µg/ml of the *Bonnaya brachiate*.

S. No	Plant species	Antioxidant IC ₅₀ (µg/ml)	Flavonoid	Phenol	Alkaloid	Saponin	Tannin
L	Bonnaya brachiata	36	20.905	17.86	30	30	9.427

The values are mean of three observation.

Table 2: Composition of Mineral elements of Bonnaya brachiata.

Plant species	K	Ca	Mg	Р	S	Fe	Zn	Cu	Mn	Со
Bonnaya brachiata	10.4	6.48	4.90	0.02	1.12	2.5	0.65	0.06	0.25	ND*

The values are mean of three observations.

 $ND^* = Not detected$



Fig 1: Phytochemical composition of Bonnaya brachiata.







Fig 3: Mineral composition of Bonnaya brachiata.

Further table1depict the estimated antioxidants^[11] upto 36 ug/ml, under free radical scavenging activity technique and affirmed the unique presence of antioxidants in the plant extract and vividly patented in the present test herbal Bonnaya brachiata. Antioxidants being compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite., its imbalance with reactive oxygen species results in oxidative stress leading to cellular damage by oxidizing nucleic acids, protein, lipids or DNA. Oxidative stress normally linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). In cohort studies under epidemiological investigation have been claimed that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks. Antioxidant and its ability to balance or trap highly reactive free radicals and oxygen are very essential in biological systems from a wide variety of sources for longer life of cells. Further antioxidant compounds like phenolic acid, polyphenols and flavonoid scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism which leads to degenerative disease. The present finding concord to earlier report of antioxidants from various therapeutic plants by numerous workers. ^[17,18,19,20] Further there are number of clinical studies suggesting that the antioxidant in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers.^[21] The phenolic compounds content in the leaves of *Bonnava brachiata* ranged upto 17.86 mg/g make obvious the higher status among the 5 phytochemical compounds detected from the plant (Table 1, Fig. 1.) Recent studies showed that phenolic

phytochemicals from botanical sources are natural inhibitors of α -amylase and β -glucosidase 3-6 with a strong inhibitory effect on β -glucosidase, but a mild inhibitory effect on α amylase and thus can be used as an effective measure to prevent postprandial hyperglycemia with minimal side effects.^[22] Eventually phenolic antioxidant-mediated inhibition of these enzymes can significantly decrease the postprandial hyperglycemia after ingestion of a mixed carbohydrate diet and could be an effective strategy in the control of type 2 diabetes.^[23] Phenol is also used as an antiseptic, a general disinfectant, and a slimicide (chemicals that kill bacteria and fungi in slimes), in medical preparations including lotions, ointments, mouthwashes, salves. Phenol is also the active ingredient in some over-the-counter oral anesthetics sprays used as a treatment for sore throats. ^[24, 25] Phenol is well absorbed from the gastrointestinal tract and through the skin. It is metabolized principally by conjugation (by sulfation and glucuronidation) with a minor oxidation pathway leading to quinone-related reactive intermediates which bind covalently to protein and are detoxified by conjugation with glutathione. Most of the absorbed phenol and its metabolites are excreted in the urine, with trace amounts of excreted in expired air and the feces. Phenol may be eliminated in the feces and some may pass to the blood. Phenol have been reported from different herbals.^[26]

The present investigation accounts flavonoid upto 20.905 mg/g in comparison with other phytochemical compounds (Table 1). Graphical representation demonstrate the unique feature of the compound (Fig. 1). The flavonoids have aroused considerable interest recently because of their potential of beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities. Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic. ^[27] anti-inflammatory, antioxidant^[28] anti-microbial antibacterial, antifungal. ^[29] and antiviral anti-cancer ^[30] and to induce DNA mutations in the mixed-lineage leukemia (*MLL*) gene in *in vitro* studies. ^[31] Flavonoid with antioxidant activity in *in vivo* studies with rats, protecting their gastrointestinal mucosa against the reactive oxygen species generated by acute and chronic stress. ^[32] The flavonoid content in asserted medical herbals was in agreement with the result of other workers. [^{13, 17]}

Perusal on the present phytochemical test, the presence of saponin accounts with 30 mg/g (Table1, Fig.1). Saponins bind with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponins cause a reduction of

blood cholesterol by preventing its re-absorption. saponins have antitumor and antimutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Saponins may help to prevent colon cancer. ^[33] The finding was in accordance with the result of other plants. ^[34, 35, 14]

The present finding revealed that the alkaloid content ranged to 30 mg/g among the phytoorganic compounds. Graphical representations illustrate the status of the compound among the phytochemicals (table 1 & fig. 1). The plant Parts which generally uses in the treatment of various diseases may be due to presence of traces of alkaloids. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents or analgesic, anti-spasmodic and bactericidal effects. ^[36]

The present test estimated the tannin content upto 9.42 mg/g (Table 1) of the test herbal (Fig.1). Plant tannins, one of the major groups of antioxidant polyphenols found in food and beverages, have attracted a lot of attention in recent years because of their multifunctional properties beneficial to human health including antioxidative, cardioprotective, antitumor, antibacterial, antiviral, anti-inflammatory and immune-modulatory effects ^{[37].} The finding was in conformity with the result of other workers. ^[26, 38]

Table 2. illustrate the *Bonnaya brachiata* with remarkable amounts viz potassium 10.4 mg/g, calcium 6.48 mg/g, magnesium 4.9 mg/g, phosphorous 0.02 mg/g, iron 2.5 mg/g, zinc 0.65 mg/g, copper 0.06 mg/g, sulfur 1.12 mg/g and manganese 0.25 mg/g, cobalt nil and reflect the precious resource full of essential minerals which readily enhance vital metabolic activities of human body. The values of all these minerals are graphically represented in figure.3 and vividly displayed the array of minerals. The finding was in agreement with the results of earlier workers in different medicinal plants from different countries. ^[18,19,20,38]

Potassium, the most important cation in human's body also called mineral of the heart regulates blood pressure, maintains normal fluid balance and impart significant for nervous system and heart muscle work. It inhibits the negative effects of free radicals in cells; potassium reduces thrombus formation in arteries[39] Consumption of more potassium decreases level of risk hypertension related diseases- stroke, heart attack and kidney failure. Potassium being an electrolyte, it enact as an essential (major) mineral and regulates membrane potential using positively charged ions and interacts with sodium (positively

charged) and chloride (negatively charged) for membrane potential. Low potassium results in low levels of stored glycogen or muscle fuel. Potassium deficiency results in fatigue and muscle weakness. ^[40] Eventhough, calcium, the most common and abundant mineral in the body primarily found in the bones and teeth, is found in the blood and soft tissues as a small but absolutely essential amount. As a result of homeostasis, the body will take calcium from the bones if there is not enough circulating in the blood and extracellular fluid. The skeleton is constantly being absorbed and remodeled. Calcium helps vitamin K function in blood clotting, functions in blood pressure regulation, and may be useful in lowering moderate hypertension, functions in many enzyme reactions inside and outside cells and is a cofactor for enzymes and proteins, functions in nerve impulse conduction, in neurotransmitter release, in hormone secretion, and in heart, smooth and skeletal muscle cell contraction. ^[41]

Magnesium, an important for muscular (especially when one's got crumps) and nervous system activity, also for the bone structure regulates the metabolism of other minerals; it also controls the uptake of such substances as: calcium, potassium, phosphorus, copper, vitamin C and zinc. Magnesium affects blood sugar regulation by stimulating the production of insulin. In the absence of magnesium, there is a higher possibility of micro clots forming in the vessels, faster progress in atherosclerosis. Furthermore, magnesium deficiency might result in increased nervous irritability, nervousness, headaches, sleep disturbances, general weakness, drowsiness, physical and emotional exhaustion, quick tiredness.^[42] Magnesium is essential for energy production, protein formation and cellular replication (e.g., DNA, RNA). Magnesium works with calcium in muscle contraction and relaxation (calcium-contraction, magnesium-relaxation).^[43]

Iron, an essential for metabolism, DNA synthesis, growth, healing, immune function, reproduction, as a cofactor in many enzyme reactions, preventing anemia, is found in hemoglobin and myoglobin, proteins which transport oxygen through the blood and muscles (important for athletic performance). Hemoglobin acquires and transports oxygen from the lungs and releases it as blood courses through the tissues.^[44]

Sulfur, the most abundant element (approximately one half percent by weight) in our body after calcium and phosphorus and the fourth most abundant anion in our plasma, helps to maintain the balance of anions (bicarbonate, chloride, and phosphate) to effectively carry oxygen to the cells. Zinc functions in cell/energy metabolism for growth and development, in cell signaling systems, in the immune system, in neurological development, and in reproduction. It is particularly important in enzyme function (over 200) and hormone function, and in vision, taste, smell, and in wound-healing. Its highest concentration is in muscles (65%), in red and white blood cells, bone, skin, liver, kidneys, pancreas, eye retina, in the male prostate gland and sperm; it helps in making cell membranes strong. ^[45] The present finding revealed the phytochemicals, minerals, antioxidants in the test herbal with accountable ranges thus draws attention to renovation of indigenous herbal which was use since immemorial time. In this regard it is stated that a large number of antioxidant proprietary preparations containing widely variable amounts of β - carotene, Vit. A acetate, Vit.E, Vit. C, selenium, zinc, copper, manganese are briskly promoted and consumed but with no credible evidence of benefit and may be some potential harm ^[46] and advised to adopt a healthy lifestyle viz. eating sufficient fruits and vegetables, doing regular exercise, avoiding overweight and smoking rather than consuming antioxidant proprietary. ^[47]

CONCLUSION

The present finding confirmed the therapeutic properties of the herbal test plant through scientifically accounts of potent phytochemicals and phytonutrients, eventually highlights the needs of hitherto hi- tech analysis for more information to medicinal chemistry and standardization of traditional health care system under the aegis of potentianization of potent compounds. The finding also focus attention on the probability of interactive activities of the precious life saving therapeutic chemical compounds if it act as synergistic towards the potentianized action of healing capabilities of human being through organ system and cells, it would renovate the traditional knowledge of health care system.

REFERENCES

- 1. Katzung B G, Basic and Clinical pharmacology, 2007; 10th Edn. Mc. Graw Hill Boston.
- Sinha S C, Notes on ethnomedicinal plants of manipur. (New Report) Curr. Pam. Letters 1990; 1: 2-3.
- 3. Sinha S C, 1996. Medicinal plants of Manipur Mass and Sinha Imphal.
- 4. Goldberg I, Functional food, Designer food, nutraceutical, Champan and Hall, London, United Kingdom, 1996.
- 5. Herrero A, Cifuentes, E. Ibanez, Food Chem, 2006; 98:136.
- 6. Fogliano V, Vitaglione P, Molecular Nutrition and Food Research, 2005; 49: 256.
- 7. Plaza M, Cifuentes A, Ibañez E, Trends Food Sci. Technol, 2008; 19: 31.

- 8. Capar S G, Tanner J T, Friedman M H and Boyer K W. Multi-element analysis of animal waste and sewage. *Studge Environ. Sc. Technol*, 1978; 12: 785.
- Gupta P K, Soil Plant, Water and Fertilizer analysis. Agrobios (India) Agro House, Behind Nasrani Cinema Chopasani Road, Jodhpur, 2006; 438.
- Tandon H L S, Method of analysis of soils, plants, waters and Fertilizers. Fertilizer development and consultation organization 204-402A Bhanot corner 1-2 Pamposh Enclave 1993; 143.
- 11. Dudonne S, Vitrax X, Coutricre P, Woilez M and Merillon J. Comparative study of antioxidant properties and total phenolic content at 30 plants extract at industrial Interest using DPPH, ABTs, FRAP, DOS and ORAC Assay J. Agrc. Chem. 2009; 57:1768-1774.
- 12. Hamid R M, Ali G and Mehrdad I. Antinociceptive effects of Peganum harmala, L. alkaloid extract of mouse formalin test. *Canadian society of pharmaceutical science*, 2004.
- Obadoni B O and Ochuko P O. Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Global J. Pure and Applied Science*. 2001; 8: 203-208.
- 14. Chang C, Yang M, Wen H and Cherr J. Estimation of total flavonoid content in protein by two complementary colorimetric methods. *J. Food Drug Analy*, 2002; 10: 178-182.
- 15. Malick C P and Singh M B. in: *Plant Enzymology and Histoenzymology*, Kalyani publication, New Delhi 1980; 286.
- Sadasiyam, S. and Manickan, A. In: *Biochemical method for Agricultural science*, Wiley Eastern Ltd. New Delhi, 1992; 189-191.
- Okwu D E. phytochemical and vitamin content of indigeneous species of south eastern Nigeria J. Sustain Agric. Environ, 2004; 6: 30-34.
- Singh S R and Devi M. N. Antioxidant, phytochemicals and minerals contents of therapeutic plant *Croton caudatus* (Geiseler) for human kind. Indian J. Environ. & Ecoplan, 2010; 17(1-2): 219-226.
- Devi M N, Singh K B, Singh, S R, Singh C B, Lukesh D, Amitabh D. Antihyperglycemic effect of Aqueous and Ethanol extract of Aerial part of *Osbeckia nepalensis* Hook in Alloxan induced Diabetic rats . International Journal of PharmTech Research, 2012 ;4(1): 233-244,
- 20. Devi M N, Singh S R and Salam J S, Antioxidant, mineral and phytochemical composition of *clerodendrum colebrookianum* walp, a well known home remedy herbal for humankind. World Journal of Pharmaceutical Research, 2014; 3(2): 2667-2676.

- Miller H E, Rigelhof F, Marquart L, Prakash A and Kanter M *Cereal foods world*, 2000; 45(2): 59-63.
- Bennick A, International of plant polyphenols with salivery protein, Crit. Rev. Oral Biol, Med, 2002; 13: 184-196.
- 23. Arts I CW, Vande Putte B and Hollman P C H. Catechin contents of foods commonly consumed in the Netherlands. J. Agric Food Chem, 2000; 48: 1746-51.
- Wallace J, "Phenol" in Kirk-Orthmer encyclopedia of chemical technology 4th Edn. John Wiley and Sons, 1996; 18: 592-602.
- 25. Jordan W, "Phenol' In: Ullmann's encyclopedia of industrial chemistry, 5th Ed. VCH Verlagsgesellschaft, A, 1991; 19: 299-312.
- Igboko D O. Phytochemical studies on *Garcinia kola* Heckel M. Sc. Thesis University of Nigeria Nsukka Nigeria, 1983; 202.
- 27. Yamamoto, Yumi; Gaynor, Richard B. "Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer". *Journal of Clinical Investigation*, 2001; 107(2): 135–42.
- 28. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, Pizzolatti MG, Silva FR "Flavonoids: Prospective Drug Candidates". *Mini-Reviews in Medicinal Chemistry* 2008; 8 (13): 1429–1440.
- 29. Cushnie TPT, Lamb AJ "Antimicrobial activity of flavonoids". *International Journal of Antimicrobial Agents* 2005; 26(5): 343–356.
- 30. Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, Peppelenbosch MP, Ferreira CV, Aoyama H. "Phosphoprotein levels, MAPK activities and NFkappaB expression are affected by fisetin". *J Enzyme Inhib Med Chem*, 2007; 22 (4): 439–444.
- 31. Barjesteh van Waalwijk van Doorn-Khosrovani S, Janssen J, Maas LM, Godschalk RW, Nijhuis JG, van Schooten FJ "Dietary flavonoids induce MLL translocations in primary human CD34+ cells". *Carcinogenesis*, 2007; 28 (8): 1703–9.
- 32. Bagchi, Manashi; Milnes, Mark; Williams, Casey; Balmoori, Jaya; Ye, Xumei; Stohs, Sidney; Bagchi, Debasis "Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract". *Nutrition Research*, 1999; 19 (8): 1189–1199.
- Francis G, Zohar K, Harinder P S, Makkar and Klaus, B. "The biological action of saponins in animal systems: a review". *British Journal of Nutrition*, 2002; 88(6): 587– 605.

- 34. Mizui F, Kasai R, Ohtani K and Tanaka O. Saponin from brans of quinoa, Chenopodium quinoa Willd, I. *Chem. Pharm. Bul*, 1988; 36: 1415-1418.
- 35. Sodipo O A and Akiniyi J A. Studies on certain characteristics of extracts from bark of Pansinystalia macruceras. *Pierre Exbeille Global J. Pure and Applied Sc*, 2000; 6: 83-87.
- 36. Stray F. The national guide to medicinal herbs and plant. Tiger books International London, 1998; 12-16.
- 37. Liu Y Z, Cao Y G, Wang W G Song, K.J.; Wang, C.H. and Deng, X.M. Immunomodulatory effects of proanthocyanidin A-1 derived in vitro from Rhododendron spiciferum, Fitoterapia, 2009; 81: 108-114.
- 38. Devi M N, Singh S R and Salam J S. Antioxidant, mineral and phytochemical composition of *clerodendrum colebrookianum* walp, a well known home remedy herbal for humankind. World Journal of Pharmaceutical Research, 2014; 3(2): 2667-2676.
- 39. Joseph E, Zerwekh A, Clarita V, Odvinna L W, Charles A and Paka Y C. Centre for mineral metabolism and clinical research, University of Texas Southwestern medical centre at Dallas, 2012.
- 40. Christian JL, Greger JL. *Nutrition for Living (4th Ed.)* Reading, MA: The Benjamin/Cummings Publishing Company, Inc, 1994.
- 41. McArdle W D, Katch F I, Katch V L. *Sports and Exercise Nutrition (2nd Ed.)* Philadelphia: Lippincott Williams and Wilkins. 2005.
- 42. San Antonio. Journal of Clinical Pharmacology, Nov 1998; 38: 1035 1041.
- 43. Natow AB, Heslin J-A. *The Vitamin and Mineral Food Counter*. New York: Pocketbooks, 2004.
- 44. USDA Nutrient Database for National Reference. Available at: http://www.nal.usda.gov/fnic/foodcomp/search. Accessed April 13, 2006.
- 45. USDA Food and Nutrition Products and Services. Available at: http://www.ars.usda.gov/Services/docs.htm?docid=7783. Accessed April 17, 2006.
- 46. Brunton L L, Lazo J S, Parker K L. (Goodman and Gilmaris The pharmacological Basis of Therapeutics 2006); 11th edn. Mc. Graw Hill. New York.
- 47. Tripathi K D. Essentials of medicinal pharmacology, 6th Edition Joyree Brothers mrdical Publishers. New Delhi, 2006; 940.