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Pharmacognostic and phytochemical investigations of *Dioscorea bulbifera*

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Abstract

In India, you may find a lot of Dioscorea bulbiferea L., sometimes known as Yam (Dioscoreaceae). According to recent pharmacological findings, it tubers possess significant activities like -purgative, deflatulent, aphrodisiac, rejuvenating and tonic, anthelmintic, and is used in haematological disorders, scrofula, syphilis, hemorrhoids, flatulence, diarrhoea, dysentery, worm infestations, general debility, diabetic disorders, polyuric, and skin disorders. However, there has been no clear pharmacognostic investigation of the tubers. This study focuses on establishing the quality characteristics, such as physiochemical and phytochemical analysis, and performing both qualitative and quantitative microscopic evaluations of the tubers. Chief microscopic character comprises periderm, ground tissue, vascular bundle, exomorphic characteristics of bulb and triangular starch grains. Such a research would serve as a valuable gauge standardization of tubers material and assuring the quality formulations.

Key-Words: Dioscoreaceae, Microscopy, Pharmacognostical parameters

Introduction

It is frequent in India to come across Dioscorea bulbiferea L. Eng: Yam. (Family - Dioscoreaceae). 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. Ulcer and sinus infections may be treated by using an oil made from a decoction of the tubers. In order to determine the identification and purity of medicinal plants, the World Health Organization (WHO, 1998) recommends first performing a macroscopic and microscopic description of the material.Gonth, Kolkand, and Varaheekand are all names for the same tribal plant, Dioscorea bulbifera. It's a tuberous-rooted climber. Dioscorea is a vast genus of twining annual plants that may be found in both the wet tropics and some milder parts of the planet. India is home to around 50 different species. Many of them are found in their natural habitats. Species of Dioscorea may be found almost everywhere in India, with the exception of the arid northwest. They thrive at altitudes between 20,000 and 30,000 meters in the Himalayas. It's really bitter when it's in its natural form. The bitterness of the plant disappears with cultivation, and the tubers are produced specifically for roasting and consumption. The tribal people of central India, especially those living in the states of Madhya Pradesh, Chattisgarh, Jharkhand, and Orissa, rely on the tuber for sustenance.

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Herbaria collection and identification

D. bulbifera tubers were dug up 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, Tiruchirapalli are only few of the many locations where this plant has been verified as genuine. Palayamkottai. The voucher sample and accompanying herbarium specimen were archived at the Department of Pharmacognosy & Phytopharmacy at Sastra University in Thanjavur (Voucher No. DB - 0062).

Specimen Accumulation

For histological research, transverse sections (T.S) of the various organs of the plant materials were cut and taken from the plant and fixed in FAA (Formalin 5ml + Acetic acid 5ml + 70% Ethyl alcohol 90 ml). After being fixed for 24 hours, the samples were dehydrated using a graduated series of tertiary butyl alcohol (TBA), following the protocol laid forth by sass (1940). Paraffin wax was added little by little until the TBA solution was supersaturated, then used to infiltrate the specimens. Paraffin was used to cast the specimens.

Sectioning

Using a rotator microtome, sections were cut through the paraffin-embedded specimens. The sections ranged in thickness from 10 to 12 um. The pieces were dewaxed using standard protocol (Johansen, 1940). Toludine blue was used to stain the sections as described by O'Brien et al., 1964. Saffranin and quick -green were used to stain the parts where they were deemed required. Olympus DP12 digital camera was linked to Olympus BX 40 microscope for taking microphotographs of the sections.

Physical and Chemical Criteria

The percentages of total ash, acid-insoluble ash, water-soluble ash, extractive values, and loss on drying (LOD) were determined in accordance with the Indian Pharmacopoeia (Anonymous, 1985), as were the physical and chemical constants consistency and organoleptic features.

Analysis of the Phytochemical Content of Dried Bark Extracted Using Several Methods

The extracts were evaluated for the presence of alkaloids, flavonoids, glycosides, phenols, resins, saponins, tannins, volatile oils, carbohydrates and amino acids using conventional technique.

Estimating the Quantity of Phytochemicals

Using a UV spectrophotometer (Lambda 25), the phenolics, tannins, carbohydrates, vitamin C, and vitamin D of raw DB were measured.

AAS for elemental analysis

We clean and dry the samples in the shade. After that, you bake the samples at 40-50 degrees Celsius until they reach a consistent weight. Then, using an agate pestle and mortar, the dried samples are reduced to a powder. Pre-cleaned containers are used to label and store samples. 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, thesurface 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 rootand root scars present in tubers, outer surface darkbrown, 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 thenodular 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 ofthe 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 theperiderm 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 hydroalcoholicextract of DB although having a lot ofphytoconstituents, is devoid of the main compoundfamilies indicated in the table. The 85 %hydroalcoholic extract showed to be a rich source ofglycosides, proteins, fats, sterols, alkaloids,polyphenols and tannins, flavonoids and saponins arequalitatively 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) andvitamin E (Jayasree et al., 1985) were estimated in bothraw herb and 85 % of hydroalcoholic extract (Table 2).The raw herb contained higher concentration ofphenolics followed by carbohydrates, whereas 85 % of hydroalcoholicextracts possessed higherconcentration 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 itis 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 aretabulated in Table 3. The results of the physiochemicalanalysis prove the stability, purity and firmness of the plant drug for use and are helpful to standardize for theuse as a potential drug (Indian Pharmacopoeia 1996).

Heavy metal analysis

After calibrating the instrument with prepared working standard, the digested liquid samples solution issubjected 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 dependingupon 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, theconcentration 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 andtrace 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 isno 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 onthe 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 usingAAS 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 bulbiferais used in diarrhoea, dysentery, piles, as a tonic, alternative, aphrodisiac, stomachic, anthdmintic, improves appetite, dyspepsia, leucoderma, bronchitisand applied to ulcer (Chopra et al, 1956). Macroscopic and Microscopic evaluation is an indispensable tool foridentification 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 aremore useful to man than being harmful. Human body requires mineral elements to certain extent. At the sametime, 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 usesfertilizer containing cadmium and organic mercury or lead based pesticides, and contaminated irrigation water (Abou Arab et al., 1999). Hence, analysis of

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Fig. 1*: Dioscorea bulbifera*

Fig. 2*: Exomorphic features of bulbs*

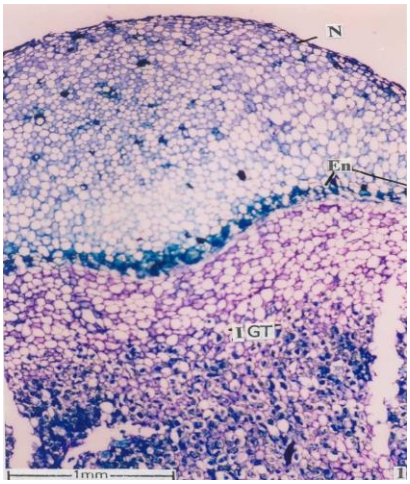

Fig. 3: T.S of bulb a sector enlarged

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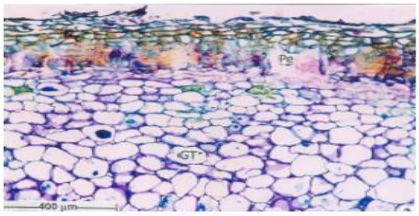


Fig. 4: Periderm and Ground Tissue

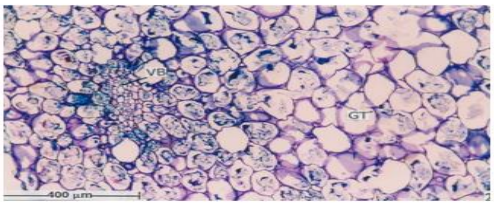
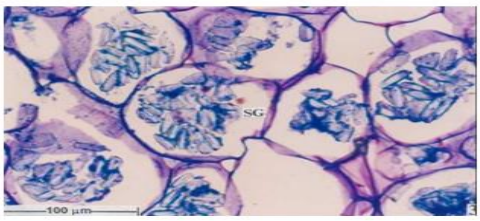


Fig. 5: Ground tissue and Vascular bundle



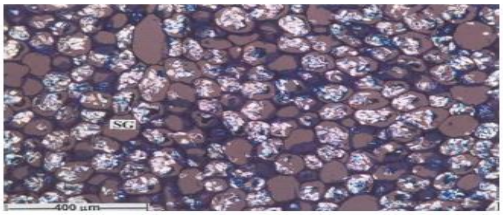


Fig. 6 & 7: T.S of the bulb showing starch grains

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