

Pharmacognostic Study of *Dioscorea villosa* Leaves

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Abstract

Context: Yam (*Dioscorea* spp.) is an important edible tuber plant used for medicinal purposes to promote health and longevity in Chinese tradition. Steroidal saponins were reported to be the major physiologically active constituents in yams. Therefore, this study was designed to investigate its pharmacognostic characters for its standardization. **Materials and Methods:** Macroscopic, microscopic, and physiochemical evaluation of *Dioscorea villosa* was performed using standardized procedures as mentioned in the WHO guidelines. Thin-layer chromatography (TLC) was done to check the purity and identification of the drug. **Results and Discussion:** Morphology of *D. villosa* showed that the upper surface of leaves was green in color, having bitter taste and some characteristic odor. The presence of calcium oxalate crystal, thin long fibers, xylem vessels, and epidermal cells was observed in powder microscopy. Phytochemical analysis of crude extracts indicated the presence of alkaloids, glycosides, saponins, and flavonoids. The presence of saponins was also confirmed by foaming index. TLC was done for all the extracts which showed a number of spots indicating the presence of number of chemical constituents. **Conclusion:** All the above pharmacognostic parameters could be useful for the authentication and preparation of monograph for *D. villosa*.

Key words: Diosgenin, extraction, standardization, thin-layer chromatography

INTRODUCTION

India is a vast repository of medicinal plants that are used in traditional medical treatments. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines.^[1] This led to sudden intensification in a number of herbal drug manufactures.^[2]

Standardization refers to the confirmation of identity, determination of quality and purity, and detection of the adulterant if any.^[3] Methods of standardization have to include all aspects that contribute to the quality of the herbal drugs, correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation such as ash values and extractive values, phytochemical evaluation, microbial load testing, toxicity testing, and biological activity.^[4]

The genus *Dioscorea* (family of Dioscoreaceae), known as yam, comprises of about 600 species distributed throughout the world. Most species contain steroid saponins and also sapogenins, such as *Diosgenin*, which is the starting material

of industrial interest in the synthesis of many steroids which are sold in the market as anti-inflammatory, androgenic, estrogenic, and contraceptive drugs.^[5,6]

Dioscorea villosa (Dioscoreaceae) is a tuberous, twining vine with pale-brown, knotty, woody, cylindrical tubers. The leaves are symmetrical and heart-shaped, gradually tapering to a sharp, acuminate point, and are born on leaf stalks.^[7] *Diosgenin* [Figure 1] is the primary active ingredient in *Dioscorea*. It is structurally similar to cholesterol.^[8] After oral administration, it is metabolized in the liver and eliminated through the bile. Over 50 steroid saponins of furostan-, spirostan-, and pregnane-type skeletons have been isolated and characterized from various *Dioscorea* species, and these compounds have been reported to be the major physiologically active constituents in yams.^[9]

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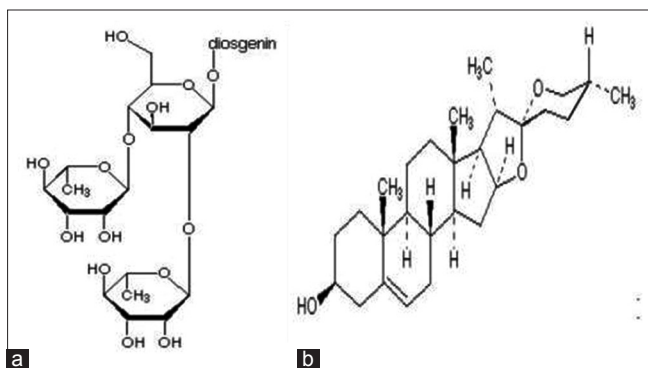


Figure 1: Chemical structure of (a) dioscin (b) diosgenin

D. villosa invites the attention of the researchers worldwide for its pharmacological activities. In the traditional system of medicines, it is used in rheumatoid arthritis, inflammation, irritable bowel syndrome, gallbladder inflammation, etc.^[10,11] Diosgenin is used as a raw material for the formation of female sex hormones. It is such a good antispasmodic that it can be used for cramps, coughs, and hiccoughs and for muscular spasms, croup, and gas. It is considered good for loosening phlegm, inducing vomiting, and increasing urine flow.^[12] As this plant has multifarious biological activities and used traditionally to treat various ailments, therefore, it must be authenticated and standardized to avoid any kind of adulteration. Taking in view its importance, this study was designed to evaluate pharmacognostic parameters and develop its monograph.

MATERIALS AND METHODS

Plant Material

The leaves of *D. villosa* were collected from Herbal Garden of Lovely Professional University. Plant materials were authenticated at the National Institute of Science and Communication Research, New Delhi. A Voucher Specimen No. 147 has been deposited in the same department herbarium.

Pharmacognostic Evaluation

Macroscopic characters

Morphological characteristics such as color, odor, and taste of the dried leaves of *D. villosa* were observed with the naked eye or with the aid of a magnifying lens.

Powder characteristics

The powder of leaves of *D. villosa* was cleared with the chloral hydrate and stained with respective reagents such as phloroglucinol, hydrochloric acid, iodine solution, and ruthenium red. The sample was mounted on glass slide and observed under the microscope ($\times 10$ and $\times 45$) to determine the type of cells, phloem fibers, and lignified tissues.^[13]

Physicochemical Evaluation

Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ash values of vegetable drugs is to determine the inorganic matter present in the drug. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates, sulfates, and silicates of sodium, potassium, calcium, and magnesium.^[14]

Extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Varieties of chemical compounds are available in crude drugs having variable properties. Various solvents are used for the extraction of various chemical compounds in a particular amount.^[15]

Foaming index

Many medicinal plant materials contain saponins that cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of the foaming index.^[15]

Determination of Water and Volatile Matter

Loss on drying

The moisture content of a crude drug will be responsible for the decomposition of crude drugs either producing chemical change or microbial growth. Hence, the moisture content of a drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.^[16]

Karl Fischer titration

A specified quantity (50 mg) was weighed and added to the methanol in the beaker. The start titration button was pressed, and the sample was titrated.^[17] The moisture content was calculated by the following formula:

$$\text{Moisture content} = \frac{100 \times \text{Karl Fischer reagent reading} \times \text{Factor}}{\text{Weight of sample (mg)}}$$

Extraction

Successive solvent extraction was performed on small scale with coarsely dried powdered plant material (50 g). The plant material was extracted in a Soxhlet apparatus with petroleum ether (400 ml) at 60°C for 18 h, and then, the plant material was dried at room temperature and the dried plant material was soxhleted with chloroform (400 ml) at 63°C for 18 h and dried at room temperature. The same process was repeated for methanol at 64°C and water at 100°C. The extracts after

filtration were evaporated to dryness under reduced pressure and made to a specified volume.

Phytochemical screening of extracts

The extracts prepared from the powdered leaves of *D. villosa* were subjected to preliminary phytochemical screening using standard methods for different classes of phytoconstituents using specific standard reagents.^[13,18] The phytochemical screening helps in identifying the chemical constituents belonging to a particular class as each class has its own pharmacological importance.

Thin-layer chromatography (TLC) of extracts

TLC is a method for analyzing mixtures and determining the number of components present in it. It is also used for the identification and to check the purity of a compound. The chromatograms were taken on precoated silica plates (alumina base, 0.2 mm thickness, E. Merck). About 10 µl of these extracts were applied on precoated plates for developing TLC profile. The chosen solvent system was ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.6). Ethanolic sulfuric acid (4%) was used as a spraying agent.

RESULTS

Pharmacognostic Evaluation

Macroscopic evaluation

Macroscopy of the leaves of *D. villosa* was carried out, and the characteristics observed are briefed in Table 1.

Powder microscopy

Powder microscopy of *D. villosa* leaves revealed the presence of crystals and epidermal cells. Phloem fibers were observed in bundles as well as in individual. Xylem vessels were also present [Figure 2].

Physicochemical Evaluation

Ash values

Total ash, acid insoluble ash, and water soluble ash of *D. villosa* leaves were determined using standard procedure as mentioned in the WHO guidelines. Drug used was 2 g. The total ash, acid insoluble ash, and water-soluble ash were found out to be 450 mg/g, 30 mg/g, and 37 mg/g, respectively.

Extractive value

Extractive value was found out by both the methods, i.e., hot extraction and cold maceration. Amount of drug used was 4 g. Maximum extractable matter was found in the aqueous extract (69.5 mg/kg) by cold maceration method followed by alcoholic extract (37.5 mg/kg) by hot extraction.

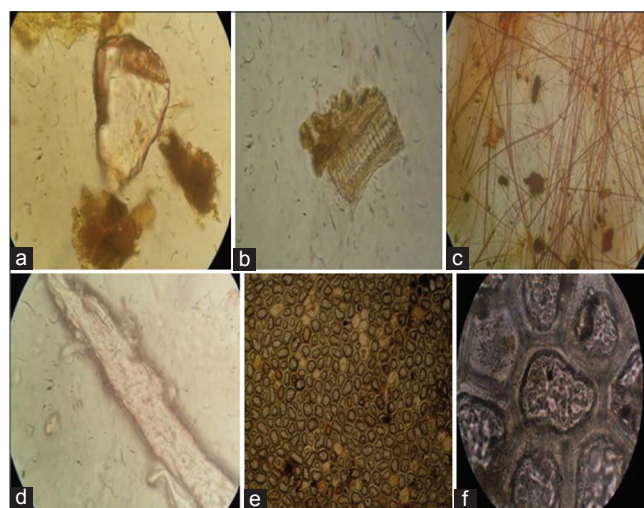


Figure 2: Powder microscopy of the leaves of *Dioscorea villosa* showing (a) calcium oxalate crystal (b) xylem vessel (c) fibers (d) phloem fiber (e) epidermal cells (×10) (f) epidermal cells (×45)

Table 1: Macroscopy of the leaves of *D. villosa* was carried out, and the characteristics

Characters	Leaves
Color	Dark green
Odor	Odorless
Taste	Characteristics
Surface characteristics	Heart-shaped smooth leaves

D. villosa: *Dioscorea villosa*

Foaming Index

As in all test tubes, the height of foam was more than one. Hence, foaming index was found out to be more than 1000, which indicates the presence of saponins (Table 2).

Determination of water and volatile matter

Loss on drying was found to be 86 mg/kg and moisture content was found out to be 17.4%.

Phytochemical screening

All the extracts were subjected to preliminary phytochemical screening, and the results obtained are shown in Table 3. Alkaloids, glycosides, and flavonoids were found to be positive in the methanolic and aqueous extract. Tannins and saponins were present in chloroform, methanolic, and aqueous extracts. Steroids were found to be positive in methanolic and petroleum ether extracts.

TLC

The solvent system ethyl acetate:Formic acid:Acetic acid:water (10:1.1:1.1:2.6) gave the best resolution and maximum number of resolved components for methanol and aqueous extracts. Different components were separated as shown in Figure 3.

Table 2: Foaming index

Test tube	1	2	3	4	5	6	7	8	9	10
Ratio of water: Extract	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9	0:10
Height of foam (cm)	1.2	1.2	1.5	1.6	1.6	1.4	1.7	1.7	1.8	1.9

Table 3: Qualitative chemical examination of extracts

Chemical constituent	Tests	Extracts			
		Petroleum ether	Chloroform	Methanolic	Aqueous
Alkaloids	Mayer's test	+	-	-	+
	Dragendorff's test	-	+	-	+
	Wagner's test	-	-	+	+
	Hager's test	-	-	-	+
Carbohydrates	Molisch's test	-	-	-	-
	Benedict's test	-	-	-	-
	Fehling's test	-	-	-	-
Glycosides	Modified Borntrager's test	-	-	+	+
	Legal test	-	-	+	+
Saponins	Froth test	-	+	+	+
Phytosterols	Salkowski's test	-	-	+	+
Steroids	Acetic anhydride test	+	-	+	-
Fixed oils	Stain test	+	-	-	-
Phenols	Ferric chloride	-	+	+	+
Tannins	Gelatin test	-	+	+	+
Flavonoids	Alkaline reagent test	-	-	+	-
	Lead acetate	-	-	+	+
	Zinc hydrochloride test	-	-	+	-

(-) sign indicates the absence of constituent in the respective screening test; (+) sign indicates the presence of constituent in the respective screening test

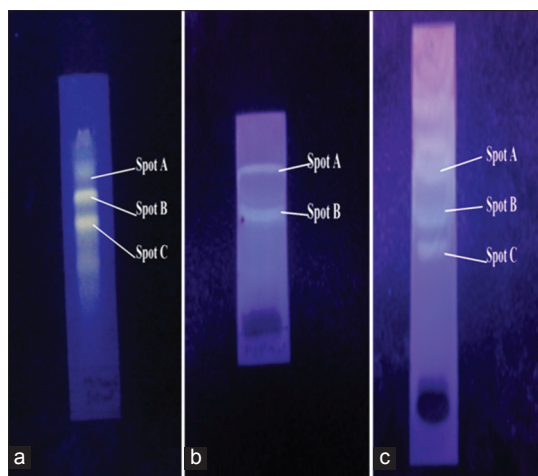


Figure 3: Thin-layer chromatography profile of leaf extracts of *Dioscorea villosa*, (a) methanol extract; (b) chloroform extract; (c) aqueous extract

In case of methanolic extract, the R_f values of separated constituents ranged from 0.4 to 0.6, and in case of aqueous extract, the R_f values ranged from 0.49 to 0.69, while the R_f values ranged from 0.73 to 0.82 in case of chloroform extract. As can be seen in the images, the type of constituents present is quite similar in three extracts, but their amount is different as observed from different intensities of bands.

DISCUSSION

Herbal drugs are an integral part of the Indian system of medicine (Ayurveda) which is an ancient and mainstream system. India has one of the richest plants of medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine, and known to rural communities in India. Medicinal plants

play a central role not only as traditional medicines but also as trade commodities.^[19]

Authentication of plant material is a precondition before using it as a research material. Therefore, it was planned to establish pharmacognostic standards of *D. villosa*. Pharmacognostical studies on the plants, namely determination of physicochemical constants and development of TLC profiles, provide suitable standards for the identification of plant materials. The phytochemical screening of *D. villosa* confirmed the presence of alkaloids flavonoids, glycosides, saponins, phytosterols, tannins, and steroids which may be the responsible candidates for its pharmacological activities. TLC was done for all the extracts which showed a number of spots indicating the presence of number of chemical constituents.

CONCLUSION

All the above diagnostic, macroscopic, and microscopic features and pharmacognostic parameters could be a useful tool for the identification, authentication, and preparation of suitable monograph of *D. villosa*. This study would also help to check the adulteration of this important medicinal plant and is of much importance for further research on this plant.

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