

Investigations on the nutritional and medicinal potentials of an under exploited food plant *Alocacia indica*

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Abstract:

The present study investigated the nutritional and bioactive components of *Alocacia indica* stem of the popularly consumed vegetable in some part of Iran. The proximate analysis and bioactive constituents were determined by standard methods of AOAC, while vitamin E, micro/macro elements and fatty acids were determined using AAS and HPLC respectively. The results showed that the stem contained $5.7 \pm 1.60\%$ protein, $72.66 \pm 2.10\%$ carbohydrate, $3.29 \pm 0.60\%$ fat, $7.3 \pm 0.46\%$ ash, $11.05 \pm 0.05\%$ crude fiber, and vitamin E not detected. The bioactive compound found were 0.87 ± 3.09 mg/g phenolics, saponnins and steroids were detected. All these results indicate that the stem of this *Alocacia indica* contained nutrients and mineral elements that may be useful in nutrition, while the bioactive compounds explained the medicinal action of the plant stem encountered in its therapeutic uses.

Keywords: *Alocacia indica*, nutritional, medicinal potentials and proximate

INTRODUCTION

Alocacia indica is a plant that is normally found in the wild, it belongs to the family of Liliaceae. It is a perennial growing to 1.5m by 0.75m. It is hardy to zone and is not bearing frost tender. It bears flower in August, and the seeds ripen from September to October. The flowers are dioecious (individual flowers are either male or female, but only one sex is to be found on any one plant so both male and female plants must be grown if seed is required) and are pollinated by Bees. The plant is not self-fertile. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils and can grow in *Alocacia indica* is a dioecious perennial herb with scale-like leaves and an erect, much-branched stem that grows to a height of up to 3 meters. Asparagus is native to Europe and Asia and is cultivated widely. The part used as a vegetable consists of the aerial stems, or spears, arising from rhizomes. The fleshy roots and, to a lesser degree, the seeds have been used for medicinal purposes. *Asparagus* spears are used widely as a vegetable and frequently are blanched before use. Extracts of the seeds and roots have been used in alcoholic beverages, with the maximum levels averaging 16 ppm. The seeds have been used in coffee substitutes, diuretic preparations, laxatives, remedies

for neuritis and rheumatism, to relieve toothache, to stimulate hair growth, and as cancer treatments. Chinese medicine has used them to treat parasitic diseases. Extracts are said to have served as contraceptives. Home remedies have employed the topical application of preparations containing the shoots and extracts to cleanse the face and dry acneform lesions. very acid, very alkaline and saline soils. It can grow in semi-shade (light woodland) or no shade. It requires moist soil. The plant can tolerate maritime exposure. *Alocacia indica* is packed with nutrients and low in calories, sodium and cholesterol. It is an excellent source of folic acid and is a source of vitamin C, thiamin, and vitamin B6. *Alocacia indica* contains no fat or cholesterol of dietary significance. It is an important source of potassium and many micronutrients. According to the National Cancer Institute, asparagus is the highest tested food containing glutathione, one of the body's most potent cancer fighters also quoted as most potent anti carcinogens and antioxidants. Additionally, asparagus is high in rutin, which strengthens blood vessels [1-5]. Aim of this study was screening of nutrients and anti-nutrients for assessment of nutritional and medicinal potentials of *Alocacia indica* stem.

MATERIALS AND METHODS

Collection of plant sample and identification

The *Alocacia indica* stem were collected from two years ago at the farm located in the Dezful Research center, Iran. The stem was identified and authenticated by Dr. Deokule, a taxonomist in the Department of Botany, Pune University, India.

Sample preparation

Collected plant stems were cleaned and spread on the already cleaned laboratory bench for two weeks in the Food Science Department, Isfahan University, Iran. The dried stems

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were pounded into powder with laboratory mortar and pestle. A portion (50 g) of the powdered sample was processed for various parameters according to the following procedures:

Proximate analysis

The proximate analysis (carbohydrates, fats, protein, and ash) of the plant stems were determined by the method described by AOAC, 1990. Carbohydrate was determined by subtraction difference methods ($100 - (\text{crude protein} + \text{crude fats} + \text{crude fiber} + \text{moisture} + \text{ash})$). The total crude protein was determined by micro Kjeldahl method. The nitrogen value was converted to protein by multiplying a factor of 6.25. The “moisture and ash” were determined using weight difference method while determination of crude lipid content was done using Soxhlet type of the direct solvent extract method. The solvent used was petroleum ether (40 to 60°C) analar grade [6].

Elemental analysis

The sample was investigated for element composition by using atomic absorption spectrophotometer (AAS), bulk scientific model AVG 210. Appropriate working standard solution was prepared for each element. The calibration curves were obtained for concentration versus absorbance. The data were statistically analyzed by using fitting of straight line by least square method. All elements were determined in the medicinal plant (*Alocasia indica*,) under this investigation procedure. Laboratory procedures for the preparation and determination of macro and micro nutrients were used as outlined by Shah et al. [35] for plant samples.

Vitamin E and fatty acid profile determination

Higher performance liquid chromatography was used to determined fatty acid profile, vitamin (V) E content.

Preparation of sample for HPLC

A portion (0.50 g) of sample was weighted into 10 ml capped bottle and 10 ml of N-hexane (BDH, HPLC grade) was added and shaken to dissolve and left to stay overnight. The content was then centrifuged and the supernatant (hexane layer containing the extracted oil) removed with pipette and kept for (V) E and fatty acids analysis by HPLC.

For vitamin E

A portion (2 ml) of the aforementioned extract was

measured into test tube and hexane was evaporated through nitrogen gas. A portion (2 ml) of methanol (HPLC grade) was added to dissolve the vitamin E (fat soluble). A portion (20 μ l) of it was injected into HPLC (AKPA HPLC) with column ODS 2 C18, detector: UV 290 nm and F/R = 10 μ l/min.

For fatty acid

A portion (2 ml) of the aforementioned extract measured into a test tube and 0.3 ml of 1 M Na-methoxide, it was mixed thoroughly and left overnight and centrifuge. The clear solution decanted and evaporated to dryness. A portion (2.0 ml) of “acetonitrile” (BDH and HPLC grade) was added and shakes to dissolve the precipitate. Then 20 μ l was injected into the HPLC with column ODS 2 (C18), detector UV 215 nm and F/R = 1 μ l/min [7]. The “fatty acids”, vitamin E was calculated with reference to the standard using this formula:

$$\text{Conc. of sample} = \frac{\text{Peak area (in AU min) of sample}}{\text{Peak area (in AU min) of standard}} \times \text{Conc. of standard}$$

Phytochemical analysis

Total phenolics were determined by method described by Mole and Waterman (1987), saponins by the spectrophotometric method of Brunner as described by Akinmutimi (2006), steroids by gravimetric method of Harbone as described by Onwuka (2005), and Phytate by Lucus and Markakas method as described by Akinmutimi [7].

Statistical analysis

All data were expressed as mean \pm SD and GraphPad Instat (Data set 1.SD) were used.

RESULTS

The proximate composition and vitamin of young stem of *Alocasia indica* are summarized in Table 1. While Table 2 shows micro and macro mineral elements. The results show the concentration of element in the highest order in the mg/100 g of plant stem of 3.4, 0.88, 4.4, 0.48, and 1.21, in K, Ca, Na, Fe, and Zn respectively. The Table 3 shows the result of bioactive component of the *Alocasia indica*. The results showed that the young stem of *Alocasia indica* has 3.17 ± 3.09 mg phenolics and Saponins and steroids were detected. The average contents of the fatty acids in *Alocasia indica* stem are presented in Table 4.

Table 1. Proximate composition of *Alocasia indica*

Protein(%)	carbohydrate(%)	Fat (%)	Ash(%)	crude fiber(%)	Vitamin E content
(mg/100g)					
5.7 \pm 1.60	72.66 \pm 2.10	3.29 \pm 0.60	7.3 \pm 0.46	11.05 \pm 0.05	ND

Values are expressed as mean \pm S.D (n = 3). ND means not detected.

Table 2. Macro and Micro Element composition of *Alocacia indica* in mg/100g

Fe	Zn	Ca	K	Na
0.48	1.21	0.88	3.4	4.4

Values are expressed as mean \pm S.D (n = 3).

Table 3. Bioactive constituents of *Alocacia indica*

Phen(mg/g)	Phy mg/100g	TUI (unit/mg)	Sap	Ste
0.87 \pm 3.09	312.4 \pm 0.02	7.9 \pm 0.50	++	ND

n = 3 \pm SD. Phen = Total Phenolic, Sap= Saponnin, Phy=phytate, TUI= Trypsin inhibitor, Ste= Sterpoids. ++ means high level, ND means not detected.

Table 4. Fatty acids composition of *Alocacia indica*

Myristic acid (%)	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Stearic acid (%)
ND	9.24 \pm 0.17	68.16 \pm 0.06	ND	17.97

Values are expressed as mean \pm S.D (n = 3), ND = not detected

DISCUSSION

Nutritional composition

Green vegetable are generally acceptable as good source of nutrients and supplement for staple food in a World faced with problem of food scarcity. They have known to be excellent source of nutrients such as mineral and vitamins. The moisture content of *Alocacia indica* was very high although within the range of moisture content for fruits and vegetables of 60 to 83 g/100 g [15]. However the high moisture content may underscore its high perishability and susceptibility to microbial infection [8]. The high moisture content makes to aid the digestion of food. The values of carbohydrate >crude fiber> ash > protein > crude fat obtained were higher in this order. Fiber is useful for maintaining bulk, motility and increasing intestinal peristalsis by surface extension of the food in the intestinal tract [9]. It is necessary for health condition curing nutrition disordered and for food digestion. The young stem of *Alocacia indica* is extremely high in fiber content as revealed by this result. This was indicative of its high soluble fiber (pectin) [10]. Soluble dietary fibers have health-promoting properties as they have been implicated in lowering plasma and liver cholesterol concentration [11] diarrhea treatment and detoxification of poisonous metals [12]. The high value of carbohydrate and protein suggest its nutritional quality of the *Alocacia indica* stem and this may be a veritable tool been used by the villagers for source of body nourishment. The moderate high value of the ash content is an indicative of high mineral value especially the macro minerals in the young stem of *Alocacia indica*. The value obtained was close to that of some leaf vegetables commonly consumed in Iran such as *Talinum triangulare* (20.50%) however higher than *Occimum gratiukum* (8%), *Hibiscus esculentus* (8.00%) [13]. The crude fat value is moderate as compared to those of *T. triangulare* (5.90%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%) [14]. Dietary fats function in increase of palatability of food by absorbing and retaining

flavours [15]. A diet providing 1 to 2% its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain, cardiovascular disorder such as atherosclerosis, cancer and aging [16]. The results of selected vitamin E composition of the young stem of *Alocacia indica* are shown in Table 1. The reasonable values obtained for vitamin E suggest that the plant may be of help in solving or reducing the prevailing micronutrient deficiency diseases ramphaging poor shrinking community especially Sub- Sahara Africa such as blindness, cancer, heart diseases etc. Tocopherol (vitamin E), and carotenoids (pre-cursor of vitamin A) are anti-oxidants which have been associated with prevention of nutritional related diseases such as cancer, diabetes mellitus, coronary heart diseases and obesity. [17-19], hence this young leaf of *Alocacia indica* have these health promoting potentials.

Macro and micro elements

Nutritional experts and medical doctors now recognize and are emphasizing the important roles of mineral and trace elements to human health and well being [20]. It is estimated that 70 biological trace elements are needed by all living things for the normal function of their metabolism, reproductive and immune system [21]. The selected macro/micro elements found in *Alocacia indica* are shown in Table 2. These results showed that *Alocacia indica* stem is rich in essential minerals and trace elements that promote well being in humans. Iron and “copper” for example are essential in blood formation and copper is also involve in normal carbohydrate and lipid metabolism, and zinc for its part is a multifunctional nutrient involved in glucose and lipid metabolism, hormone function and wound healing [22] and is also associated with proper hair growth [23]. Sodium and potassium are important for chemical reaction within the cells and regulates the transfer of nutrients to the cells. Sodium works in conjunction with potassium for extracellular fluid balances [24]. As can be seen in the results, daily consumption of this leaf can add values to recommended

dietary allowance (RDA) of mineral element thereby improving health and well being.

Bioactive compounds

The present research has provided first hand information on bioactive and anti-nutrient constituent studies on young stem of *Alocacia indica*. The bioactive component studied revealed that *Alocacia indica* has substantial amount of phenolics compound, alkanoid, sponnin, phytate, and trypsin inhibitor. These bioactive compounds (phenolics and saponnins) are known to exhibit medicinal activity as well as physiological activity [25]. Various studies have shown that saponnin although non toxic can generate adverse physiological responses in animals that consume them, they exhibit cytotoxic effect and growth inhibition against a variety of cell making them have anti-inflammatory and anticancer properties [26].

They also show tumor inhibiting activity on animals [27]. The presence of saponnins from various studies indicate their importance and interest in pharmacy due to their relationship with such compounds such as sex hormones especially in development of the female contraceptive pill [28]. This may be the reason why the infusion of the stem of *Alocacia indica* is given to expectant mothers in Guinea to ensure hormonal balance. Phenolics compounds are class of antioxidant agents which act as free radical terminators [29]. Polyphenols constitute the main bioactive phytochemicals that have been proven to be effective in the prevention of certain chronic diseases such as coronary heart diseases, cancers and diabetes [30].

The result of those bioactive constituent in the *Alocacia indica* further suggest the reason for usage of the plant to cure many diseases such as colic in man, dressing on sores for maturate tumours, whitlow, inflammatory, cancer, mental illness, fatigue, lumbago, gonorrhoea, dysentery, anti-microbial and anti-fungal effect [31]. The anti-nutrient content (trypsin inhibitors, phytate) as shown in Table 1 shows that the plant has low content value of various antinutrients determined. This implies that the plant stem is very safe and good for human health.

Fatty acids composition

As shown in Table 4, the most abundant fatty acid found was Oleic acid, palmitic Acid (c) and stearic acid. Other (myrusic acid and linoleic acid (C18.2). were not detected in the sample. However, the values obtained were close or higher compared to other vegetables only reported for example spinach 16.3% (C18), lettuce 20.2% (C18.2), broccoli 16.9% (C18.2), 25.0% (C16.0) [32]. For the fact that the sample contain substantial amount of Oleic acid, thus indicate good nutritional qualities, because the Oleic acid is part of the essential fatty acid and it is known to be present in many plants [33], along with C16:3n-3 in some plant species [34] linolenic acid can also act as a precursor acid of long chain PUFAs. Although linolenic acid had has been shown to increase the synthesis of long chain PUFAs [35]. According to Indu and Ghafoorunissa [16], high long term intake of linolenic acid provide only modest benefit compared to fish oils. It has also been shown that supplementation of high doses of linolenic acid in form of linseed oil, produced

antiggregatory effects [36]. A high doses of linolenic and have been linked to a possible increased risk for prostate cancer or muscular degeneration. Epidemiologic report indicate that both polyunsaturated fatty acids (PUFA) and vegetables can protect from cardiovascular diseases (CVD), but concern has arisen that the unbalance between dietary PUFA and bioactive vegetable compounds may lead to oxidative stress [37] linolenic acid is one of the omega 3 fatty acids, omega-3 fatty acid reported to reduce inflammation. The positional distribution of fatty acids in dietary triglycerides, as well as the fatty acid composition, is important factor of fat digestion and absorption [38]. Triglycerides in human breast milk contain appropriately 20 to 25% palmitic acid (C16.0) with over 70% of the (C16.0) esterifies to the Sn-2 positioning the milk triglyceride [39]. The specific positioning of (C16.0) at the 2-position of human breast milk triglycerides has been suggested a one of the reasons for high efficiency of fat absorption from human milk and calcium. Therefore, it has been discussed to humanize the fat source stereospecifically by fortifying with Sn-2 palmitic acid in the infant formula to increase absorption rate of lipids and calcium [40-41].

CONCLUSION

In the light of this investigation, *Alocacia indica* has been found to contain some nutrients and phytochemicals and this supports its ethno medicinal uses and therefore a good source of nutrients and medicine that require special attention for development.

REFERENCES

- [1] Ali M, & Tsou S, 2000. The integrated research approach of the Asian Vegetable Research and Development Center (AVRDC) to enhance micronutrient bioavailability. *Food and Nutrition Bulletin* 21: 472-481.
- [2] Honnavally P R, & Rudrapatnam NT. 2004. Carbohydrates, The Renewable Raw Materials of High Biotechnological Value, *Critical in review in Biotechnology*, 23: 149-173.
- [3] Joshipura H. 2001. The effect of fruits and vegetables intake on risk for coronary heart disease. *Annual International Medicine*. 134: 1106-1114.
- [4] Milner A.1999. Functional foods and health promotion. *Journal of Nutrition*. 129: 1395-1397
- [5] Van T.P., Jansen M.C.J.F., Klerk M., & Kok F.J., 2000. Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutrition* 3:103-107.
- [6] Akindahunsi A.A., & Salawu S.O. 2005. Phytochemical Screening and nutrient – anti-nutrient composition of selected tropical green leafy vegetables. *African Journal of Biochemistry*, 4: 97-501.
- [7] Akinmutimi A.H. 2006. Nutritive value of raw and processed Jack fruit seeds (*Artocarpus heterophyllus*); *Agricultural Journal* 1 (4): 266-271.
- [8] AOAC 1990. Official Methods of Analysis (15th Edition). Helrich, K. Ed; Association of Official Analytical Chemists, Washington D.C.p.068-069.

- [9] Asami D.K., Hong Y.J., Barett D.M., & Michell A.E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and sir-dried marionberry, strawberry, and corn grown using conventional, organic and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry*, 51: 1237–1241.
- [10] Antia B.S., Akpan E.J., Okon P.A., & Umoren I.U. 2006. Nutritive and Antinutritive Evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pakistan Journal of Nutrition*, 5: 166–168.
- [11] Behall K. 1986. Chemistry and function of pectin. In: ACS System Symposium Series, Ed. Fishman ML and Jen JJ. Am. Chem. Soc., Washington D.C. USA. P.248–250.
- [12] Budowski P. 1988. W-3 fatty acids in health and disease. *World Review Nutrition Dietetics*, 57: 214–274.
- [13] Cohn R., & Cohn A.L. 1996. The by-products of fruit processing in fruit processing. Ed. Arthey D, Asthurst PR, Chapman and Hall, London, UK, p.196–220.
- [14] Edeoga H.O., Omosun G., & Uche L.C. 2006. Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* legbrids from Nigeria. *African Journal of Biotechnology*, 5(10): 891–895.
- [15] FAO (Food and Agricultural Organization) 1968. Food composition table for use in Africa food and Agric. Organization of the United Nations, Rome, Italy.
- [16] Indu M., & Ghafoorunissa P.J. 1992. N-3 fatty acids in Indian diets comparison of the effects of precursor (linlenic acid) vs product (long chain n-3 polyunsaturated fatty acids). *Journal of Nutrition Research*, 17: 569– 582.
- [17] Iniaghe O.M., Malomo S.O., & Adebayo J.O. 2009. Proximate composition and phytochemical constituents of leaves of some Acalypha species. *Pakistan Journal of Nutrition*, 8 (3): 256–258.
- [18] Larrauri J.A., Goni I., Martin–Carron N., & Ruperez, P., 1996. Measurement of health-promotions in fruit dietary fibres. Antioxidant capacity, fermentability and Glucose retardation index. *Journal of Science and Food Agricultural*, 71: 515–519.
- [19] Lious Y.A., King D.J., Zibrik D., & Innis S.M. 2007. Decreasing linileic acid with constant -linlenic acid in dietary fats increase (n-3) eiccosapentaenoic acid in plasma phospholipids in health men. *Journal of Nutrition*, 13: 945–952.
- [20] Lious L., Howe P., Zhou Y., Hocart C., & Zhang R. 2002. Fatty acid profiles of leaves of nine edible wild plants: An Australian study. *Journal of Food Lipids*, 9: 65–71.
- [21] Martin J.C., Bougnux P., Antoine J.M., Lanson M., & Couet C.R. 1993. Triacylglycerol structure of human colostrums and mature milk. *Journal of Food Lipids*, 28: 637–643.
- [22] Mathenge L. 1997. Nutrition value and utiliszation of indigeous vegetables in Kenya. In: Quarino L (Ed.), Traditional African Vegetables: Proceeding of the IPGRI International Workshop on Genetic Resources of Traditional vegetables in Africa. Conservation and use. KRAF – HQ, Nairobi, Institute of plant Genetic and Crop Plant Research, Rome, p.76–77.
- [23] McDougall G.J., Morrison I.A., Stewart D., & Hillman J.R. 1996. Plant cell walls as dietary fibre: Range, structure, processing and function. *Journal of Science of Food and Agriculture*, 70: 133–150.
- [24] Misikangas M., Freese R., Turpeinen A.M., & Mutanen M. 2001. High linolenic acid, low vegetable and high oleic acid, high vegetable diets affect platelet activation similarly in health women and men. *Journal of Nutrition*, 131: 1700–1705.
- [25] Mole S., & Waterman P.G. 1987. A critical analysis of techniques for measuring tannin and phenolics for ecological studies, *Oecologia*, 72: 137–147.
- [26] Mongrand S., Bessoule J.J., Cabantous F., & Cassagne C. 1998. The C16:3\C18:3 fatty acid balance in photosynthetic tissues from 468 plant species. *Phytochemistry* 46: 1049–1064.
- [27] Muhammed A., Javid H., Muhammed T.S., Zabta K.S., Farman U., Ali B., Naeem K., Abdullatif K., & Takashi W. 2010. Proximate and nutrient composition of medicinal plants of human and sub-human region in North–west Pakistan. *Journal of Medicinal Plants Research*, 4(4): 339–345.
- [28] Murphy G.M., & Signer E. 1974. Bile acid metabolism in infants and Children. *Journal of Gastroenterol*, 15: 151–163.
- [29] Ness A.R., Khaw K.T., Brngham S., & Day N.E. 1996. Vitamin C status and serium lipids. *European Journal of Clinical Nutrition*, 50: 724–729.
- [30] Nikolova–Damyarova B. 1997. Reversed-phase HPLC general principle and application to the analyses of fatty acids and triacylglycerols. In *advances in lipid methodology* (Ed W.W. Christie oil), p.193–251.
- [31] Obiajunwa E.I., Adebisi F.M., & Omoda P.E. 2005. Determination of essential Minerals and Trace elements in Nigerian Sesame seeds, using TXPF Technique. *Pakistan Journal of Nutrition*, 4(6): 393–395.
- [32] Okaka J.C., Enoch N.T., Akobundu A., & Okaka N.C. 2001. Human Nutrition: An integrated approach, second edition. Academic Publisher, Enugu, p.126–139.
- [33] Oladejo T.A. 2009. Proximate composition and micronutrient potentials of three locally available wild fruit in Nigeria. *Journal of Agricultural Research*, 4(9): 887–892.
- [34] Onwuka 2005. Food Analysis and instrumentation. Theory and practical. Naphihali Prints Lagos, Nigeria, p.148.
- [35] Shah M.I., Begun S., & Khan S. 2009. Ped and Biogeochemical studies of mafic and ultra mafic rocks in the Mingora and Kabal areas, Swat. *Journal Pakistan Environmental Science*, 0253 – 8.
- [36] Shahidi F., & Wanasundara P.K.J.P.D. 1992. Phenolic antioxidants. *Critical Review Food Science Nutrition*, 32:

- 67–103.
- [37] Sofomora L.A. 1993. Medical plants and traditional medical in Africa. Spectrum Books Ltd, Ibadan, p.55–71.
- [38] Tressler D.R., Van Arsdell W.B., & Copley M.J. 1980. The freezing preservation of food. 4th Edition, Avi Publishing Co. West Port, Conn. P.23.
- [39] Tolonen M. 1990. Vitamins and Minerals in Health and Nutrition, Ellis Horwood Limited, Chichester, England, p.148-187.
- [40] Vidrih R., Filip S., & Hribar J. 2009. Content of Higher fatty acid in Green vegetables. *Czech Journal of Science*, 27: 125.
- [41] Wang C.F., Chan H.E., & Yang J.Y. 1985. Essential and toxic trace elements in Chinese medicine. *NUCC Chemistry Journal Radional*, 211: 333– 347.