Research Article
CODEN (USA): IJPLCP

[Subasini *et al.*, 4(5): May, 2013]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Pharmacognostic and phytochemical investigations of *Dioscorea bulbifera* L

U. Subasini[1]*, S. Thenmozhi[1], D. Sathyamurthy[1], S. Vetriselvan[2], G.Victor Rajamanickam[3] and G.P. Dubey[4]

1, Swamy Vivekanandha College of Pharmacy, Thiruchengode, Namakkal, (TN) - India
2, Masterskil Medical University and Health Sciences, Malaysia
3, Sri Sairam Group of Institutions, Medical Campus, West Tambaram, Chennai, (TN) - India
4, Banaras Hindu University, Varanasi, (UP) - India

Abstract

Dioscorea bulbiferea L. syn: Yam. (Family – Dioscoreaceae) is found commonly in India. Recent pharmacological findings indicate that it tubers possess significant activities like -purgative, deflatulent, aphrodisiac, rejuvenating and tonic, anthelmintic and is used in haematological disorders, scrofula, syphilis, haemorrhoids, flatulence, diarrhoea, dysentery, worm infestations, general debility, diabetic disorders, polyuric and skin disorders which comply with the claims made in the traditional medicinal texts. However, no conclusive pharmacognositc study of its tubers has been performed yet. The present investigation deals with the qualitative and quantitative microscopic evaluation of the tubers and establishment of the quality parameters including physiochemical and phytochemical evaluation. Chief microscopic character includes periderm, ground tissue, vascular bundle, exomorphic features of bulb and triangular starch grains. Such a study would serve as a useful gauge standardization of tubers material and ensuring the quality formulations.

Key-Words: Dioscoreaceae, Microscopy, Pharmacognostical parameters

Introduction

Dioscorea bulbiferea L. Eng: Yam. (Family – Dioscoreaceae) is found commonly in India. Recent pharmacological findings indicate that it tubers possess significant activities like -purgative, deflatulent, aphrodisiac, rejuvenating and tonic, anthelmintic and is used in haematological disorders, scrofula, syphilis, haemorrhoids, flatulence, diarrhoea, dysentery, worm infestations, general debility, diabetic disorders, polyuric and skin disorders. The tubers are crushed and decoction is emulsified into oil, which is used in infected ulcers and sinus. According to the world Health Organization (WHO, 1998) the macroscopic and microscopic description of medicinal plants is the first step towards the establishing the identity and degree of purity of such materials and should be carried out before any tests are undertaken.

*** Corresponding Author**
E.mail: oviya_subashini@hotmail.com,
vrajamanickam@hotmail.com

Dioscorea bulbifera also known as Gonth, Kolkand, Varaheekand, a tribal plant belonging to the family of Dioscoreaceae. It is a climber plant with tuberous root. Dioscorea is a large genus of annual twining herbs, distributed throughout the moist tropics of world and extending into warm temperate regions. About 50 species are found in India. A large number among them occur in the wild state. Dioscorea species are distributed nearly throughout India except in the dry north-western regions. They are found growing at elevations of 8000-15000 ft. in Himalayas. In its wild state, it is extremely bitter. Under cultivation the plant loses its bitterness and is much grown for the tubers, which are roasted and eaten. The tuber is used by the tribal population of central India as a food particularly in Madhya Pradesh, Chattisgurh, Jharkhand and Orissa.

Material and Methods

Collection and authentication of plants

Tubers of D.bulbifera were collected from the Thovalai, Trichy district in May 2007. This plant are authenticated in multicentres such as Rabinat Herbarium, St. Joseph College, Trichy, St. Xavier's College, Palayamkottai and Botanical survey, CCRAS Unit, Chennai and Govt. Medical College,

Palayamkottai. Herbarium and voucher sample were prepared and deposited in Department of Pharmacognosy & Phytopharmacy, Sastra University (Voucher No. DB – 0062) Thanjavur.

Collection of Specimens

The different organs of this plant were cut and removed from the plant and fixed in FAA (Formalin 5ml + Acetic acid 5ml + 70 % Ethyl alcohol 90 ml) for histological studies, transverse sections (T.S) of the different organs of the plant materials. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the schedule given by sass, 1940. Infiltration of the specimens was carried out by gradual addition of paraffin wax until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were section with the help of rotator microtome. The thicknesses of the section were 10-12 um. Dewaxing of the sections was performed by customary procedure (Johansen, 1940). The sections were stained with toludine blue as according to the method prescribed by O Brien et al., 1964. Wherever necessary, the sections were also stained with saffranin and fast –green. The microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-chemical Standards

Physico-chemical constants such as consistency and organoleptic characters and the percentage of total ash, acid-insoluble ash, water-soluble ash and extractive values and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia (Anonymous, 1985).

Phytochemical Screening of DB Extracted with Different Solvents

The extracts were tested for the presence of alkaloids, flavonoids, glycosides, phenols, resins, saponins, tannins, volatile oils, carbohydrates and amino acids using standard procedure.

Quantitative Estimation of Phytochemical compounds

Various phytochemical compounds of the raw herb like phenolics, tannins, carbohydrates, vitamin C and vitamin D of DB were estimated using UV spectrophotometer (Lambda 25).

Elemental analysis using AAS

The samples are cleaned and dried under shade. Then, the samples are dried in an oven at 40°-50° C till a constant weight is obtained. The dried samples are then, ground and powdered with agate pestle and mortar. Samples are labeled and stored in pre-cleaned polyethylene bottles for further analysis. The prepared solutions are directly subjected to flame photometry and AAS for the estimation of various elemental concentrations.

Results and Discussion

The external features of the plant

Twining perennial herb, tubers solitary and globose to pyriform covered with long roots and prominent eyes of unique in nature. Stem is 10 to 20 m (66 ft) or more in length and freely branching above. The internodes are round or slightly angled in cross section without wings (Fig 1). Bulb axillary's, sessile, spherical, and green when young, rusty brown when matured, the surface is warty with closely aggregated hemispherical nodules, each nodule with nipple

(Fig 2). The bubbil is fairly hard and heavy. Dish shaped with to 12 cm (5") x 10 cm (4") brown with prominent numerous, uniformly distributed tubercle like eyes. Bulbils abundant and of different sizes and shapes; in certain cultigens the tuber is suppressed in favour of rather large bulbils, having all the reserve food; small bulbils are, as a rule warted, but they may be smooth when large. Tubers are usually small and round, but large under cultivation. They are weighing up to 1 kg. Their skin is purplish black or earth coloured, usually coated with abundant small feeding roots, but smooth in some cultivated varieties having flesh of white to lemon yellow, sometimes marked with purple flecks and very mucilaginous (Fig 2) Drugs occur in cut pieces, 0.5 to 0.7 cm thick, 2 to 3 cm dia. in size are used as raw material for drug. A few root and root scars present in tubers, outer surface dark brown, inner yellow to light brown; odour- indistinct; taste – bitter.

Microscopical features of the Tubers

A cross section of the bulb shows a darker region of the nodular part, an endodermoid layer and inner homogeneous parenchymatous ground tissue (Fig 3). The surface of the nodule has an intact epidermis made up of thick walled rectangular cells. (Fig 3) The cells of the nodular part are fairly thick walled compact and darker. Solitary cells containing dark, amorphous content are seen diffusely distributed in the nodule. The inner boundary of the nodule has a thin wavy layer. Ground tissue, forming major portion of drug composed of oval to polygonal cells having a few scattered closed vascular bundles; starch grains found both in cortex and ground tissues, but abundant in ground tissue, rounded to oval, three sided with rounded angles or rod-shaped, simple, solitary or in groups, 11-28 µ in diameter; hilum present at the narrower extremity.

Rectangular cells are forming the endodermoid layer. The outer part of the nodule has four or five layers of thick walled seleroids that are tubular in shape. These layers represent the periderm. The surface of the bulb in between the nodules has four or five layers of their walled tubular cells followed by four or five layers of tubular scleroids. These four are the continuation of the periderm of the nodules (Fig 4). The periderm zone is 150 micrometer wide. The ground tissue within the boundary of the endodermoid layer has many scattered vascular strands distributed in the parenchymatous tissue. The outer zone of the ground tissue has no cell indusious and vascular strands (Fig 5). The inner zone has vascular strands as well as heavy load of strand grains.

The vascular strands are oblong and collateral with a few angular thin walled xylem elements and a cluster of phloem, distinct bundle sheath is not evident.

Starch grains are most courpieuos features of the ground tissue (Fig 6 and 7) these two characteristic types of starch grains Type-I: the starch grains elongated or rectangular with semicircular ends (Plate 6). When viewed under the polarized light microscope, these starch grains have X or Y shaped. The elongated (Cylindrical) starch grains are 20.22 micrometer long.

ii) When viewed under the polarized light, no dark lines are evident. Usually, the triangular type of starch grains occurs in separate cell (Fig 7). Second type of starch grains are triangular (Fig 8) and are equally abundant as the elongated grains. They are 30 micrometer long.

The results of Table 1 show that the hydroalcoholic extract of DB although having a lot of phytoconstituents, is devoid of the main compound families indicated in the table. The 85 % hydroalcoholic extract showed to be a rich source of glycosides, proteins, fats, sterols, alkaloids, polyphenols and tannins, flavonoids and saponins are qualitatively analyzed Trease and Evans (1958). Various phytochemical compounds like phenolics (Bray and Thorpe 1954), tannins(AOAC, 1980), proteins (Lowry's et al 1951), , carbohydrates (Dubois, et al., 1956) , vitamin C (Sarojini, et al., 1999) and vitamin E (Jayasree et al., 1985) were estimated in both raw herb and 85 % of hydroalcoholic extract (Table 2). The raw herb contained higher concentration of phenolics followed by carbohydrates, whereas 85 % of hydroalcoholic extracts possessed higher concentration of tannins followed by Vitamin C.

Physiochemical analysis

Analysis of the three herbal plants for the various physiochemical parameters such as total ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive gives an idea to use the same as a pharma-therapeutic agent. It is computed to be of 7 % when all the chosen parameters are added together. If it is so, it is presumed to possess promising biological activity. Such characters enable one to recognize the sample taken is fit for using it as a drug. The results are tabulated in Table 3. The results of the physiochemical analysis prove the stability, purity and firmness of the plant drug for use and are helpful to standardize for the use as a potential drug (Indian Pharmacopoeia 1996).

Heavy metal analysis

After calibrating the instrument with prepared working standard, the digested liquid samples solution is subjected to analysis of Fe, Cu, Mn, Zn, Ni, Mg, Mo, etc., by AAS flame/Graphite furnace with specific instrumental conditions as given by instruments' manufacturer. Introduce the solution into flame, record the reading, using the mean of the three readings and quantified the concentration of the metals in the given samples against the standard calibration curve obtained from Concentration vs. Absorbance of the prepared known concentration on the day of the analysis. The various mineral elements are generally being imbibed into the plants from the soil, water and atmosphere. The level of mineral elements in plant varies depending upon the environmental factors and the type of plant itself. Among plant types growing in the same environment, fungi lichen and mosses accumulate more metals than the others. For a particular species, the concentration level generally decreases in the order root >stem > leaves > fruit > seed when the source of the mineral element is only the soil. Moreover the concentration of elements also varies with the age of the plant.

Inorganic micronutrients include Fe, Cu, Zn, Mn, Co, Mo, V, B, Cl, I. Br and Na. They are important as catalyzing metabolic reactions and in osmoregulation. They are required in optimum quantities for better growth of the plant but when supplied in excess, it is turning to be harmful. Results of the micronutrients and trace elements are given in the Table 5. In view of the criticism provided for the traditional drugs on the ground of metal toxicity, the extract, which is going to be tested for the drug is brought under the observation of elemental analysis. The values are very much within the limits of W.H.O. except aluminum that are also an element of useful one for the metabolism. As there is no alarming presence of heavy metals in the extracts, the extract has been taken up for further acute toxicity studies. Any plant is likely to have some elements or others in low or high quantity. The quantity depends on the soil nature and the environmental conditions. In the present study, the concentration of various elements in

raw plants, the ashes of different plants, the aqueous extracts and in hydroalcholic extracts has been determined by using flame photometry in Table 5 using AAS and the same is tabulated in Table 6. The whole plant of raw material has been analyzed for iron, copper, manganese, nickel, zinc, cobalt, chromium, aluminum, vanadium, molybdenum, lead, cadmium, mercury, arsenic and selenium (Sahito et al., 2001).

Conclusion

This study reveals that sulphonylurea are safer with reference to lactic acidosis as they do not increase lactate level, while phenformin & metformin has the tendency for hyperlactatemia eventhough there is a slight variation in the degree of rise. When prescribed for the patients without having a tendency for precipitating lactic acidosis due to hypoxia, both the drugs are safer & equally effective in Type II diabetic patients. The ethanomedicinal practices the traditional healers use Dioscorea bubifera in treatment of various ailments, especially, the tuber of Dioscorea bulbifera is used in diarrhoea, dysentery, piles, as a tonic, alternative, aphrodisiac, stomachic, anthdmintic, improves appetite, dyspepsia, leucoderma, bronchitis and applied to ulcer (Chopra et al, 1956). Macroscopic and Microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameters in Ayurveda monograph. In this regard the important microscopic features of the parts of the tubers have been documented. The T.S of tubers showed wide, well developed periderm, vascular bundles and triangular starch grains. Studies on preliminary phytochemical by qualitative and quantative methods, physiochemical standards, elemental analysis can serve as a valuable source of information and provide suitable standards to determine the quality of this plant material in future investigations or applications. The present study on Pharmacognostical characters of tubers of Dioscorea bulbifera L will be providing useful information for the future identification of this plant. Mineral elements are more useful to man than being harmful. Human body requires mineral elements to certain extent. At the same time, when it crosses the limit, it becomes toxic and degenerate the system. High level of toxic elements occur in medicinal preparations either when they are used as active ingredients as in the case of Pb and Hg in some Chinese, Mexican and Indian medicines (Levit and Lovett, 1984) or when the plants are grown in polluted areas fertilizers, such as near roadways, metal mining and smelting operations and when one uses fertilizer containing cadmium and organic mercury or lead based pesticides, and contaminated irrigation water (Abou Arab et al., 1999). Hence, analysis of various mineral/metal elements is imperative in the use of plants as drugs

Acknowledgements

The authors are thankful to Dr. P. Jeyaraman, M.Sc., Ph.D. Plant Anatomy Research Centre (PARC), Chennai Tamil Nadu, India for providing technical assistance during the studies.

References

1. Anonymous 1Indian Pharmacopoeia. Voll II, 3rd ed., Government of India. Ministry of Health, Controller of Publications. New Delhi, India. 1985, 74.
2. AOAC, Anonymous Official methods of analysis, Association of Official Analytical Chemist, Washington DC, 1980 10th Edition.
3. Chopra RN., Nayar SL., Chopra IC. Glossary of Indian Medicinal plants Council of Scientific and Industrial Research (CSIR), New Delhi, 1956, 218, 32.
4. Johansen DA, Plant Microtechnique. New York, McGraw-Hill, 1940, 523.
5. O'Brien TP, Feder N. McCully M. Polychromatic staining of plant cell walls by Toluidine Blue-O. Protplasma, 1964. 368-373.
6. Sass JE. Elements of Botanical Microtechnique. Mc Graw Hill Book Co; New York: 1940, 222. World Health Organization. Quality control methods for medicinal plant materials. WHO Library, Geneva. 1988, 1-115.
7. Bray TM, Taylor CG, Tissue glutathione, nutrition, and oxidative stress. Can J Physiol Pharmacol, 71, 1993, and 746-5.
8. Chopra RN, Chopra IC, Handa KL, et al. Indigenous Drugs of India, 2nd edition. U.N. Dhur and Sons Pvt. Ltd., Calcutta, 1958, 309.
9. Indian Pharmacopoeia, Chemical Tests and Assays, Publ. The Controller of Publications, Govt. of India, New Delhi, 1996, A34-A89.
10. Indian Pharmacopoeia, Physical Tests and Determination, Publ. The Controller of Publications, Govt. of India, New Delhi, 1996, A85-A124.
11. Jayashree V, Solimabi W, Kamat SY. Distribution of tocopherol (Vitamin E) in marine algae from Goa, West coast of India. *Indian J. Mar. Scie*, 14, 1985, 228-229.
12. Kokate CK. In: Practical Pharmacognosy. 8th Ed, Vallab Prakasan Publications, Delhi, 2000, 1-186.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 5th Ed., Nirali Prakashan Publications, Pune 1997, 109-137.

14. Lowry OH, Rosenbrough NJ, Farr AJ, et al. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem*, 193, 1951, 265- 75.
15. Sarojini Y, Nittala SS, Vitamin C content of some macroalgae of Visakhapatnam, East coast of India. *Indian J, Mar, Sci*, 28, 1999, 408-12.
16. Subashini Uthrapathy, Mohamed M. Shabi, Gayathri K, Victor Rajamanickam and G.P. Dubey et al. Analgesic and anti-arthritic effect of Corallocarpus epigaeus, *Acta Bioquim Clin Latinoam*, 45 (4), 2011, 749-56.
17. Levitt J, Lovett JV. *Datura stramonium* L.: alkaloids and allelopathy. *Australian weeds*, **3**(3), 1948, 108-112.
18. Abou-Arab AAK, Kawther MS, Tantawy ME, et al. Quantitative estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food chem*., 7 1999, 357-63.
19. Sahito SR, Kazi TG, Kazi GH, et al., Trace Elements in Two Varieties of Indigenous Medicinal Plant Catharanthus roseus (Vinca rosea). *The Sciences*, 1(2), 2001, 74-77.

.

Fig. 1*: Dioscorea bulbifera*

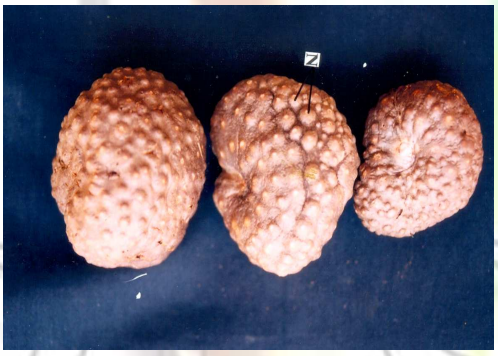


Fig. 2*: Exomorphic features of bulbs*

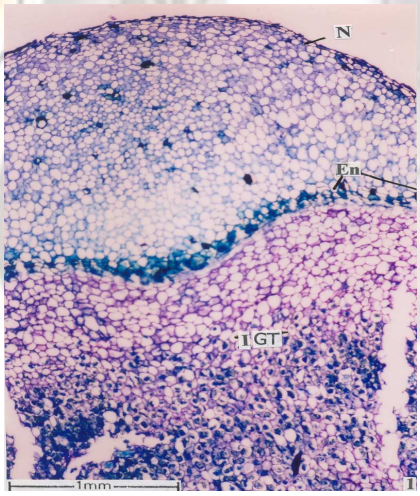


Fig. 3: T.S of bulb a sector enlarged

[Subasini *et al.*, 4(5): May, 2013]
ISSN: 0976-7126

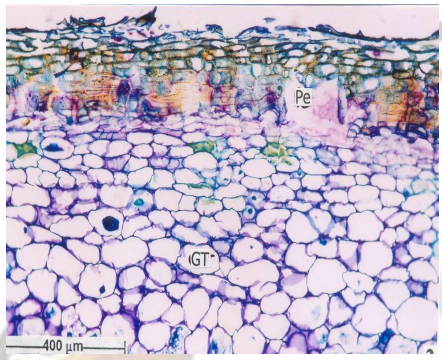


Fig. 4: Periderm and Ground Tissue

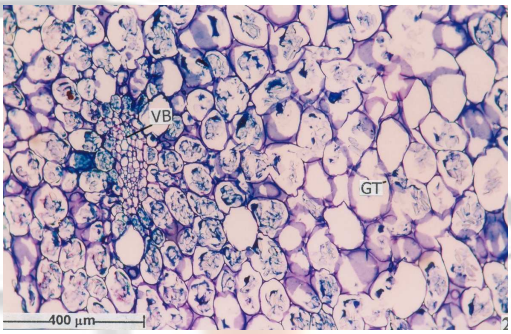
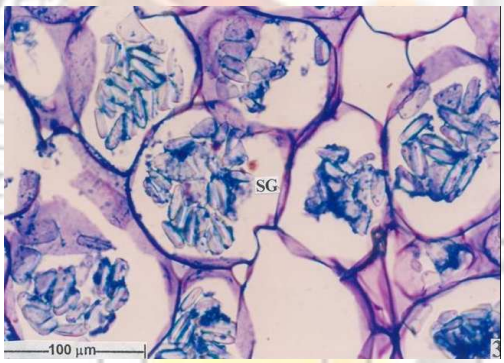


Fig. 5: Ground tissue and Vascular bundle



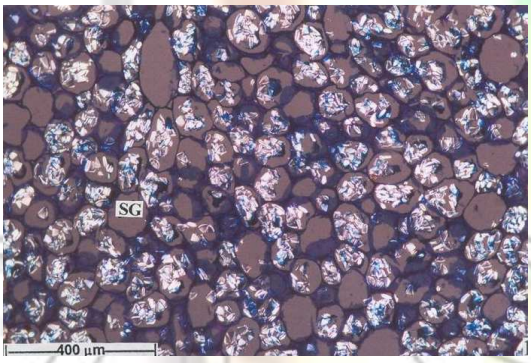


Fig. 6 & 7: T.S of the bulb showing starch grains

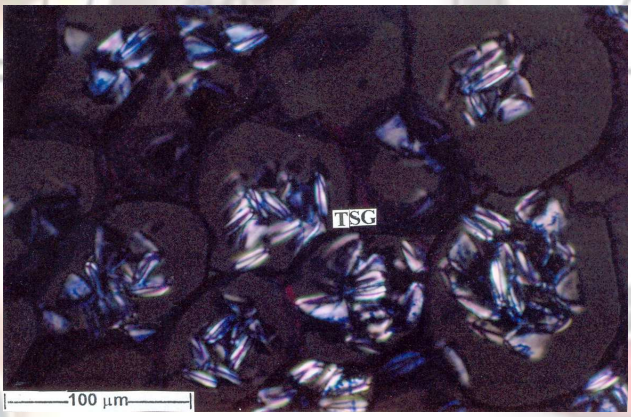


Fig. 8: T. S. of bulb showing triangular starch grain

Table 1: Qualitative Phytochemical Analysis of Hydroalcoholic Extract

Plant	DB
Alkaloids	+
Carbohydrate	+
Phytosterol	-
Protein	+
Glycosides	+
Flavonoids	-
Saponins	-

Table 2: Quantitative Estimation of Phyto-Constituents in Raw Drugs

S.No.	Plants	Total Phenolics (mg /100 gm)	Total Tannins (mg /100gm)	Total Proteins (µg/100gm)	Vitamin C (µg/100gm)	Vitamin E (µg/100gm)	Total Carbohydrate (mg/100gm)
1	DB	14.20±0.1	4.32±0.3	33.0±1.2	0.36±0.2	0.40±0.2	685±2.3

(values are mean±SD)

Table 3: Proximate analysis

S.No	Raw drug	Total Ash (%w/w)	Water Insoluble Ash (% w/w)	Acid Insoluble Ash (% w/w)	Crude Fibre Content (%w/w)	Water Soluble Extract (%w/w)	Ethanol Soluble Extract (%w/w)
1	DB	4.7	2.3	1.5	3.8	7.84	15.19

(Average of triplicate)

Table 4: Estimation of active constituents of DB

Name of the Phytoconstituents	*Dioscorea bulbifera L*
Glycosides	5.3015 %
Alkaloids	0.3703 %
Flavanoids	39.6284 %
Tannins	34.1624 %
Fixed oil	-
Resins	-
Bitters	1.2029 %
Vitamin C	8.4351 mg.

Table 5: Elemental Analysis using Flame Photometry

Plant	Na (mg/l)	Ca (mg/l)	K (mg/l)	Li (mg/l)
DB	1.81	79.05	160.30	0.55

Table 6: Distribution of elements from raw drug, ashes, aqueous extract & HAE

(Units in ppm)

Drug Code	Fe	Cu	Mn	Ni	Zn	Co	Cr
Raw drug	0.4740	0.2635	0.1220	0.0990	1.2825	0.1780	0.4435
Ashes	0.7410	0.1412	0.1412	0.0399	0.0880	0.0034	0.0379
Aqueous extract	0.7410	0.1412	0.1412	0.0399	0.0880	0.0034	0.0379
Hydroalcoholic extract	0.1495	0.0840	0.0170	0.0225	0.4900	0.0950	0.1235

(Units in ppm)

Drug Code	Al	V	Mo	Pb	Cd	Hg	As
Raw drug	7.3500	3.4350	0.3615	0.3780	0.1660	0.0400	0.0087
Ashes	9.5800	0.2880	1.8440	0.5720	0.1124	0.0063	0.0006
Aqueous extract	0.0036	0.0359	0.0356	0.0058	0.0224	0.0005	0.0003
Hydroalcoholic extract	3.6915	2.5000	0.0910	0.2265	0.0675	0.0096	0.0010