ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

Volume5 Issue3,Aug2017

Pharmacognostic and phytochemical investigations of *Dioscoreabulbifera* L

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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:

Common in India is the Dioscorea bulbiferea L., sometimes known as yam (Dioscoreaceae). It has been used to treat haematological disorders, scrofula, syphilis, hemorrhoids, flatulence, diarrhoea, dysentery, worm infestations, general debility, diabetes, polyuria, and skin disorders, as well as other conditions, according to recent pharmacological findings. Ulcer and sinus infections may be treated by using an oil made from the decoction of the tubers. The first stage in verifying the identification and degree of purity of such materials, according to the World Health Organization (WHO, 1998), is to perform a macroscopic and microscopic description of medicinal plants.Gonth, Kolkand, and Varaheekand are all names for the same tribal plant, Dioscorea bulbifera. It has tuberous roots and grows like a climber. Twining annuals belonging to the genus Dioscorea may be found all over the globe, from the wet tropics to the warmer subtropics. There are around 50 different species in India. There are a lot of them that live in the wild. Except for the arid northwestern areas, Dioscorea species may be found practically everywhere in India. Elevations in the Himalayas between 20,000 and 50,000 feet are ideal for their growth. It's really bitter when it's in its natural form. The bitterness of the plant disappears when cultivated in a controlled environment, and the roasted tubers are a popular crop. In the states of Madhya Pradesh, Chattisgarh, Jharkhand, and Orissa, the tuber is a staple meal for the indigenous people.

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Substances and Techniques

Herbaria collection and identificationD. bulbifera tubers were gathered in the Thovalai area of the Trichy district in the month of May, 2007. Rabinat Herbarium, St. Joseph College, Trichy, St. Xavier's College, Palayamkottai, Botanical survey, CCRAS Unit, Chennai, and Govt. Medical College all verify the authenticity of this plant. Palayamkottai. Herbarium and voucher sample were prepared and deposited in Department of Pharmacognosy & Phytopharmacy, Sastra University (Voucher No. DB – 0062) Thanjavur.Collection of SpecimensThe 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.SectioningThe 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 StandardsPhysico-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 SolventsThe 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 compoundsVarious 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 AASThe 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-cleanedpolyethylene 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 plantTwining 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 ofDBalthoughhavingalot of ytoconstituents, is devoid of the main compound familiesindicatedin Thetable.The85% hydroalcoholic extract showed to be a rich source of glycosides,proteinsfats,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 hydroalcoholicextractsPossessedhigher concentration of tannins followed by Vitamin C. Physiochemical analysisAnalysis of the three herbal plants for the various physiochemical parameters such as total ash, acid insoluble ash, water soluble extractive and alcoholsoluble 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 analysisAfter 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 inraw 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

Based on the findings of this research, sulphonylurea are less dangerous in terms of lactic acidosis since they do not raise lactate level, but phenformin and metformin have a propensity for hyperlactatemia, although to varying degrees. Both medicines are safe and similarly effective in treating Type II diabetes, provided they are given to individuals who are not at risk of developing hypoxic lactic acidosis. The tuber of Dioscorea bulbifera is used in the treatment of a wide range of medical conditions, including diarrhea, dysentery, piles, as a tonic, alternative, aphrodisiac, stomachic, anthdmintic, improved appetite, dyspepsia, leucoderma, bronchitis, and ulcers (Chopra et al., 1956). One of the most important criteria in an Ayurveda monograph is the use of macroscopic and microscopic examination in the identification of therapeutic plants. Here, the salient microscopic characteristics of the tuber components have been recorded. There was a lot of periderm, vascular bundles, and triangular starch granules visible in the T.S of tubers. Researchers and practitioners may benefit greatly from having access to appropriate standards for determining the quality of this plant material based on the results of preliminary phytochemical studies conducted using qualitative and quantitative methodologies, physiochemical standards, and elemental analysis. Future identification of Dioscorea bulbifera L will benefit from the current study's pharmacognostical feature analysis of tubers. Man can benefit from minerals much more than they may do damage. Minerals have an important role in human health. Yet beyond a certain point, it becomes poisonous and degenerates the system. Some traditional Chinese, Mexican, and Indian medicines contain high levels of lead and mercury because these elements are used as active ingredients (Levit and Lovett, 1984) or because the plants were grown in polluted areas with fertilizers containing cadmium and organic mercury or lead based pesticides, and irrigated with contaminated water (Abou Arab et al., 2010). Therefore, a dissection ofThe utilization of plants as medicines relies heavily on their accumulated mineral and metal content.

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Fig. 1: *Dioscorea bulbifera*

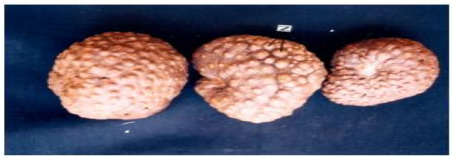


Fig. 2: Exomorphic features of bulbs

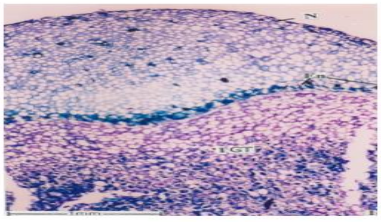


Fig. 3: T.S of bulb a sector enlarged

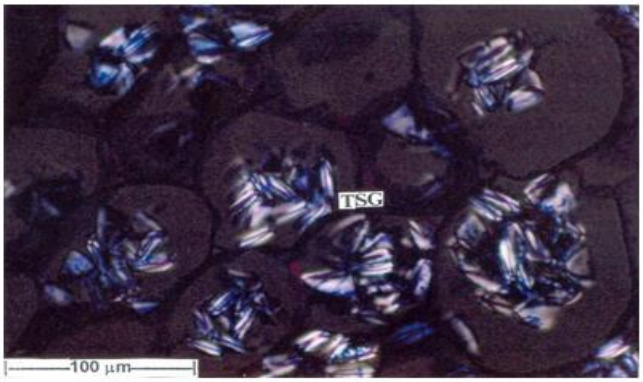


Fig. 8: T. S. of bulb showing triangular starch grain

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

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.

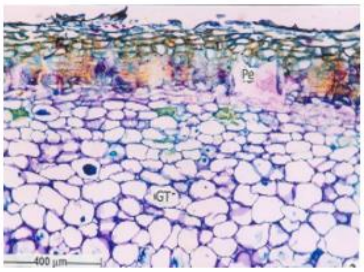


Fig. 4: Periderm and Ground Tissue

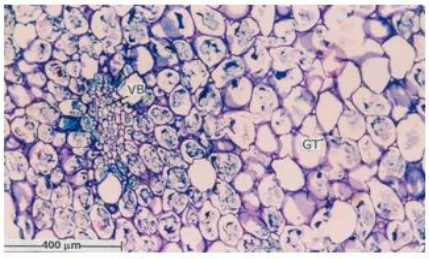
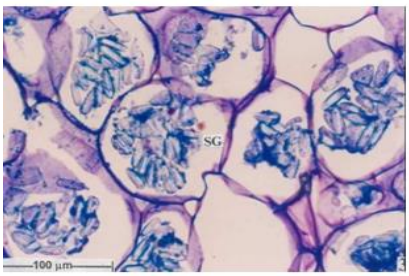


Fig. 5: Ground tissue and Vascular bundle



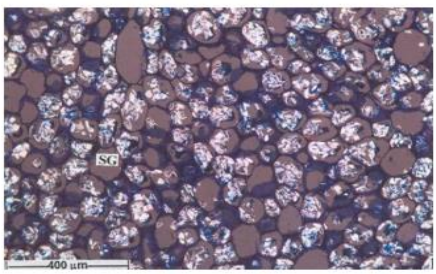


Fig. 6 & 7: T.S of the bulb showing starch grains