Nature & Environment
Vol. 19 (2), 2014: 151-157
Website: www.natureandenvironment.com

ISSN (Print) : 2321-810X
ISSN (Online) : 2321-8738

RESEARCH ARTICLE

Purity Testing of the Raw and Processed Plant Origin Drugs for Phytoformulation

Ishrat Alim[1], Ayesha Fatima Rizvi[2], Saba Khan[3] and Mohammad Rafi[4]

[1] Quality Assurance Laboratory, M.P. Council of Science and Technology, Vigyan Bhavan, Science Hills, Nehru Nagar, Bhopal (M.P.), India

[2] School of studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur (Chattisgarh)

[3] Department of Biological Science, College of Science & Arts, Rabigh, King Abdulaziz University, Saudi Arabia

[4] ICFAI Institute of Science and Technology, Medchal, Hyderabad (A.P.)

Email: ishrat_alim@yahoo.com

Received: 28th Feb. 2014, Revised: 7th May 2014, Accepted: 10th May 2014

ABSTRACT

Madhya Pradesh is a reservoir of biodiversity as it has rich and very diverse forest resources. Medicinal plant materials which are used as home remedies and offcourse essential element supplement represent a substantial proportion of the global dru market. Henceforth, it is necessary to establish a data base regarding the proximate analysis and elements as supplementary constituents. The methods related to sample treatment and preparation for pre-treatment solubilisation of the samples are essential for trace and heavy metal analysis in the herbal drugs which are being formulated by using such plants parts. In this way, we may established the protocol and standard operating procedure internationally on the basis of recognized guide lines which are applied on the quality assurance of the particular drugs. Level of essential and toxic metals in the selected plants is a matter of great concern all over the world. Here we report that whether the selected plant species viz., Chlorophytum borivillianum (Safed Musli), Emblica officinalis (Awla), and Withania somnifera (Ashwagandha) used as raw and processed phytoformulation are safe or not in regard to physico-chemical characteristics and the metal contaminants having permissible range of the trace essential elements. Present study has been under taken to establish the methods which are technically viable and feasible.

Key Words: *Food and Agri-produce, Quality assurance, Physico-chemical characteristics, Nutritional constituents, Metal contaminants.*

INTRODUCTION

Plant material is used throughout the world whether it is developed or developing countries as domestic remedies. The World Health Assembly has emphasized the need to ensure the quality of medicinal plants cultivation process and their pharmaceutical products by using modern control techniques applying suitable standards and definite norms. The home remedies over the counter drug products and raw materials for pharmaceuticals preparations represent substantial proportions of the global drug market. It is therefore testing the purity of raw material and their products are very essential for direct or indirect value addition (Bose B. C. *et al.,* 1961; AOAC 2000; API 2001).

There is a need for a scientific approach for propagation of medicinal plants and to collect relevant information regarding agro technology, genuine planting material, economics of field cultivation, high yielding varieties etc. One has to explore wild medicinal plant species and to bring them under cultivation. Sometimes plants selected from the wild population may be suitable for the cultivation and there is no immediate necessity for any improvement programs in it (Caius 1986). Safed Musli grows wild in thick forests and is a traditional medicinal plant. Mainly its tuberous roots are used in Ayurvedic medicines.

Safed Musli is an annual herb with Tubers, Crown, Leaf and Flowers as different parts. Naturally occurs in forests of Gujarat, Madhya Pradesh and Maharashtra States which are listed in the endangered species of India. *Emblica officinalis* or Awla is a natural, efficacious,

an antioxidant with the richest natural source of vitamin C. The fruit contains the highest amount of vitamin Ca in natural form.

Withania somnifera, commonly known as Ashwagandha, is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years. In view of its varied therapeutic potential, it has also been the subject of considerable modern scientific attention. *W. somnifera* is native to drier parts of India and the Old World. All plant parts are used including the roots, bark, leaves, fruit and Ashwagandha grows prolifically in India, Nepal, Pakistan, Sri Lanka and Bangladesh but it is commercially cultivated in Madhya Pradesh State (CIMAP, Lucknow 1988; Nadkarni 1993; Orient Longman 1997; Satyavati *et al.*, 2000). Testing of heavy and trace metals in herbal medicine has become compulsory and their labeling for heavy metals within permissible limits would be mandatory January, 1st, 2006. The Department of Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homeopathy (AYUSH) has issued a notification under the Drugs and Cosmetics Act 1940, stating that testing should be made compulsory and the container for processed herbs or medicines meant for export or within country must clearly display "Heavy Metals within permissible limits". The testing of heavy metals has become mandatory for export purposes on the basis of permissible limits for heavy metals will be recommended by WHO on Quality Control Methods for Medicinal Plants and Materials. Keeping the view in mind and the importance of these above mentioned plants, present study has been carried out to cater the needs of various herbal phytoformulation and to explore the necessity of purity testing of the raw and processed plant origin drugs.

MATERIAL AND METHODS

Present study has been under taken to examine the purity of the raw and processed herbal drugs formulation. Collection of the samples of raw material as tubers of *Chlorophytum borivillianum* (Safed Musli), fruits of *Emblica officinalis* (Awla), and the roots of *Withania somnifera* (Ashwagandha) from different sources was done. Phytoformulations available in different ayurvedic drug store was also procured for the present study. Sample storage, preservation and coding of the samples were done. Physico-chemical examination of the samples of selected or target plants and then standardization of the methods related to quality assurance of herbal drugs were performed as per the standard operating procedures of herbal drugs and raw material[1-2]. In accordance with the objectives, protocol for sample preparation was established for the detection of metals i.e. Cu, Zn, Ni, As and Pb by using flame atomization technique of Atomic Absorption Spectrophotometer (Make: ECIL, Hyderabad Model: AAS 4141). The details of the procedure as per prescribed methods (DGHS Manual 2005; ECIL Manual, Hyderabad 2007).

1. SAMPLE TREATMENT

For mineral determination, digestion of all the samples by using Microwave digester (Milestone ETHOS lab station with WAVE or CONTROL software HPR1000/10S) high pressure segmented rotor was carried out for sample analysis. This method provides for the acid digestion of the plant leaves dried powder sample in a closed vessel device using temperature control microwave heating for the metal determination by Atomic Absorption Spectrophotometer. The selected plant leaves were shade dried and then crushed using pestle and mortar. They were crushed and sieved repeatedly to pass through a 2 mm sieve. The leaves powder prepared during the study period were stored at room temperature in dry, air-tight containers for analysis. All the solvents and reagents used were of Analar grade. Demineralize water was used as solvent for solution preparation and all glassware were washed, cleaned and dried in an oven at 105°C.

2. SAMPLE PREPARATION FOR PRE-TREATMENT SOLUBILISATION

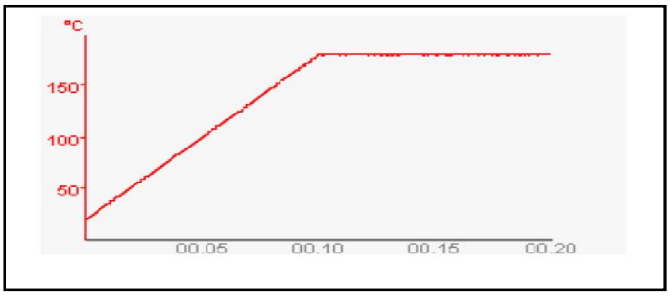
Plant leaves powdered sample amount (0.5 g) was poured into 7 ml of HNO_3 65% and 1 ml of H_2O_2 30% for pre-treatment solubilisation of samples for the present study. Placed a TFM vessel on the balance plate, tared it and weighed of the sample than introduced the TFM

vessel into the HTC safety shield. Added the acids; if part of the sample stays on the inner wall of the TFM vessel, wet it by adding acids drop by drop, then gently swirled the solution to homogenize the sample with the acids. Closed the vessel and introduced it into the rotor segment, then tighten by using the torque wrench. Inserted the segment into the microwave cavity and connected the temperature sensor. Cooled the rotor by air or by water until the solution reaches room temperature than opened the vessel and transfer the solution to a marked flask. Microwave program for plant leaves powder sample was as time 10 minutes, temperature 180^0 C up to 1000watt microwave power prescribed under first and second step set for sample preparation of raw and processed material and the formulations available in the local pharmacy shops for first and second step (Table 1 and Figure 1).

Table 1: Microwave programme of samples preparation

Step	Time	Temperature	Microwave power
1	10 minutes	180°C	Up to 1000 Watt*
2	10 minutes	180°C	Up to 1000 Watt*

Fig 1: Temperature profile of sample



RESULTS AND DISCUSSION

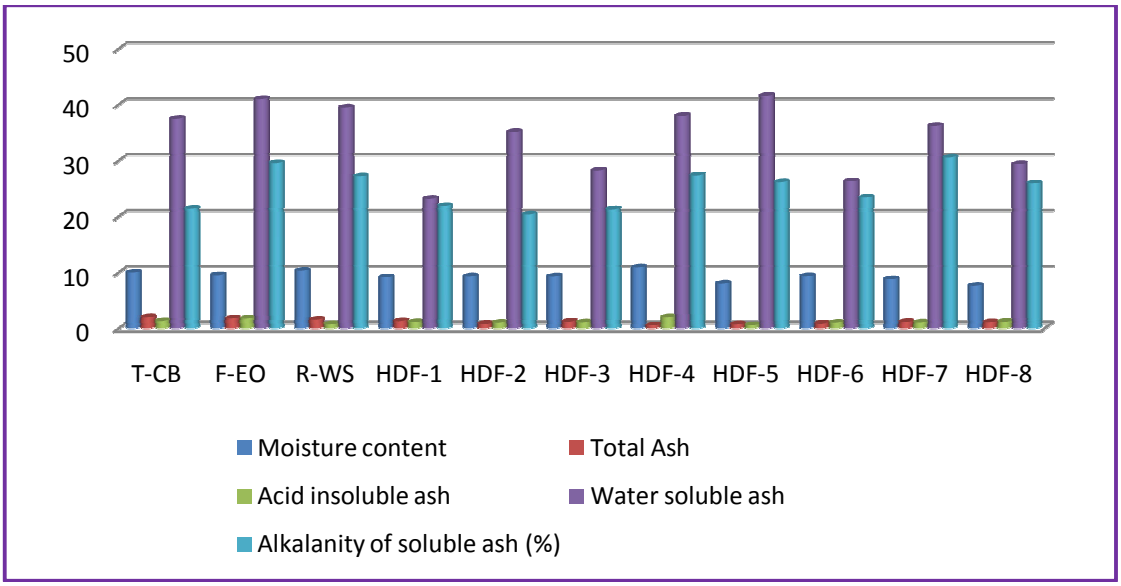
C. borivillianum, *E. officinalis*, and *W. somnifera* have been analyzed for physical and chemical parameters and all the data regarding these parameters has been expressed in Table-2 and Figure 2.

Table- 2: Physico-Chemical Profile of raw and processed phytoformulation

S. No.	Samples	MC (%)	TA(%)	AIA (%)	ASA(%)	WSA(%)
1.	T-CB	9.9607	1.9950	1.2959	41.59	30.57
2.	F-EO	10.893	1.7571	1.7371	41.02	29.57
3.	R-WS	10.290	1.5808	1.9803	39.48	27.16
4.	HDF-1	9.1330	1.2862	1.1001	23.20	21.90
5.	HDF-2	9.2925	0.7990	1.0143	35.13	20.38
6.	HDF-3	9.2851	1.1866	1.0501	28.25	21.28
7.	HDF-4	9.5113	0.5221	0.7371	38.01	27.34
8.	HDF-5	8.0117	0.7302	0.5803	37.51	26.15
9.	HDF-6	9.3135	0.8701	1.0131	26.27	23.38
10.	HDF-7	8.8116	1.1335	1.0332	36.23	21.39
11.	HDF-8	7.6341	1.0732	1.1651	29.37	25.92

*MC-R & F : Moisture content, TA: Total ash, AIA: Acid Insoluble Ash, ASA: Alcoholic soluble ash, WSA: Water soluble ash.. **T- CB: Tuber *C. borivillianum*, F-EO: Fruit *E. officinalis*, R-WS: Roots *W. somnifera*. ***(pH: 6.5 to 8.5)

Fig-2: Physico-Chemical Profile of raw and processed phytoformulation



The moisture content of the selected raw and finished phytoformulations samples was determined by measuring the number or mass of water molecules present in a known mass of sample. The data of the proximate composition of the samples has been presented in the Table 2 reflected the mean moisture content of all the samples was observed in a range from 9.5113% (*E. officinalis*) to 10.291% (*W. somnifera*) in plant (as raw material) whereas the moisture content appeared in a range of 7.6341 % 10.893 % in the phytoformulation samples HDF-8 and HDF-4 respectively. It is evident that the total ash of the plant samples was found in a range from 1.5808 in *W. somnifera* to 1.9951 % in *C. borivillianum*.

In the present study ash content was estimated as insoluble in dilute hydrochloric acid, water soluble ash and then alkalinity of soluble ash was done for all the selected samples of raw and finished phytoformulations collected from the different sources. From the data obtained during the study period indicated in the Table 2 and Fig., 2 it is cleared that the overall minimum of these characteristics mentioned above were found as 0.7371% in *W. somnifera*, 37.51% and 21.39% in *C. borivillianum.* However the maximum values were observed as 1.7371%, 41.02% and 29.57% in *E. officinalis* respectively for all these parameters. On the other hand phytoformulations procured during the study period revealed that the ash content as insoluble in dilute hydrochloric acid was observed in a range of 0.5803% (HDF-5) to 1.9803% (HDF-4), water soluble ash in a range of 23.2% (HDF-1) to 41.59 (HDF-5) and alkalinity of soluble ash was observed in a range of 20.38% (HDF-2) to 30.57 (HDF-7).

The physiochemical properties of raw and processed herbal drug formulations determine their perceived quality and behavior during production, storage and consumption. The stability of a produce is a measure of its ability to resist changes in its properties over time. These changes may be chemical, physical or biological in origin. Chemical stability refers to the change in the type of molecules present in the product with time due to chemical or biological reactions, e.g., fat rancidity or non-enzymatic browning. Physical stability refers to the change in the spatial distribution of the molecules present in the product with time due to movement of molecules from one location to other. That's why such herbal drugs must therefore be carefully formulated, so that they have the required physicochemical properties over the range of environmental conditions that they will experience during processing, storage and consumption, e.g., variations in temperature or mechanical stress. Consequently analytical techniques are needed to test the plant product or phytoformulation considered under the present investigation to ensure that they have the appropriate physicochemical properties.

Evaluation of most chemical parameters like moisture content is based on dry mass and therefore water content must be measured. Also, water content affects microorganism growth and enzymatic activity, affecting the stability and shelf life of product. The moisture contents are very important as this content limits the storage of the material. Higher amount represent that the formulation has lower shelf life. High moisture content aids microbial activities, oxidation- reduction processes and fungal growth. The variation in the moisture may be attributed to the degree of drying type and nature of formulation involved (Kumar *et al.,* 2005). A literature revealed that less moisture keeps the product microbiologically safe by preventing bacterial, fungal and yeast growth. If moisture content is very high, enzymatic activation may occur and result in loss of therapeutically active substance. In the present study the moisture content (LOD) appeared as within permissible limits which showed sufficient. The deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high or insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principle (Sharma *et al.,* 2011). The ash content is a measure of the total amount of minerals present within a food and Agri-produce. Determination of the ash is important for a number of reasons i.e. nutritional labeling. The concentration and type of minerals present must often be stipulated on the label of food, the quality of food depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability, microbiological stability, high mineral content are sometimes used to retard the growth of certain microorganism's nutrition. In addition of these some minerals which are essential to a human health (e.g., copper and zinc) whereas others can be toxic (e.g., lead, arsenic) and finally processing and development of product as it is often important to know that minerals content of foods during processing because this affects the physicochemical properties of food and agri-produce as well as phytoformulations. The ash content of any samples is an indication of its mineral content and it does not necessarily indicate high quality except when there is a favorable balance of the essential minerals.

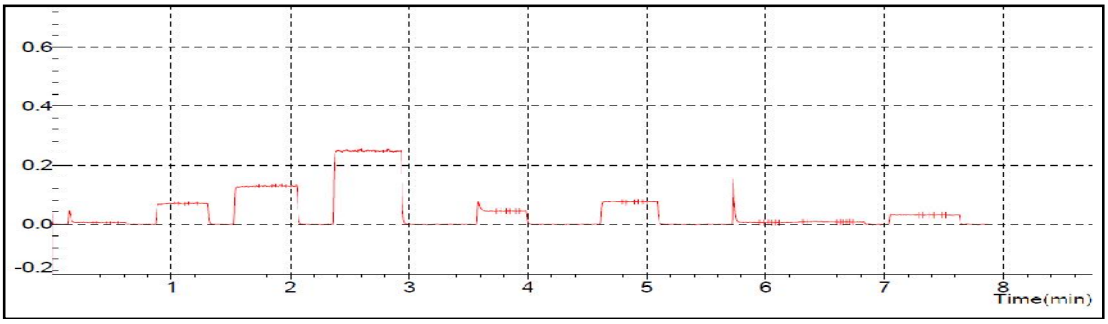
Physico-chemical standardization for quality assurance of different raw and finished phytoformulations covering the parameters like pH, which was also carried out and the data revealed that the pH of raw and finished phytoformulation's solution was observed in the normal range in aqueous solution systems as 6.5 to 8.5. Alkalinity is a measure of the capacity of the aqueous solution to resists a change in pH that would tend to make the aqueous solution more acidic. In the present study, pH of 1% w/v of aqueous solution of phytoformulation was found as minimum 6.7 in HDF-8 and the maximum pH of aqueous solution was found as 8.13 in HDF-3 which revealed the slightly acidic condition of the aqueous phytoformulations.

Trace and heavy metals level in the selected drugs have been detected by using Atomic Absorption Spectrophotometer to find out the facts that some minerals are essential to a healthy diet (e.g., copper and zinc) whereas others can be toxic (e.g., nickel, , arsenic and lead). Detection of trace and heavy metals was performed by using instrumental methods of analysis as per the standard conditions (Table 3) indicated under application part of the Manual. Flame absorption standardization during the analysis by AAS has been expressed in the Figure 3 reflected the metal analysis by Atomic Absorption Spectrophotometer.

Table 3: Standard conditions of Cu, Zn and Ni by Atomic Absorption Spectrophotometer

Instrumental Parameters	**Copper**	**Zinc**	**Nickel**	**Arsenic**	**Lead**
Wave Length (nm)	324.8nm	213.90	232.00	193.7	217.0
Slit (nm)	0.7	0.7	0.2	0.2	0.2
Lamp Current (mA)	2.00	2.00	2.00	2.00	2.00
Recommended Flame	Air -C_2H_2 (lean, blue)				
High Voltage (V)	194.88	285.70	273.37	194.88	285.70
Analysis parameters	**Copper**	**Zinc**	**Nickel**	**Arsenic**	**Lead**
Sampling Speed	50	50	50	50	50
Integral Time (s)	2.00	2.00	2.00	2.00	2.00
Smooth Curve Factor	50	10	10	50	10

Fig 3: Flame Absorption of AAS during the analysis



Present study reports that the concentration of heavy metals in all the samples taken during the study period. From the data as expressed in the Table 4 and Figure 4, it is revealed that the copper and zinc concentration was observed as minimum 15.4933 mg. Kg^{-1} in *C. borivillianum* and 37.6191mg. Kg^{-1} in the samples of *E. officinalis* whereas the maximum copper and zinc concentration was observed as 16.8539 mg. Kg^{-1} and 38.5089 mg. Kg^{-1} in the samples of *W. somnifera*. On the other hand the samples collected from different ayurvedic phytoformulations procured from local supplier were found with a level of copper and zinc concentration as 15.3871 mg. Kg^{-1} (HDF-6) and 37.5863 mg. Kg^{-1} (HDF-2).

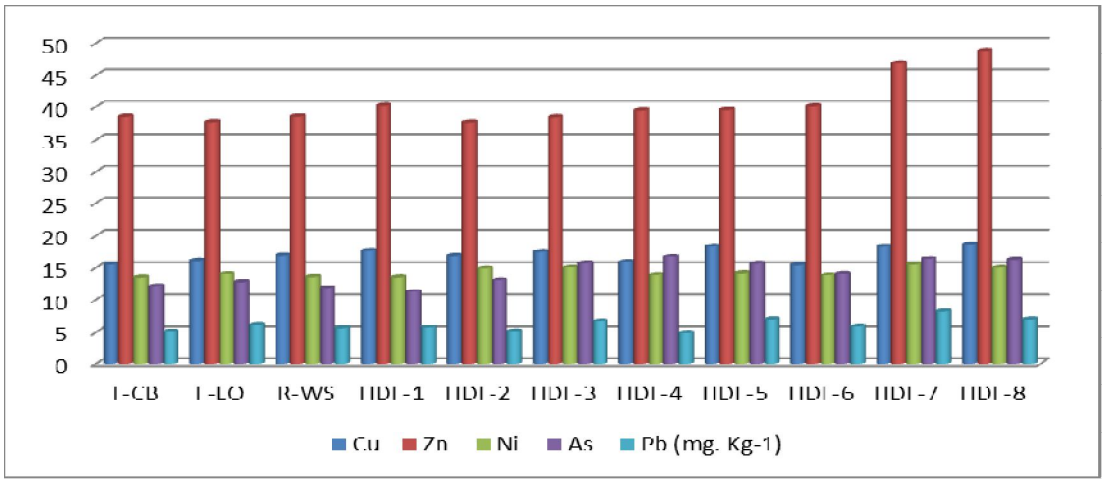
Similarly heavy metals like Nickel, Arsenic and lead was also estimated in the samples of raw and processed material and the formulations which were observes as in a range of 13.5107 mg. Kg^{-1} to 14.0277 mg. Kg^{-1} in *C. borivillianum* and *E. officinalis* for nickel bioaccumulation whereas 11.883 mg. Kg^{-1} (*W. somnifera*) to12.759 mg. Kg^{-1} (*E. officinalis*) for arsenic and 5.0809 mg. Kg^{-1} (*C. borivillianum*) to 6.1168 mg. Kg^{-1} (*E. officinalis*) for lead. On the contrary Nickel and arsenic concentration was observed in a range of 13.5187 and 11.269 in formulated sample HDF-1 to15.4933 mg. Kg^{-1} in HDF-7 and 16.63 in HDF-4 whereas lead was found in a range of 4.8885 mg. Kg^{-1} in HDF-4 to 8.2109 mg. Kg^{-1} the phytoformulations HDF-7 respectively (Table 4 and Figure 4).

Table 4: Status of trace and heavy metals in raw and processed phytoformulations

Raw	**Cu**	**Zn**	**Ni**	**As**	**Pb**
T-CB	15.4933	38.4897	13.5197	12.1667	5.0809
F-EO	16.0098	37.6191	14.0277	11.2691	6.1168
R-WS	16.8539	38.5089	13.5867	11.8829	5.6129
Formulations	**Cu**	**Zn**	**Ni**	**As**	**Pb**
HDF-1	17.5278	40.1759	13.5187	12.7589	5.7186
HDF-2	16.8099	37.5863	14.8698	13.0886	5.0979
HDF-3	17.3883	38.4099	15.0101	15.6693	6.7008
HDF-4	15.8189	39.4186	13.9087	16.6298	4.8885
HDF-5	18.2099	39.4882	14.1887	15.6193	6.9979
HDF-6	15.3871	40.1096	13.8695	14.0752	5.8708
HDF-7	18.2001	46.8088	15.4933	16.3488	8.2109
HDF-8	18.5199	48.8467	14.9858	16.1694	6.9806

*All the values are expressed in (mg. Kg^{-1}). **T-CB: Tuber *C. borivillianum*, F-EO: Fruit *E. officinalis*, R-WS: Roots *W. somnifera*.

Figure 4: Determination of elements by Atomic Absorption Spectrophotometer



Many elements present in medicinal plants, product and food at major, minor and trace levels are reported to be essential to man's well-being. However, their ingestion in excessive amount can cause severe health problems. The optimum concentration needed for this purpose varies widely depending on the kind of element and the age of consumers (Ahmad *et al.,* 1989*).* Human body requires both metallic and non-metallic elements for healthy growth, development and the proper functioning of the body. The determination of these elements in plant and product is thus of utmost importance and is currently the subject of studies. Difference between mineral contents was due to their agronomic practices their botanical and genetic factors as well as product formulation influencing on their contents. Difference among the copper content may be due to the difference in the agronomic application, fertilizer utilization and due to product specific genetic variation. Influence of climate and region also hold significant position on that regard.

ACKNOWLEDGEMENT

Thanks are due to Prof. Pramod K. Verma, Director General and Dr. D.K. Soni, Sr. Scientist and Incharge QAL, Madhya Pradesh Council of Science and Technology for extending support and constant encouragement. The authors are also thankful to all the staff of QAL offered selfless support and assistance to perform experimental work.

REFERNCES

1. Ahmed I., Zaidi S.S.H and Khan Z.A. (1989): The determination of major, minor and trace elements in different samples. Pak. J. Sci Ind. Res., 32(8): 513-515.
2. AOAC (2000): Official methods of AOAC International, Volume 1 Agricultural chemicals: Contaminants: Drugs. No. 999.11, 17th Ed. USA.
3. Ayurvedic Pharmacopoea of India, API (2001): Government of India, I-V.
4. Bose B. C. et al., (1961): Ind. J. Med. Sci., 1961; 15: 888
5. Caius J. F. *et al.*, (1986): Medicinal and Poisonous Plants of India, Scientific Publishers, Jodhpur.
6. CIMAP, Lucknow (1988): Dictionary of Indian Medicinal plants, p. 340.
7. Directorate General of Health Services- DGHS, Ministry of Health and Family Welfare Govt., of India (2005): Manual for analysis of metals, 1-34.
8. ECIL, Hyderabad (2007): Manual Atomic Absorption Spectrophotometer, 1-60.
9. Kumar A., Nair A.G.C., Reddy A.V.R. and Garg A.N. (2005): Availability of essential elements. Food Chem., 89: 441-448.
10. Nadkarni K.M. (1993): Indian Materia Medica, Vol. 1, Popular Prakashan.
11. Orient Longman (1997): Indian Medicinal Plants - a compendium of 500 species Part 3.
12. Satyavati G.V. *et al.*, (2000): Medicinal Plants of India, Vol. 1, ICMR.
13. Sharma P.K., Mohammad Ali and Yadav D.K. (2011): Journal of Applied Pharmaceutical Science, 01(03): 121-124.