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Original Article

Pharmacognostical Standardization of Goraksha pods: an important Nutritive and antidiabetic Plant

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ABSTRACT

Objective: Goraksha or cluster beans are an annual legume found throughout India. It is also extensively cultivated for its pods used as vegetable for its high nutritive value, antidiabetic properties and for the source of guargum. **Methods:** Macroscopy, microscopy, powder microscopy, histochemical studies and preliminary phytochemical studies on the pods of Goraksha has been carried out highlighting its importance along with important diagnostic characters, which will help in the identification of pods both in fresh form as well as in dry form. **Results:** Study revealed presence of distantly arranged elongated uniseriate warty trichomes in the outer most layer of epidermis, presence of stone cells, simple starch grains and very small calcium oxalate crystals in the hypodermal region. Presence of unevenly thickened row of palisade layer of macro sclereids in the outer most layer of cotyledon region. Phytochemical studies revealed the presence of alkaloids carbohydrates, phenols, proteins, saponins, starch, steroids, and tannins. **Conclusion:** The pharmacognostical standardization revealed the presence of various specific characters which are important for the identification of the genuine pods of *Cyamopsis tetragonoloba* (L.) Taub.

Key words: Cluster beans, Goraksha, Pharmacognosy, Pods. Histochemical studies.

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INTRODUCTION

Cyamopsis tetragonoloba (L.) Taub. is sold in the name of Cluster beans and also known as *Goraksha*/*Dridhabija* (in Sanskrit) and Guar (in Hindi) in the local market of southern India. The botanical synonyms of *Goraksha* are *C. Psoralioides* (Lamk.) DC.; *Psoralea tetragonoloba* L. belonging to the family Fabaceae, under Sub family Papilionaceae. It is a moderate sized annual herb found throughout India as a cultivar for its pods and for the source of vegetable, seed-gum, forage and green manure. The stem is grooved; leaves are generally 3-foliolate, sometimes simple; leaflets are elliptic with hair on both sides. Flowers are small, white or purple in color, typically papilionaceous, 6-3 flowers in axillary with close racemes. The pods are linear, stiff, erect clusters with double ridge compression on dorsal side and single ridge below. The tender green pods are popular vegetable in many parts of our country.[1] The plant is recognized for its food price and possess different nutrients like proteins, fat, fibre, carbohydrates, minerals like calcium, phosphorus & iron, fluoride, zinc, molybdenum, manganese, copper cobalt etc; vitamins like thiamine, riboflavin, niacin, vitamin C, carotene, β-carotene, folic acid, ascorbic acid nicotinic acid etc.; amino acids like methionine, cystine, trptophan, valine, arginine, aspartic acid etc.; polyphenols like kaempferol, quercetin, chrysene, 1,2,5,6-dibenzanthracene etc. and globulin.[1, 2] The plant is traditionally used as vegetable, improves appetite and removes biliousness and night blindness.[3] Different properties in Ayurveda are described in the name of Goraksha such as **Rasa** (Taste): *madhura* (Sweet); ***Guna*** (Properties): *guru, ruksha, sara*; ***Doshakarma*****:** *kaphavatavardhaka, pittanasaka*; ***Karma*** (Action): *ruchikara, balya*.[4] *Goraksha* exhibited different therapeutic activities such as anti hypercholesterolaemic,[5] anti helminthic,[6] hypolipidemic and hyoglycemic activity,[7, 8] antiulcer and cytoprotective activity.[9] The pods of this plant have been recommended in traditional literature as a remedy for treatment of diabetes and it is believed that the antihyperglycemic effect of aqueous extract of *C. tetragonoloba* beans may be due to presence of tannins, coumarin and flavonoids.[10]

However, there is not much data available on botanical standardization of the plant. In view of this Pharmacognostical standardization on the basis of microscopical studies like histochemical studies with different chemicals have been reported. The study was carried out to ascertain the diagnostic characters through pharmacognostical tools which will be beneficial for the identification of the genuine crude drug.

MATERIALS AND METHODS

Plant Material

The fresh pods of *Goraksha* {*Cyamopsis tetragonoloba* (L.) Taub.} were collected from different vegetable markets of Bangalore (K. R. Puram, Krishna Rajendra and Jayanagar vegetable markets) and the plants were identified with help of Bangalore Flora and from survey of Medicinal plant unit, NADRI, Bangalore and processed in the form of Herbarium, and deposited in RRCBI Herbarium Bangalore for the future reference with voucher specimen no. 12362.

Microscopic analysis

The pods were soaked in 70% alcohol for 24 hours, and free hand sections were taken, cleared with chloral hydrate solution and water, stained with different staining reagents according to the standard prescribed methods. Photomicrographs were captured with Cat cam camera. Further pods were also shade dried and powdered for powder microscopy. Powder studies were also carried out by following the standard methods.[11,12]

Micrometric and Histochemical Studies

Micrometric details of pods were carried out and the measurements were recorded with the help of cat cam software and histochemical

studies were conducted by using different reagents as per the standard methods.[13]

Physico-chemical analysis

Physico-chemical analysis such as ash values, extractive values, were carried out according to the standard procedures prescribed in Ayurvedic Pharmacopeia of India.

Preliminary phytochemical analysis

Preliminary Phytochemical screening was carried out for different extracts by using standard procedures.[12,14]

Thin Layer Chromatography (TLC)

Shade dried pods coarse powder was extracted with petroleum ether, chloroform and methanol at room temperature with the help of rotary shaker. TLC studies of these extracts were carried out by using, commercially available precoated silica gel plates at room temperature by following standard procedures.[15]

RESULTS AND DISCUSSION

Macroscopical characters

Pods green when fresh and pale green in dry condition, compressed, grooved and fibrous at the edges, mature Pods slightly hard, with prominent seeds, can be easily break up by hand, surface smooth, and winged, 4 angular. Petiole glabrous, fibrous, 0.5 cm long, with pleasant odour and bitter taste (Figure 1).

Microscopic characters

The transverse section of the pod showed pericarp layer where exocarp consists of epidermis made up of rectangular shaped parenchyma cells with intercellular spaces covered by thin cuticle with distantly arranged small papillae followed by epidermis, 6-9 layers of lignified stone cells with small lumen and filled with brown cell content of tannin were present. The mesocarp region of the pod ridges showed 15-17 layers of tightly arranged thin walled parenchymatous cells without any intercellular spaces were present. Endocarp region consists of 2 to 4 layers of lignified stone cells with wide lumen (Figure 2).

Seed showed outer integument consists of 1- 2 layers of unevenly thickened row of palisade cells, which are made up of macro sclereids. The palisade layer was followed by a single layer of thick walled sub epidermis arranged by elongated coiled cells with abundant simple starch grains. This was followed by 4 to 6 layers of thin walled tangentially elongated parenchymatous cells with abundant simple rounded starch grains. This is followed by 1to 2 layers of endosperm loaded with oil gloubles and aleurone grains. Endosperm region is followed by cotyledon region made up of many layers of compactly arranged thick-walled parenchymatous cells, encumbered with aleurone grains and oil globules (Figure 3). Micrometric details of pods were carried out and the measurements were recorded in Table 2.

Powder

Fruit powder light green in colour with prominent small pieces of fibers, smell agreeable and taste bitter, when observed under the microscope, it showed different fragments of tissues like rectangular epidermal cells covered by a thin cuticle and thin walled parenchymatous cells, endosperm and parenchyma cells in groups, epidermal cells with thin walled tangentially elongated parenchymatous cells, lignified fibers with pits, undulated fibers, macro sclereides, thickened helical xylem vessels, epidermal cells with stomata, pitted xylem vessels, tracheid, single fibers, epidermal cells with stomata, etc. (Figure 4).

Figure 1: Fresh Plant of *Cyamopsis tetragonoloba* with pods; **A**-fresh pods, **B**-dried pods.

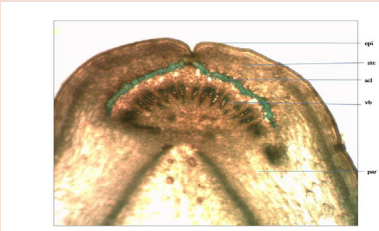

Figure 2: T.S. of Pod 4x; epi-epidermis, scl-sclerenchyma, stc-stone cells, vb-vascular bundle, par-parenchyma.

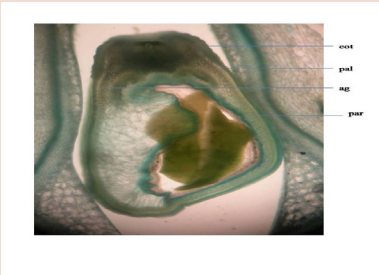

Figure 3: T.S. of seed 10x, cot-cotyledon, pal-palisade cells, ag-aleurone grains, par-parenchyma.

Histochemical Studies of the Fruit

Histochemical studies of the fruit showed the presence of calcium in epicarp, mesocarp and seed; calcium oxalate, calcium pectate in different regions, protein content, phenols, lignin, starch grains, magnesium, silica contents in different regions of tissues (Figure 5 and Table 1).

Physico-chemical analysis

Physico-chemical analysis such as loss on drying, total ash, acid-insoluble ash, water & alcohol soluble extractive values and pH of 5% aqueous solution were carried out according to the standard procedures and the results were recorded in Table 3 and 4.

Preliminary phytochemical analysis

Preliminary phytochemical screening was carried out for different extracts by using standard procedures and the results were recorded in Table 5. The result indicated the presence of tannins, saponins which

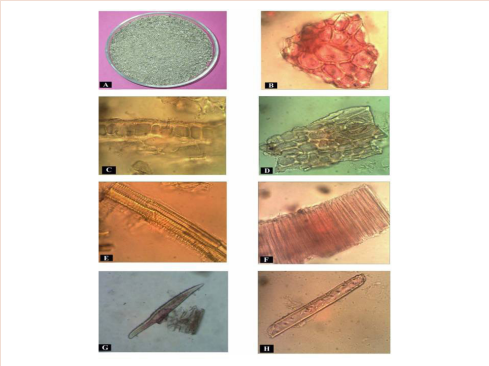

Figure 4: Powder studies: A. Powder; **B.** Group of Parenchyma cells 40x; **C.** Epidermal cells covered with thin cuticle10x; **D.** Epidermal cells with stomata 40x; **E.** Thickened helical Xylem vessels; **F.** Macro sclereides 40x; **G.** Single fiber and parenchymatous cells 40x; **H.** Single Tracheid 40x.

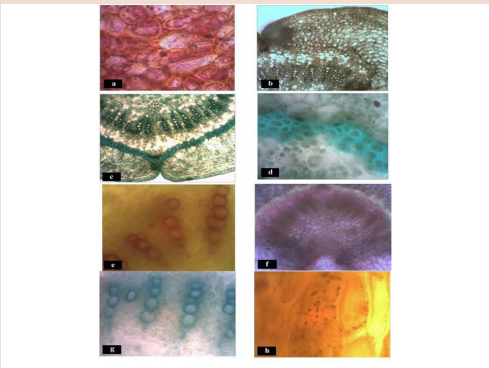

Figure 5: Histochemical studies: a. Calcium oxalate contents 40x; **b.** Calcium contents in seed 10x; **c.** Protein contents; **d.** Phenol contents 10x; **e.** Lignin contents in vascular bundles 40x; **f.** Magnesium contents; **g.** Phenol contents in vascular bundle region; **h.** Starch contents 40x.

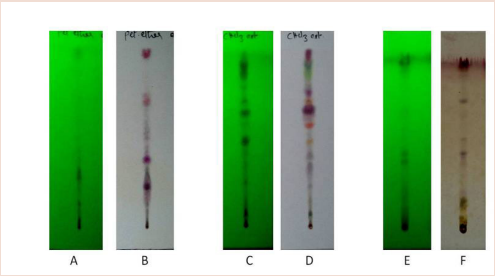

Figure 6: TLC of *Cyamopsis tetragonoloba* (pods): **A** & **B**-Pet. Ether extract UV-254 and After spraying; **C** & **D**- Chloroform extract UV-254 and after spraying; E & F-Methanol extract UV-254 and after spraying.

may be considered as an indicative of its antidiabetic potential as it was claimed by previous researchers that these phytoconstituents may be responsible for the antihyperglycemic effect of aqueous extract of *C. tetragonoloba*.

Thin Layer Chromatography (TLC)

Shade dried pods coarse powder was extracted with petroleum ether, chloroform and methanol with rotary shaker. TLC studies of these extracts were carried out by using, commercially available precoated plates at room temperature by using the mobile phase toluene: hexane: ethyl acetate- 6:3.5:0.5 for petroleum ether extract; Toluene: Ethyl acetate: Chloroform- 6:3.5:0.5for chloroform extract and ethyl acetate: methanol: water- 7.7:1.5:0.8 for methanol extract. The Rf values for different extracts were found as follows:

Petroleum ether extract-Under UV-254: 0.14, 0.31., after spraying with Anisaldehyde-Sulphuric acid (H_2SO_4): 0.14, 0.24, 0.40, 0.55, 0.725. Chloroform extract- under UV-254: 0.26, 0.50, 0.675, 0.725, 0.81, 0.88, 0.94, after spraying with anisaldehyde-sulphuric acid: 0.24, 0.35, 0.41, 0.50, 0.59, 0.675, 0.725, 0.78, 0.88, 0.94. Methanol extract-under UV-254: 0.375, 0.44., after spraying with anisaldehyde-sulphuric acid: 0.16, 0.44, 0.525 and 0.725 (Figure 6).

DISCUSSION

Goraksha - Cyamopsis tetragonoloba (L.) Taub is found throughout India as a cultivar for its pods and for the source of guargum. Fruit decoction is drunk or fried as a curry or chutney and taken for 40 days is used for diabetes disease, fruit fried with ghee are given to get relief from general weakness during the disease.[16] Pods are used for cooling, laxative digestive, appetiser, tonic and galactagogue, constipation, dyspepsia, anorexia, agalactia, nyctalopia and vitiated conditions of *pitta*.[17] Guar gum is used as a laxative. It is also used for treating diarrhoea, irritable bowel syndrome (IBS), obesity, and diabetes; for reducing cholesterol; and for the prevention of atherosclerosis. In foods and beverages, guar gum is used as a thickening, stabilizing, suspending, and binding agent. Guar gum is widely used as an excipient specifically as binding agent in tablets, and as a thickening agent in lotions and creams.[18] The gum is used as a laxative and appetite depressant and in peptic ulcer, seeds are rich in protein and can be used for human consumption and for medicinal purposes to cure sprains and swellings, boiled seeds as poultice in plague, enlarged liver, head-swellling due to broken bones etc. The meal obtained as by-product during gum manufacture is also rich in crude protein and essential amino acids and it can be serve as a nutritious feed for cattle, toxic constituents present in meal should be inactivated by appropriate heat treatment. The gum obtained from seed is useful in paper manufacturing, oil-well drilling, mining industry, explosives, pharmaceuticals, cosmetics etc.[1]

The study resulted with the outcome of some basic data regarding the genuine crude drug. Pods pale green in dry condition, fibrous at the edges, microscopically it revealed the presence of uniseriate warty trichomes in the outer most layer of epidermis, macrosclereids in the outermost layer of cotyledon region, lignified polygonal stone cells with wide lumen, in the distal end of the fruit, Rubiaceous type of stomata in the surface view of the epidermal region and presence of abundant fibers in the powder. Histochemically pods showed the presence of phenols, protein, calcium oxalate, magnesium, starch, cellulose, calcium pectate, lignin, sulphated muco polysaccharides, tannin, silica contents in different locations. Qualitative phytochemical studies revealed the presence of alkaloids, carbohydrates, phenols, proteins, starch, tannins and absence of flavanoids and resins.

CONCLUSION

Pharmacognostical studies carried out with different parameters on Goraksha pods helps in utilizing as a potent herbal ingredient for diabetes along with its important nutrients. Further studies also help in preservation of health, traditional system, therapeutics and revalidation.

ACKNOWLEDGEMENT

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Table 1: List of stains and reagents used for the analysis of various histochemicals in different regions of fruit

Stain/Reagent	Histochemical	Result	Locality of the cells
TBO	Poly Phenols	Cells contents turn turquoise green to blue green colour.	Sclerenchyma cells and Vascular bundles.
Fast green	Protein	Proteins appear bright green.	Cuticle and Sclerenchyma cells and Vascular bundles.
10 % Urea, Acetic acid, Sodium Nitrite mixture (Nitroso reaction).	Phenol	Phenol substance will turn into cherry red colour.	Sclerenchyma cells and Vascular bundles.
Alkaline pyrogallol method	Calcium	Yellowish brown colour.	Epidermis, trichome, parenchyma cells in the hypodermis, cell walls of vascular bundles, cell walls of Sclerenchyma cells, Cotyedon portion.
Silver hydrogen peroxide	Calcium oxalate	Balck in colour.	Calcium oxalate crystals in the distal end of the fruit.
Titian yellow	Magnesium	Brick red 7 colour.	hypodermis cells, Sclerenchyma cells, Vascular bundles in distal end of fruit.
Methyl red	Silica	Yellowish orange.	Cuticle, Sclerenchyma cells, Vascular bundles and cotyledon portion.
Iodine-potassium iodide reaction	Starch	Blue to black in colour	Starch grains in the hypodermis of parenchyma cells and distal end of fruit parenchyma cells.
Tannic acid-ferric chloride method	Calcium pectate	Blue to black in colour	Epidermal cells, parenchyma cells in the hypodermis, Sclerenchyma cells, , vascular bundles and cotyledon portion
Phloroglucinol method	Lignin	Red in colour.	Sclerenchyma cells and vascular bundles.
Sulphated muco polysaccharides	TBO	Pink to reddish purple colour.	The cell wall of phenolic content cells are become change to reddish purple color.
Ferric chloride method	Tannin	Black in colour.	Almost present all parts of the body and rich in hypodermis and cotyledon portion.

Table 2: Micrometric details of Fruit

Type of Cell (Measurements are average of 75 readings)	Minimum Measurements in µm	Maximum Measurements in µm	Average with standard Error Measurements in µm	Standard deviation in µm
Epidermal cell width	54.23	90.43	69.18	11.49
parenchyma cells width	72.72	277.77	157.85	52.71
Sclerenchyma cells width	38.01	101.12	63.20	18.03
Sub epidermis	242.3	389.08	313.19	64.73
Cotyledon length	904.21	1100.92	981.17	92.09
Cotyledon width	323.36	476.21	438.20	65.12

Table 3: Physicochemical parameters

Name of the parameter	Values (%) w/w
Description	Light green coarse powder
Foreign matter	Less than 1.0%
pH (5% w/v aq. solution)	6.45
Loss on drying at 105^{0}C	11.09
Total ash	5.16
Acid-insoluble ash	0.26
Water-soluble extractive	28.13
Alcohol-soluble extractive	7.85

Table 4: **Extractive values by Soxhlet extraction**

Solvent	Values (%) w/w
Petroleum ether (40-60^{0}C)	0.55
Chloroform	8.63
Ethanol	11.01

Table 5: **Preliminary Phytochemical tests for *Cyamopsis tetragonoloba* pods**

Natural product group	Test for natural products	Presence (+)/ Absence (-)
Alkaloids	(a)Dragendorff's test	++ve
	(b) Hager's test	+ve
	(c) Mayers's test	+ve
	(d) Wagner's test	++ve
Carbohydrates	(a)Anthrone test	+ve
	(b) Benedict's test	++ve
	(c) Fehling's test	++ve
	(d) Molisch's test	++ve
Flavonoids		-ve
Phenols	(a) Ferric chloride test	+ve
	Lead acetate test	+ve
Proteins	Biuret's test	++ve
	Millon's test	++ve
Saponins		++ve
Starch		+ve
Steroids	Salkowski reaction	+ ve
Tannins	(a) Ferric chloride test	++ve
	(b) Lead acetate test	++ve
Resins		-ve

New Delhi, for the sanction of the project and for providing the facilities to carry out the work successfully.

CONFLICT OF INTEREST

The author declare no conflict of interest.

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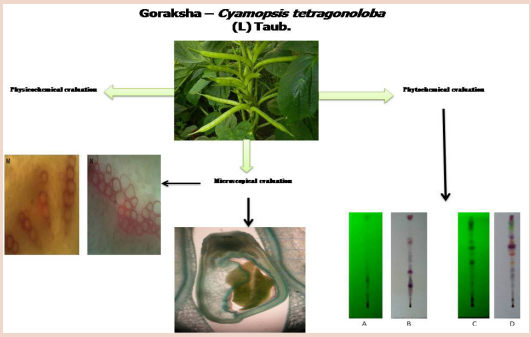
PICTORIAL ABSTRACT



SUMMARY

- The different standardization parameters studied are very useful to establish the Pharmacopoeial standards which is need of the hour not only for the survival and encouragement of the age old traditional systems of medicine but also in the view of the fact that these systems are attaining Global importance.
- The study resulted with the outcome of some basic data regarding the genuine crude drug. Pods pale green in dry condition, fibrous at the edges, microscopically it revealed the presence of uniseriate warty trichomes in the outer most layer of epidermis, macrosclereids in the outermost layer of cotyledon region, lignified polygonal stone cells with wide lumen, in the distal end of the fruit, Rubiaceous type of stomata in the surface view of the epidermal region and presence of abundant fibers in the powder.
- Histochemically pods showed the presence of phenols, protein, calcium oxalate, magnesium, starch, cellulose, calcium pectate, lignin, sulphated muco polysaccharides, tannin, silica contents in different locations.
- Qualitative phytochemical studies revealed the presence of alkaloids, carbohydrates, phenols, proteins, starch, tannins and absence of flavanoids and resins.
- The studies carried out with different parameters contribute towards utilizing *Cyamopsis tetragonoloba* (L.) Taub. as a potent herbal ingredient and nutritional supplement for Diabetes and other ailments.

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