

Standardization of Seeds of *Dolichos Biflorus* Linn.

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Seeds of *Dolichos biflorus* Linn. are considered to be very useful for removing kidney stones. These are used as astringent, diuretic and tonic. Seeds have been identified by their macroscopic and microscopic characters, cell contents, behaviour of powdered drug with different reagents and preliminary phytochemical analysis.

Key Words: Standardization, Seeds, *Dolichos biflorus* Linn.

INTRODUCTION

Dolichos biflorus Linn. (Fam. Leguminosae) is also known as horse gram and *Kulthi* in Hindi. It is a native of Southeast Asia, throughout the tropics, India, Malaysia and West Indies. About 14 species occur in India, of which *D. biflorus* and *D. lablab* are extensively cultivated and used either as human food (beans or seeds) or as animal fodder (leaves and stem). Seeds extract seem to be useful for the patients suffering from urinary or kidney troubles, eye troubles, piles, enlargement of the spleen and pain in the liver¹⁻⁵.

EXPERIMENTAL

The seeds of *Dolichos biflorus* were procured locally from Modinagar market and identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

Macroscopic and microscopic studies were made from free hand. Seeds were powdered by crushing in electric grinder. Behaviour of powdered drugs was studied by treating with different chemical reagents. Foreign organic matter, loss on drying, ash values, extractive values and other physical parameters on seeds of *D. biflorus* Linn. were determined as per I.P. Methods⁶. Preliminary investigations on fluorescence behaviour of ethanol extracts under long (365 nm) and short (257 nm) UV radiation were also studied.

RESULTS AND DISCUSSION

Macroscopic Characters: Fruits contain 5–7 seeds, compressed, hard, surface smooth, ellipsoid, flattened, 4–6 mm long and 4 mm wide, micropyle prominent, greyish to reddish brown in colour (Fig. 1).

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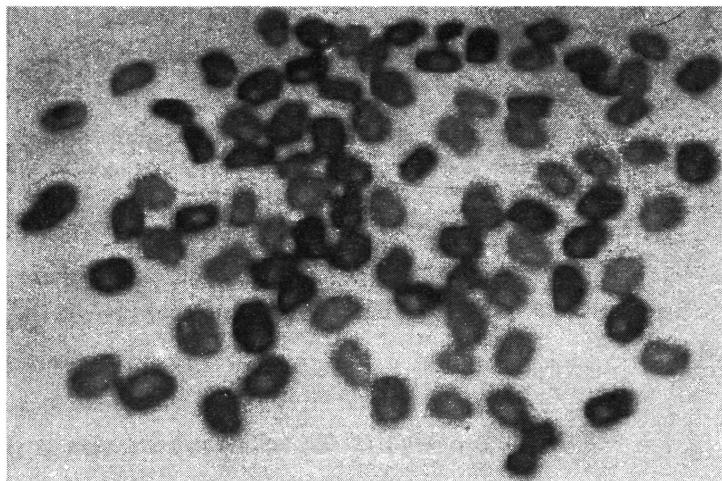


Fig. 1. Seeds of *Dolichos biflorus* Linn.

Microscopic Characters: Transverse section of seed shows testa consisting of a single layer of columnar, thin-walled, parenchymatous, palisade like cells covered with a thin cuticle followed by single layer of rectangular to square bearer cells and 3–4 layers of thin-walled rectangular parenchymatous cells, more wide at micropylar region; cotyledon consisting of single layer of upper and lower epidermis covered with a thin cuticle; epidermal cells thin-walled, rectangular and parenchymatous followed by mesophyll, consisting of angular parenchymatous cells, filled with numerous simple starch grains and protein bodies also present. Powder is whitish in colour, consisting of broken pieces of testa, parenchymatous cells and starch (Fig. 2).

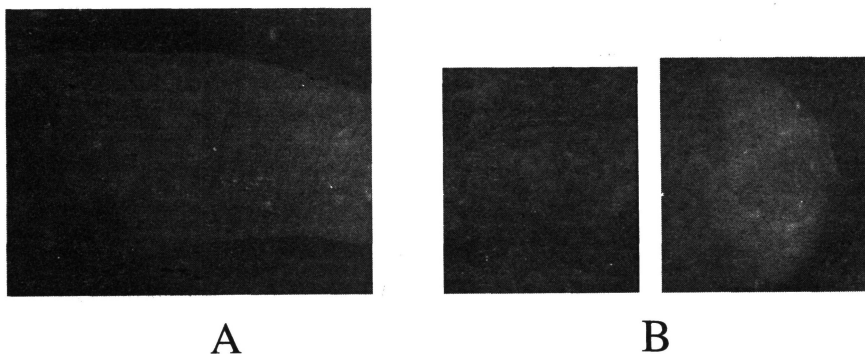


Fig. 2. Microscopic characters of seeds of *Dolichos biflorus* Linn.: (A) T.S. of seed (cellular) $\times 100$, (B) Powder characteristics $\times 100$

Physical Constant Values

Foreign organic matter : Nil, Loss on drying : (10.9%); Total ash : (4.07%); Acid-insoluble ash : (0.80%); Sulphated ash : (8.38%); Water-soluble ash : (2.97%); Ethanol-soluble extractive : (0.48%); Water-soluble extractive : (5.16%); Petroleum ether-soluble extractive : (1.56%); Chloroform-soluble extractive : 0.23% Volatile oil content : (Nil); Fluorescent analysis : Very faint fluorescence in short and long UV light

Cell Contents: Fats and oil present in the form of globules in the thin-walled cells of the seed when treated with conc. HCl fat globules are liberated.

Reaction of powdered drug with different reagents

Water	:	Powder settles at the bottom producing dark grey brown coloured turbid solution with very little frothing on the surface.
5% KOH	:	Powder settles at the bottom producing greenish coloured turbid solution.
Dil. HCl	:	Powder settles at the bottom producing clear solution
Dil. H ₂ SO ₄	:	—do—
Dil. HNO ₃	:	—do—
FeCl ₃ soln.	:	Powder settles at the bottom producing clear orange liquid
Dragendorff's soln.	:	—do—
KI and I soln.	:	Powder settles at the bottom producing reddish brown clear liquid.

Preliminary phytochemical analysis

Qualitative examination of the various solvent extracts of seeds indicates the presence of fixed oil, carbohydrate, protein, fat and sterols⁷.

Thin-layer chromatography

Part I: Seeds powder was defatted with petroleum ether (60–80°C) in soxhlet extractor. 1.0 g of defatted seed powder was warmed with 10 mL ethanol (70% v/v) for 30 min and centrifuged. The residue was re-extracted with ethanol and centrifuged. This process was repeated (8–9 times) till the supernatant was negative to ninhydrin test. All the supernatants were combined and evaporated to dryness *in vacuo*, dissolved in 0.5–1.0 mL distilled water and centrifuged. The clear supernatant was subjected to thin-layer chromatography by using TLC aluminium sheets (Merck). *n*-Butanol : acetic acid : water and 96% ethanol : water were used as mobile phase. The chromatograms were sprayed with ninhydrin (0.1% w/v) in butanol. Observations are given in Table-1.

TABLE-1
SOLVENT SYSTEM

S.No.	<i>n</i> -Butanol : acetic acid : water (8 : 2 : 2)		96% ethanol : water (7 : 3)		Amino acids identified
	R _f reported ⁸	R _f found	R _f reported ⁸	R _f found	
1.	0.22	0.22	—	—	Alanine
2.	0.05	0.06	0.33	0.33	Histidine
3.	0.09	0.09	0.39	0.39	Cystine
4.	0.17	0.17	0.55	0.55	Aspartic acid
5.	0.44	0.45	0.61	0.60	Leucine
6.	0.18	0.18	0.43	0.42	Glycine
7.	—	—	0.48	0.48	Serine
8.	0.03	0.03	0.03	0.03	Lysine

Part II: The defalted seeds were extracted with water and concentrated to dark brown mass. It was found to respond to positive tests for sugars which were identified by thin-layer chromatography on silica gel G, impregnated with sodium acetate buffer using (i) chloroform : methanol, (ii) acetone : water as solvent system and aniline hydrogen phthalate as spraying reagent. Observations are given in Table-2.

TABLE-2
SOLVENT SYSTEM

S.No.	Chloroform : methanol (6 : 4)		Acetone : water (9 : 1)		Sugars identified
	R _f reported ⁸	R _f found	R _f reported ⁸	R _f found	
1.	0.54	0.53	0.71	0.72	Rhamnose
2.	0.41	0.41	0.53	0.53	Arabinose
3.	0.30	0.29	0.47	0.48	Fructose
4.	0.27	0.27	0.45	0.45	Galactose
5.	0.37	0.36	0.55	0.56	Glucose

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