

**PHYSICO-CHEMICAL AND PHYTO-CHEMICAL ANALYSIS OF DOLICHOS BIFLORUS  
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**ABSTRACT**

**Introduction:** *Dolichos biflorus* Linn. (Family Fabaceae) commonly known as horse gram is used as a vegetable in India and the seed is known as the poor man's pulse in southern India. It is a well known medicinal plant in folklore for its medicinal properties. **Objectives:** In the present study an attempt has been made to standardize this ethnopharmacologically useful seed on the basis of pharmacognostical, physico-chemical and phytochemical characteristics. **Methods:** The external morphology of seed was observed by using simple microscope. The percentage of foreign matter, loss on drying, Total ash, water soluble ash, acid insoluble ash, sulphated ash of dried seed powder were determined. Aqueous and methanolic Extraction, was done using soxhlet apparatus and the extracts were used for Preliminary phytochemical screening. **Results:** The seeds were odorless, slightly acrid and nonaromatic. The loss on drying was 1.45%, the Total Ash was 7.72 % and the Water Soluble Ash was 2.85% whereas acid insoluble and sulfated ash showed low content (3.10 and 1.71% respectively). Methanol and water soluble extractive values (hot) were 15.21 %, 8.50 % w/w respectively. The preliminary phytochemical screening results certified the presence of alkaloids, phenols, flavonoids, steroids, carbohydrates, proteins, triterpenoids, tannins, saponins and mucilage (except starch) in methanolic extract of seed. **Conclusion:** The present study established pharmacognostic, physico-chemical, phytochemical, microscopic characterizations and provided referential information for correct identification and standardization of this plant material.

**KEYWORDS:** *Dolichos biflorus* Linn., Fabaceae, standardization, physico-chemical evaluations, Phytochemical analysis.

**INTRODUCTION**

Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector, WHO recommends and encourages the use of traditional herbs/remedies because of easy availability and affordability.<sup>[1]</sup> A lack of proper documentation, stringent quality control and standardization has hindered the acceptance of the alternative medicines in the developed countries. There is a growing concern for documentation of research work carried out on traditional medicines needed for regulatory control.<sup>[2]</sup> The maintenance of herbal medicinal quality depends on both scientists and the manufacturers and consumers.

Herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants, seasonal, ecotypic, genotypic and chemotypic variations, collection, drying, processing and storage conditions. The assurance of standardization of medicinal plants in terms of safety, quality and efficacy has become

an important issue in the present scenario. It becomes extremely important to make an effort towards standardization of the plant material used for therapeutic purposes. The process of standardization can be achieved by stepwise pharmacognostic studies and minimizing the inherent variation of natural product composition through quality assurance practices applied to cultivation and manufacturing processes.<sup>[3]</sup> In the present study an attempt has been made to standardize the ethnopharmacologically useful seeds of *Dolichos biflorus* Linn. (Family Fabaceae) on the basis of physicochemical and phytochemical characteristics.

*Dolichos biflorus* Linn. commonly known as horse gram is cultivated in dry areas of Australia, Burma, India and Sri Lanka. It is an annual or perennial plant with slender pubescent stems growing in the summer. It matures in 40 days for forage and 120–180 days for seed. Leaves alternate and trifoliate having slender and hairy petiole; leaflets obliquely ovate, acute, entire margin, ciliate, pubescent, upto 31.7 cm.<sup>[4]</sup> Flowers solitary or paired,

axillary calyx campanulate, the upper two connate, other linear, hirsute, corolla of 5 pale-yellow petals, standard oblong, shortly clawed; fruit pod, recurved, sub globose or flat, beaked and straw brown. Seeds are black or brown in color, odorless, slightly acrid in taste and ovular in shape. Chemical constituents reported in the plants are streptogenin,  $\beta$ -sitosterol, bulbiformin, linoleic acid, polyphenols, oxalates and crude fiber.<sup>[5]</sup>

Consumption of horse gram seeds, after soaking/dry heating followed by cooking, along with cooked rice and soup made from the horsegram is a common practice among the rural people in India.<sup>[6]</sup> According to Ayurveda seed is bitter, acrid, hot, dry and used as astringent, anthelmintic, antipyretic and uterine stones, tumours, asthma, bronchitis, hiccup, urinary discharges, heart-troubles, disease of the brain and eyes, intestinal colic, piles, leucoderma, inflammation, liver troubles etc. Its decoction was used traditionally in leucorrhoea and menstrual dysfunctions.<sup>[7]</sup> Horse gram is an excellent source of iron and molybdenum.

## MATERIALS AND METHODS

### Plant Material

The Seeds of *Kuluttha* were collected from Godavari District, Andhra Pradesh. The plant material was taxonomically identified at Telangana State Level Drug Testing Laboratory, Hyderabad. The collected seeds were cleaned & dried in an oven at 40°C and then powdered with mechanical grinder, passing through sieve no.40 and stored in an air tight container. This coarse powder was used for the organoleptic evaluation, determination of ash values, extractive values, and preliminary phytochemical investigation as per standard methods.

### Macroscopic Study

The external seed morphology (nature, color, odour and taste) were noted. Other structural peculiarities including size, shape and texture were observed by using simple microscopy.<sup>[8]</sup>

### Extraction of plant material

100 gm coarse powdered of seeds were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with distilled water and Methanol 8 hrs separately.<sup>[9]</sup> Then each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of Methanol and the aqueous extracts were calculated. The extracts were also used for the preliminary phytochemical screening.

### Physicochemical Analysis and Preliminary phytochemical screening

Physicochemical analysis and Phytochemical screening were carried out at Telangana State Level Drug Testing Laboratory, Hyderabad and PG Department of Dravyaguna, Dr.B.R.K.R. Govt Ayurvedic college, Hyderabad. Organoleptic characters and Physicochemical characters including moisture content,

ash values, extractive values were evaluated as per the standard methods as described in Ayurvedic Pharmacopoeia of India.<sup>[10]</sup> Preliminary phytochemical screening of ethanol and aqueous was carried out for the detection of various compounds by using standard procedures described by Harborne<sup>[11]</sup> and Khandelwal.<sup>[12]</sup>

## OBSERVATION AND RESULTS

### Macroscopic characters of the seed

The drug comprises of compressed seeds which are reniform, shining, finely polished black or grey or brownish grey or reddish brown with purple and black spots; seed coat is very glistening, cotyledons two in number and whitish in color. The seeds of *Dolichos biflorus* Linn. used for the present study was of a dark black colored variety. The seeds were odorless, tasted slightly acrid and were nonaromatic. The size of the seeds was of 0.5–0.6 × 0.4–0.5 cm, ovular in shape, surrounded by the integuments and fully developed endosperm.

### Physicochemical Analysis

**Table 1: Physicochemical parameters of Kuluttha Seeds.**

Parameters	Results (% w/w)
Foreign Matter	0.82
Loss on drying at 1050C	1.45
Total ash value	7.72
Acid insoluble ash	3.01
Water soluble ash	2.85
Sulphated Ash	1.79
Water soluble extractive	8.50
Methanol soluble extractive	15.21

### Phytochemical Results

**Table 2: Phytochemical screening of Kuluttha Seeds.**

Phytochemicals	Methanol	Aqueous
Steroids	+	-
Triterpenoids	+	-
Tannins	+	+
Flavonoids	+	+
Proteins	+	+
Glycosides	-	-
Phenolic compounds	+	-
Saponins	+	+
Alkaloids	+	-
Sugars	+	+

(+) indicates presence and (-) indicates absence of that chemical constituent in the sample.

**Dolichos biflorus (Linn.)****Fig 1: Seeds.****Fig 2: Seed Powder.****Fig 3: Extraction through Soxhlet Apparatus.****Fig 4: Ash Value Determination.****DISCUSSION**

Identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameters is still more reliable, accurate and inexpensive despite the availability of analytical techniques. Macroscopic and microscopic determination of a plant is the first step towards establishing its identity and purity and should be carried out before any other tests are undertaken. To ensure batch to batch consistency, homogeneity and reproducible quality of herbal products, proper control of starting material is essential. The present study of *Dolichos biflorus* Linn. seed is supportive to know the basic characters of the drug like macroscopic characters of seed, organoleptic characters, physico-chemical parameters, phytochemical constituents of grinded seed powder. Some salient features of *Dolichos biflorus* Linn. seed studied using pharmacognostic features are discussed in this paper.

The powder is light brown in colour, odorless, tasted slightly acrid and were nonaromatic. Humidity in the

sample and extract decides the deterioration time. High water content in powder and aqueous extract are found to get deteriorated due to fungal attack. Loss in weight of seed powder on drying at 105°C was found to be 1.45 %. Analytical results like total ash value was 7.72 % which indicate the amount of minerals present in the sample. The amount of acid-insoluble siliceous matter (3.01 %) was higher than water-soluble ash (2.85%). The methanolic extractive value is comparatively higher (15.21 % w/w) than the water soluble extractive value (8.50% w/w). The extracts were subjected to preliminary phytochemical screening and the results certified the presence of alkaloids, phenols, flavonoids, steroids, carbohydrates, proteins, triterpenoids, tannins and saponins in methanolic extract of seed and presence of proteins, tannins, flavonoids, saponins in aqueous extract of seed.

**CONCLUSION**

Kuluttha is one of the important drugs used in the various indigenous medicines. The present work focuses on the phytochemical and analytical investigation of *Kuluttha* seed. The phytochemical and analytical study was carried out and their details are mentioned along with the results, observation obtained in the experiments. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample. The present work therefore, attempts to report necessary pharmacognostical and standardization parameters of *Dolichos biflorus* Linn. seed which will help to identify the drug.

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**CONFLICTS OF INTEREST**

None declared

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