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Pharmacognostic and phytochemical evaluation of *Dolichos biflorus* Linn.

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PEER REVIEW

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Comments

Study is based upon the very popular medicinal plant. Article gives the detail information regarding the various evaluation parameter of the plant like ash values, extractive values, microscopical parameter and the chemical group present in the plant which may be responsible of its various pharmacological activities.
Details on Page S101

ABSTRACT

Objective: To study in detail the micromorphology and physicochemical analysis of the seeds of *Dolichos biflorus* Linn. (Family: Papileonaceae).
Methods: Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed.
Results: The seeds are roughly trapezoidal and flattish, with quite thin cotyledons. The hylum is small and linear and located in a small depression on the seed's lateral margin. The length of archeological specimens are usually 3.0-4.0 mm, width 2.0-2.6 mm and thickness 1.4-2.0 mm. Microscopic evaluation revealed the epidermis is single layered brown in colour, thin walled and shining cells because of mucilage in this layer. Endosperm forms bulk of the seed with thick walled polygonal parenchymatous cells. Outer portion of the seed contains alueron grains which are protein in nature. In the mid of the seeds, embryo can be seen which provides nutrition. Preliminary phytochemical screening showed the presence of steroids, tannins, proteins, aminoacids, flavonoids, terpenoids, mucilage, volatileoil, saponin and carbohydrates and absence of alkaloids, fixed oil.
Conclusions: The microscopic using histological identification, microscopic constants and other physico chemical examinations of the seeds of *Dolichos biflorus* Linn. can be used as a rapid, inexpensive botanical identification technique and is useful in standardization, hence it would be of immense value in authentication of seed.

KEYWORDS

Papileonaceae, Microscopical evaluation, Physicochemical studies, Phytochemical studies, Standardization

1. Introduction

Dolichos is a genus of family Papileonaceae. A genus of twining herbs distributed in the tropics of both hemispheres. About 8 species occur in India of which *Dolichos biflorus* (*D. biflorus*) and *Dolichos lablab* are extensively cultivated and used. The plant is a common twining creeper, a branched sub-erect or trailing annual with small trifoliate leaves, bearing when mature, narrow flat curved pods 1.5-2.0 inches long tipped with a persistent style. The pods contain 5-6 flattened, ellipsoid seeds 1/8-1/4 inch long. It distributes native to most parts of India, and is found up to altitude of 5 000 m. It was reported that seed (both tender and mature) contains are poor in amino acid content, but rich in urease. A new and nonspecific lectin having the inner carbohydrate moiety as N-acetyl glucosamine, N-M-glycosidically linked to aspargine has been isolated from seed of *D. biflorus*. Constituents of grain with husk: albuminoids, starch, oil, fiber, ash, and phosphoric acid: enzyme urease[1,2].

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The plant is much more popular in India than in any other country since antiquity. Seeds possess astringent, diuretic and tonic properties. Decoction of seeds is used in diarrhoea, haemorrhage from bowels, and is given to females during parturition to promote discharge of the lochia. Pulse is a demulcent in calculus affection and coughs. A soup is diet in sub acute cases of enlarged liver and spleen. As a home remedy, kulthi has been used in dysuria, bleeding piles, vaginal bleeding, and leucorrhoea. Its use in reducing obesity is also recognized. In dysuria, it works due to its diuretic property. It is also used to reduce crystalluria and to lyse stones. The powdered seeds are used as a poultice to induce sweating[3].

In short, there is good level of traditional and experimental evidences to support various claims and advantages of this widely available plant[4].

As mentioned earlier, several reports have been published on the effects of the plant extract and chemical constituents on different biological[5] activities *in vitro* and *in vivo*. An investigation to explore its pharmacognostic examination is inevitable. This work we report an attempt on microscopic evaluation, physicochemical determination and phytochemical screening for the standardization and quality assurance purposes of this cultivar.

2. Materials and methods

2.1. Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

2.2. Plant collection and authentication

The seeds of the *D. biflorus* Linn. selected for our study was collected from local market, Jhansi, Uttar Pradesh, India and identified by Dr. Gaurav Nigam, Coordinator, Department of Botany, Bundelkhand University, Jhansi (U.P.), India. A voucher specimen of the plant has been deposited at the herbarium of Department of Botany, Bundelkhand University, Jhansi (U.P.), India (Bot/01/12).

2.3. Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste *etc*. were noted.

2.4. Microscopic analysis

Transverse section midrib region of fresh seed were cut and fixed in formalin/acetic acid/alcohol. Sections were taken using microtome. Permanent mount was prepared using saffranin fast green double staining technique. In order to supplement the descriptive part, the photomicrographs in different magnifications of all necessary cells and tissues were taken.

2.5. Powder microscopy

Course powder of seed was used to study the microscopical characters of the seed powder.

2.6. Physicochemical analysis

Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values were determined.

2.7. Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phyotoconstituents using standard procedure[6].

3. Results

3.1. Macroscopy

The seeds are roughly trapezoidal and flattish, with quite thin cotyledons. The hylum is small and linear and located in a small depression on the seed's lateral margin[7]. The length of archeological specimens are usually 3.0-4.0 mm, width 2.0-2.6 mm and thickness 1.4-2.0 mm.

3.2. Microscopy of the seed

Testa: Epidermis is single layered brown in colour, thin walled and shining cells because of mucilage in this layer.

Endosperm: Endosperm form bulk of the seed with thick walled polygonal parenchymatous cells.

Alueron: Outer portion of the seed contains alueron grains which are protein in nature.

Embryo: In the mid of the seeds, embryo can be seen which provide nutrition[8,9].

Microscopy of the seed can be seen in Figures 1, 2 and 3.

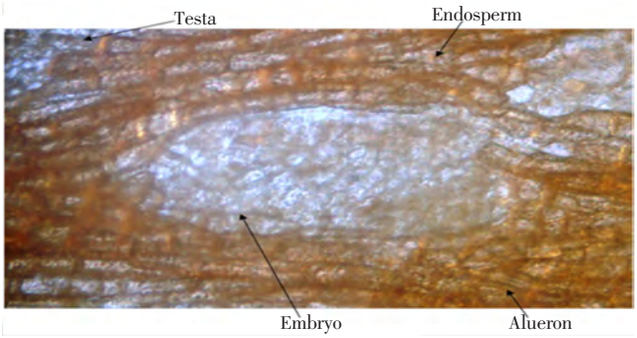


Figure 1. Microscopy of seed of *D. biflorus*.

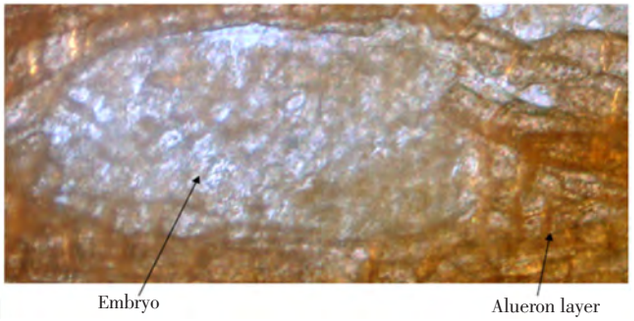


Figure 2. Magnified portions of microscopy of seed of *D. biflorus*.

3.3. Powder microscopy

The analysis of the dried powder of the seed of *D. biflorus* Linn. showed polyhedral epidermal cells of surface view with thick and straight walls, testa, endosperm, pigment layer and starch (Figure 3).

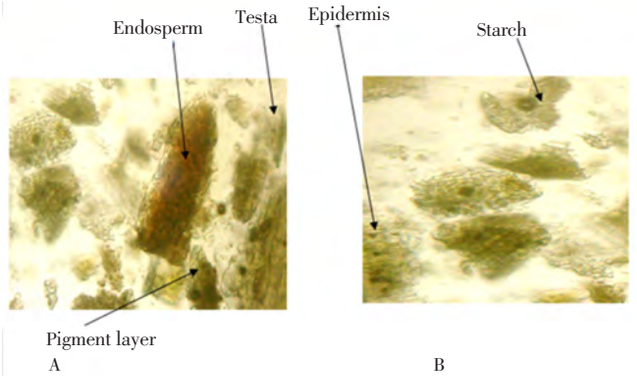


Figure 3. Powder microscopy seeds of *D. biflorus*.

3.4. Physicochemical analysis

The physical constants such as total ash value, loss on drying and soluble extractive values were detarmined (Tables 1–3).

Table 1
Determination of ash values of *D. biflorus*.

S. No.	Ash type	Percentage of Ash
1.	Total ash	4.684% w/w
2.	Acid insoluble ash	0.478% w/w
3.	Water soluble ash	5.030% w/w
4.	Sulphated ash	9.680% w/w

Table 2
Determination of loss on drying of *D. biflorus*.

S. No.	Plant	Weigh (g)
1.	Weight of drug+disc before drying (A)	68.3961
2.	Weight of drug+disc after drying (B)	67.2999
3.	(A–B)	1.0962
4.	Loss on drying (%)	10.9000

Table 3
Determination of extractive value of *D. biflorus*.

Extract	Yield (%)
50% Aqueous sodium hydroxide soluble extractive value	0.96
Water soluble extractive value	2.32
Methanol soluble extractive value	1.34

3.5. Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of carbohydrate, glycosides, saponins, tannin (Table 4).

Table 4
Preliminary phytochemical screening of *D. biflorus*.

Test	50% Aqueous sodium hydroxide	Distilled water	Methanol
Alkoaids	–	–	–
Carbohydrate	+	+	+
Flavonoids	–	–	–
Glycosides	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Proteins	–	–	–

3.6. Thin layer chromatography (TLC)

"Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding"

$$R_f = \frac{\text{Distance travelled by solute front from origin line}}{\text{Distance travelled by solvent front from origin line}}$$

Where R_f=Retention factor.

The ethanolic extract of powdered of *D. biflorus* Linn. was subjected to TLC studies, to find the presence of number of compounds which support by the chemical test.

In solvent system of toluene:ethyl acetate:acetic acid (7:2:1), colour of TLC spots can be observed in Figure 4.

3.7. High performance thin layer chromatography (HPTLC) finger printing

Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system of toluene:ethyl acetate:acetic acid (7:2:1)

was ideal and gave well resolved sample peaks (Figure 5). Given the spots of the chromatogram were visualized at 366 nm.

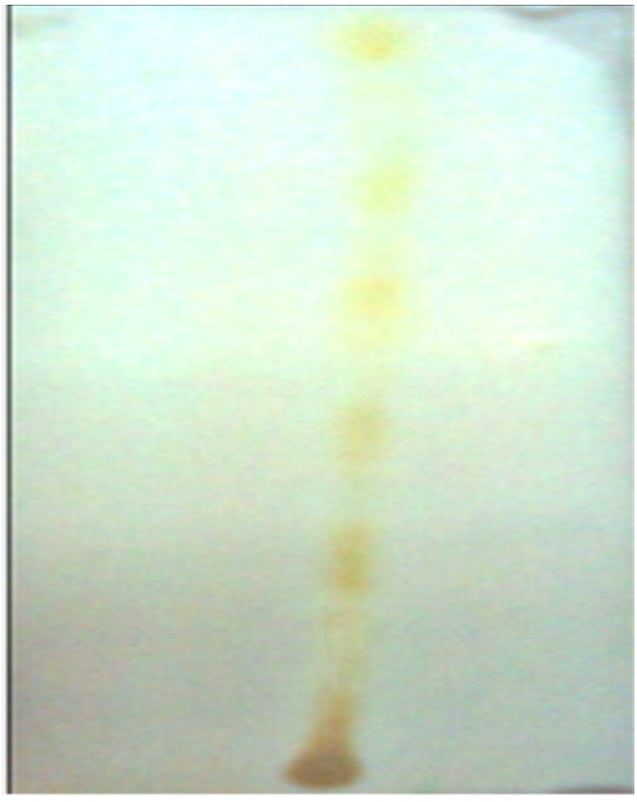
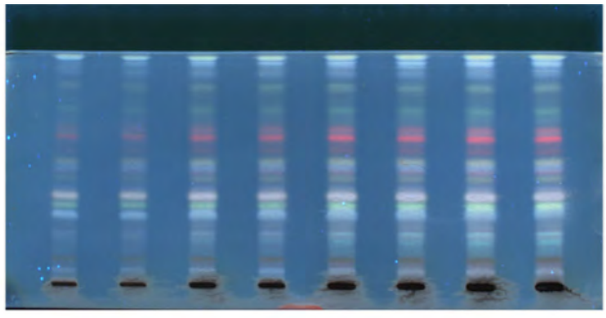
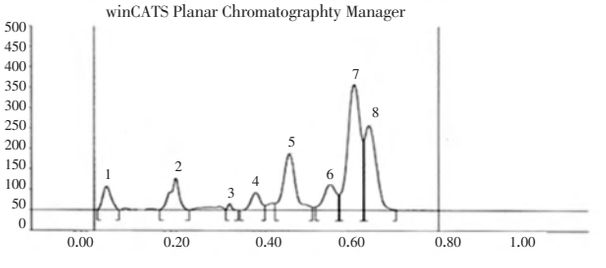


Figure 4. TLC finger printing of extract on *D. biflorus* Linn.





Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %	Assigned substace
1	0.04	0.2	0.06	57.3	6.30	0.09	0.2	878.9	4.9	unknown *
2	0.18	0.3	0.21	77.3	8.50	0.24	0.5	1203.5	6.61	unknown *
3	0.32	2.3	0.33	15.5	1.71	0.35	0.0	110.7	0.57	unknown *
4	0.36	0.1	0.39	42.5	4.66	0.41	11.4	771.9	3.94	unknown *
5	0.44	16.6	0.47	137.5	15.12	0.52	7.5	3137.1	16.03	unknown *
6	0.53	8.2	0.56	63.3	6.96	0.56	38.0	1490.5	7.62	unknown *
7	0.58	38.5	0.62	308.2	33.89	0.64	170.2	7426.6	37.95	unknown *
8	0.64	171.9	0.65	207.7	22.84	0.71	1.0	4461.7	22.80	unknown *

Figure 5. HPTLC finger printing and chromatogram of extract on *D. biflorus* Linn. 366 nm.

4. Discussion

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new good manufacturing practices in quality control of raw materials, intermediates and finished products of botanical origin. The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials. In our present work, we selected the plant *D. biflorus*. The macroscopic and organoleptic characters of the seed can serve as diagnostic parameters. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adulterated drug. Preliminary phytochemical screening will reveal the useful information about the chemical nature of the drug. Preliminary phytochemical screening showed the presence of carbohydrate, glycosides, saponins and tannin.

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Microscopical evaluation and physicochemical standards and preliminary phytochemical reports can be useful to substantiate and authenticate drug.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Natural products are main source for the various type of medicinal agent. Approximate 40% of medicinal agent still derived from natural source only directly or indirectly. The selected topic is the analysis of the plant which is widely use in the entire Indian continental.

Research frontiers

Studies are performed on the seeds of *D. biflorus*. Macroscopical, microscopical, physicochemical details and phytochemical details of aqueous and methanol extract are performed.

Related reports

The positive presence of some constituents like carbohydrate, tannins, glycosides and other are also mentioned in some other studies. The various mentioned microscopical and macroscopical parameter are also complies with other reports.

Innovations & breakthroughs

The article gives the detailed information of the various phytoconstituents which are present in the plants. The various physicochemical parameter and HPTLC fingerprint are useful for its identification.

Applications

The various pharmacological studies can be performed on the basis of phytoconstituents mentioned in the reports. Morphological, microscopical, physicochemical details are helpful for the standardization of the plant.

Peer review

Study is based upon the very popular medicinal plant. Article gives the detail information regarding the various evaluation parameter of the plant like ash values, extractive values, microscopical parameter and the chemical group present in the plant which may be responsible of its various pharmacological activities.

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