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PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTICAL STUDY OF *Dioscorea deltoidea* Wall. ex Griseb.

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ABSTRACT

The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. In the present study was planned to conduct phytochemical and pharmacognostic study of *Dioscorea deltoidea* leaf. The results of this study clearly indicate that the preliminary phytochemical analysis of *Dioscorea deltoidea* leaves revealed presence of flavonoids, phenolics, steroids, tannin, saponins, terpenoids, glycosides, steroids, alkaloids, protein and anthroquinones. Pharmacognostic study ensures plant identity, lays down standardization parameters which help and prevents adulterations. Such studies would help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. In Physico-chemical evaluation, ash values and extract values were studied. The ash and extract values are helpful in determining the quality and purity of a crude drug in the powdered form and to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvent.

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INTRODUCTION

Plant anatomy or phytotomy is the general term for the study of the internal structure of plants. While originally it included plant morphology, which is the description of the physical form and external structure of plants, since the mid-20th century the investigations of plant anatomy are considered a separate, distinct field, and plant anatomy refers to just the internal plant structures. Plant anatomy is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy (Zhao *et al.*, 2011).

Pharmacognosy is the study of medicines derived from natural sources. The word "pharmacognosy" is derived from the Greek words pharmakon (drug), and gnosis (knowledge). The term "pharmacognosy" was used

for the first time by the Austrian physician Schmidt in 1811 and 1815 by Crr. Anotheus Seydler in a work titled Pharmacognostica. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources" (AMP, 1998).

A Pharmacognostic study ensures plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

Dioscorea deltoidea leaf has been traditionally used as an anti-rheumatic and to treat popthalmic

conditions. In the western India it has been used as a source of steroid drugs. It has also been used to rid the body of intestinal worms as well as parasites, and sometimes the women use it to wash shawls and woolen cloth. Extract from the rhizomes are used to treat roundworm and have anti-rheumatic properties (Sofowara 1993). The aim of the study to investigate the phytochemical and pharmacognostical study of *Dioscorea deltoidea* leaf.

MATERIALS AND METHODS

Plant materials

The fully mature *Dioscorea deltoidea* leaves were collected in December 2015 from Vayimedu village, Nagappattinam district, Tamil Nadu, India.

Pharmacognostical study

The leaf of *Dioscorea deltoidea* was collected and rotary microtome sections were taken for leaf to obtain a thin section. The thickness of the section was 10-12 micrometers. Anatomical study invariably slides were prepared. The transverse sections of required parts (leaf) was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min. After placing a cover slip, care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip. Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper (Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue are to be confirmed by staining. To the powder a few drops of mixture of 1:1 Phloroglucinol + Conc HCl was added and after 3 to 4 minutes observed under microscope. The well known identifying characters were taken Photomicrographs by Sony digital camera under microscope (10 x & 40x) (Wallis, 1989; Dutta, 1971).

Preparation of alcoholic extract

The leaf of *Dioscorea deltoidea* was first washed well and dust was removed from the leaves. Leaf was washed several times with distilled water to remove the traces of impurities from the leaf. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Determination of physico-chemical parameters

Physico-chemical parameters of the powdered drug such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method described in WHO guidelines (WHO, 1998).

RESULTS

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for

plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

Medicinal plants are assumed greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Liu, 2003).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Dioscorea deltoidea* leaves investigated and summarized in Table-1. The phytochemical screening *Dioscorea deltoidea* leaves showed that the presence of flavonoids, Polyphenol, phlobatannins, tannin, saponins, triterpenoids, carbohydrate, glycosides, steroids, alkaloids, protein, terpenoids and anthroquinones.

Anatomical characteristics

Transversal section of the leaf

Pharmacognosy may be defined as "an applied science that deals with the biologic, biochemical and economic feature of natural drugs and their constituents." Modern aspects of science include not only the crude drugs but also their natural derivatives. Plant anatomy, in turn, has given rise to the independent science of cytology, which is the study of the cell, a rapidly developing field that plays a great role in the understanding of vital processes in general and of the phenomena of heredity and mutability in particular. Plant Anatomy is the branch of botany concerned with the internal structure of plants. It is closely related to plant physiology, the science of the vital processes which take place in plants.

Transverse section of *Dioscorea deltoidea* leaf through the midrib showed an upper and lower, single-layered epidermis that was externally covered with a thick, striated cuticle, a few epidermal cells on both lower and upper surfaces, parenchymatous cells that were thin-walled and isodiametric to circular (Plate 1). Intracellular spaces were present in ground tissue and the stele was crescent-shaped and composed of bicollateral and open vascular bundles. The xylem consisted mostly of vessels and tracheids, and a strip of cambium was present between the xylem and phloem tissues. The lamina which was dorsiventral with the mesophyll, was seen to be differentiated into a palisade and spongy tissue. The upper

and lower epidermises were covered externally with a thick, striated cuticle. Below the upper epidermis were three rows of elongated, closely arranged, palisade parenchyma. Spongy parenchyma tissues were almost radially elongated with intracellular spaces. Central cells were irregular in shape; laticifers and vascular bundles were also present scattered in this region.

In present study revealed the T.S. of *Dioscorea deltoidea* leaf containing, upper and lower epidermis, spongy parenchyma, palisade cells. The upper and lower epidermis are single cell layered with cuticle, the epidermal consist of a single layer of cubical cells with covering trichomes and emergences, which are sparsely distributed and show the presence of an external cuticular layer (Fig 1 and 2). The upper epidermal cells are somewhat larger than the lower. The mesophyll consist of a polysade and spongy parenchyma. A single layer of polysade parenchyma only present. Spongy parenchyma with large intercellular space present. The vascular bundle is in the leaf do not show the presence of bundle sheath. Ground tissue of midvein consists of collenchyma and parenchyma tissues. Collenchyma is present as a group of cells in the adaxial ridge and is three to four layered on the abaxial side, with cells angular thickened. Vascular tissue of midvein consists of an arc shaped vascular bundles, laterally.

Physicochemical analysis of *Dioscorea deltoidea*

The results of physicochemical parameters such as loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive water and soluble extractive are shown in Table 2. Present study evaluated the physico chemical analysis of *Dioscorea deltoidea* leaves powder. This work established the description loss on drying at 105 °C, moisture content, total ash, acid insoluble ash, water soluble ash and alcohol soluble extractive of *Dioscorea deltoidea* leaves were found to be 1.73 %, 1.01 %, 1.0 %, 2.0 % and 4.0 % respectively (Table 2

Table: 1 Phytochemical screening of *Dioscorea deltoidea* leaf

S.No	Phytochemical analysis	Results
1	Tannin	+
2	Phlobatannins	+
3	Saponin	+
4	Flavonoids	+
5	Steroids	+
6	Terepenoids	+
7	Triterpenoids	+
8	Alkaloids	+
9	Carbohydrate	+
10	Protein	+
11	Anthroquinone	+
12	Polyphenol	+
13	Glycoside	+

(+) Presence (-) Absence

Table 2: Physicochemical parameters of *Dioscorea deltoidea* leaves

S.No	Tests	As Per Analysis
	Colour	Pale green coloured fine powder
1	Loss on Drying at 105°C (Moister Content)	1.73%
2	Total Ash	1.01 %
3	Acid insoluble Ash	1.0%
4	Alcohol Soluble Extractive	4.0%
5	Water Soluble Extractive	2.0%

Fig 1 Transverse section of *Dioscorea deltoidea* leaf

- Ads – Adaxial side
- Ep – Epitermis
- Gt – Ground tissue
- L La – Lower lamina
- Mr – Mitrib
- Pa – Parenchymatous
- Ph – Phloem
- T – Trichomes
- U La – Upper lamina
- Vb – Vascular bundle
- X – Xylem

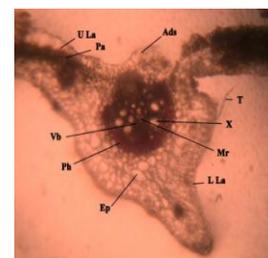
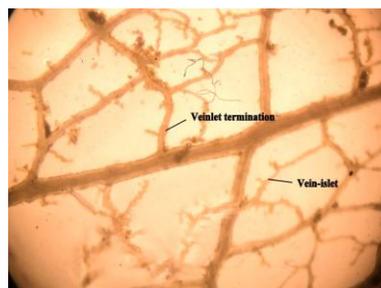


Fig 2a Vein terminal and islet of *Dioscorea deltoidea* leaf



DISCUSSION

The plants and its derivatives may considered as good sources of natural phytochemicals for medicinal uses Plant derived phytochemical therapy may be helpful for various free radical mediated diseases such as against cancer, diabetic mellitus, cardiovascular diseases and aging.

Falodun *et al.* (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al.* (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al.* (2007) extracted eight bioactive compounds from dry leaf of *Cnidioscolus aconitifolius* using water and ethanol.

Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al.* (2007) for its phytochemical properties.

Onwukaeme *et al.* (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycnanthus angolensis*. Uma Devi *et al.* (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins (Vaghasiya and Chanda, 2007). Ayoola *et al.* (2008) investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al.*, (2009) for its phytochemical and phytochemical properties.

Dinesh Kumar *et al.* (2014) examined the pharmacognostic evaluation of *Clerodendrum phlomidis* Linn. in terms of organoleptic, fluorescence analysis, macro-microscopy and physicochemical parameters. The characteristic macroscopic study showed that the root consists of 7-15 cm long, 0.2 -3.0 cm thick pieces which are cylindrical, tough and yellowish-brown externally, with hard fracture and slightly astringent taste. The main microscopic characters of the root show exfoliating cork, having 10-15 rows of tangentially elongated, thick-walled cells. Cortex consists of round to oval parenchymatous cells, a few containing rhomboid shaped calcium oxalate crystals. Endodermis consists of 3-4 layers of non-lignified, thick-walled rounded parenchymatous cells followed by a single pericyclic layer. Phloem consists of isodiametric, thin-walled, parenchymatous cells whereas xylem contains lignified pitted vessels. Medullary rays consisting of biseriate layer of lignified and radially elongated parenchymatous cells is narrower in the xylem region during wider in the phloem region.

According to WHO (1992, 1996a and b) standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. The parameters which are studied moisture content, description, loss on drying, total ash, acid-insoluble ash, sulphated ash, alcohol soluble and water-soluble extractive values, etc.

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The

compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.*2012).

The physicochemical analysis of plant drugs is an important for detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity and quality of drugs. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and nonphysiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or a, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or non-polar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles (Mukherjee, 2002).

Similarly work was done by Rajmohanan *et al.* (2014) the physicochemical studies on the *Cayratia pedata* (Lam)(Juss ex)Gagnep. Leaves showed significant total ash (9.62 – 10.50 %), acid-insoluble ash (3.2 – 3.9 %), loss on drying at 105 °C (7.23 – 8.25 %) and water soluble extractive (2.4 – 3.4 %) alcohol soluble extractive (8.95 – 9.35 %) respectively. Ash value used to determine quality and purity of crude drug.

The earlier studies of Giby Abraham (2015) showed that the physico-chemical analysis of *Vernonia cinerea* L. Physico-chemical such as total moisture content (4.6 %), total ash (7.44 %), water soluble ash (4.57 %), acid insoluble ash (0.29 %), ethanol extractive (5.29 %) and water extractive (10.24 %).

Kaskoos and Ahamad (2014) reported that the quality control parameters like extractive of *Andrographis paniculata* with different solvents, ash values, foreign organic matter and loss on drying were determined. Ujwala, (2013) reported the physicochemical analysis of the extract of *Bixa Orellana*.

CONCLUSION

The results of this study clearly indicate that the preliminary phytochemical analysis of *Dioscorea deltoidea* leaves revealed presence of flavonoids, polyphenol, steroids, tannin, saponins, terpenoids, carbohydrate, triterbenoids, glycosides, steroids, alkaloids, protein and anthroquinones. A Pharmacognostical study ensures plant identity, lays down standardization parameters which help and prevents adulterations. Such studies would help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products. In Physico-

chemical evaluation, ash values and extract values were studied. The ash and extract values were helpful in determining the quality and purity of a crude drug in the powdered form and to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvent.

Overall, the *Dioscorea deltoidea* leaves are a rich source of phytochemicals that can be important in disease prevention. These studies help in identification and authentication of the plant material. Such information can act as reference information for correct identification of particular plant and also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

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