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Original Article

Macro-microscopic and HPTLC Atlas of *Canavalia gladiata* (Jacq.) DC. Fruit

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ABSTRACT

Background: *Canavalia gladiata* (Jacq.) DC. (Family Leguminosae) is a large annual or perennial climber with a terete glabrous stem, native to Indo-Malaysian and also distributed in tropical, subtropical regions. *C. gladiata* (Jacq.) DC. is generally confused with another closely related species i.e. *C. ensiformis* (Linn.) DC (Jack bean). The tender pods of both the above species have close similarity in morphology, especially shape and size of fruit, therefore it is difficult to differentiate on their vegetative and also fruiting stages. The plant *C. ensiformis* mentioned as a synonym of *C. gladiata* in many of the earlier literature though they are 2 different taxa. Some authors published different colour seeds in the name of *C. gladiata*. It is doubtful if *C. ensiformis* (Linn.) DC. or *C. gladiata* (Jacq.) DC was studied during that analysis. **Objectives:** A systematic pharmacognostical study has been executed to identify the correct botanical source. **Materials and Methods:** All the studies were carried out by standard procedures in Pharmacopoeias and other authentic literature. **Results:** Macroscopically surface characters followed by odour and taste, anatomically TS of the pericarp, plecenta, testa, cotyledon and radical, powder microscopically epidermal cells with stomata and prismatic crystal cystolith, trichomes, testa palisade cells, sclereids with different shape and size, brownish content, starch grains, tracheids, fibres and prismatic crystals of calcium oxalate are the unique diagnostic characters observed. HPTLC with different spots having unique Rf values in the pericarp, testa, cotyledon and seeds will be helpful for critical identification of the species. **Conclusion:** The findings of the present study will be helpful in the identification and differentiation of related species as the whole drug or in powder form.

Keywords: Jack bean , Seed anatomy, Sword bean, Vegetable Crude Drug.

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INTRODUCTION

The old world tropical fruits of *Canavalia gladiata* (Jacq.) DC. (Family Leguminosae) is a large annual to perennial climber with terete glabrous stem, native to Indo-malaysian countries and distributed in tropical, subtropical regions of the world; it is cultivated in rural areas around house by allowing to trail on walls, tree, buildings and on hedges.[1-5] The tender pods and mature seeds are consumed as vegetable.[5] The *C. gladiata* has different names in Indian languages; such as Sword bean in English; *Asisimbi* and *Mahasimbi* in Sanskrit; *Lal kadsumbal, Khadsampal* and *Badi Sem* in Hindi; *Sembi Tumbekonti, Sembi avare* and *Tumakai* in Kannada, *Makhan Shim* in Bengali; *Talvardi* and *Tarvardi* in Gujarati; *Tebi* in Manipuri; *Valamara, Valvara* and *Ranavalia* in Malayalam; *Abai* in Marathi; *Kattavarai, Vaal avarai, Segapu Thampattai* and *Valari kayi* in Tamil; *Thamba kayi* and *Yerra-tamma* in Telugu and known as *Bara-sim* and *Makhan-sim* in Tripura.[5,6]

The fruits and seeds are rich in several phytochemicals as the literature revealed the presence of enzymes, amino acids, amines, phenolic compounds like flavonoids and their glycosides, sterols and lipids. Enzymes urease and arginase,[7] canavalin, con-canavalins A and B and amino acid canavanine (2.8%),[7-9] polyamines, sym-homospermidine (homoSPD) and canavalmine (CAN),[10,11] have been isolated from the seeds. A guanidinooxyamine identified as γ-guanidinooxypropylamine was isolated from seedlings.[12] Aminopropylcanavalmine, aminobutylcanavalmine,[13] phytohaemagglutinin (yield, 2.6%), have been isolated from dried mature seeds.[14] A tertiary methylated tetraamine, N^4-methylthermospermine in addition to spermidine, homospermidine, spermine, thermospermine and canavalmine,[15] have been reported. Ent-kaurane-type glycoside, canavalioside, and eight new acylated flavonol

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glycosides, gladiatosides A1, A2, A3, B1, B2, B3, C1, and C2, were isolated from the seeds together with robinin, kaempferol 3-O-b-D-galactopyranosyl-7-O-a-L-rhamnopyranoside and kaikasaponin III.[16] 5-Deoxyflavonol, gladiatin along with a known phenolic compound, methyl gallate have been isolated from the methanol extract.[17] Raffinose, stachyose, verbascose and the gibberellins, GA_{21}, GA_{22} and GA_{59} are reported.[18-20] Gallotannin and other 33 phenolic compounds were tentatively identified in seed coat ethanol extract by LC-MS/MS methods.[21] Major lipid components were triacylglycerol (43.8 to 45.7%), phospholipids (46.7 to 47.0%) and other 16 components have been found using HPLC and GC-MS methods.[22,23] Thermostable α-amylase was isolated from germinating seeds.[24] 11-Dimethyl-8 methylenebicyclo[7.2.0]undec-4-ene-4-carboxylic acid; kaempferol-7-O-a-L-dirhamnopyranosyl(1→2; 1→6)-O-b-D-glucopyranosyl(1→2)-O-a-L-rhamnopyranoside; methylgallate;(2S,3S,4E,8E)-2-aminooctadeca-4,8-diene-1,3-diol 1-O-b-D-glucopyranoside; (2S,3S,4E,8Z)-2-aminooctadeca-4,8-diene-1,3-diol 1-O-b-D-glucopyranoside; lupeol; trilinolein; 1,6-di-O-galloyl b-D-glucopyranoside; N-(2-methoxybenzoyl) homoserine; dihydrophaseic acid; dillenetin; kaempferol-7-O-[2-O-b-D-glucopyranosyl-6-O-a-L-rhamnopyranosyl]-a-L-rhamnopyranoside; kaempferol-3-O-[2-O-b-D-glucopyranosyl-6-O-a-L-rhamnopyranosyl]-b-D-glucopyranoside; kaempferol-3-O-(2,6-O-a-L-dirhamnopyranosyl)-b-D-glucopyranoside; kaempferol-3-O-rutinoside; gladiatoside A1 and gladiatoside B1were reported.[25] Amino-acids like cysteine, methionine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan and arginine were reported.[26,27]

Sword bean is eaten as a vegetable in tropical African countries like Ethiopia Tanzania, Madagascar and many parts of the Asian countries like Sri Lanka, India, Indonesia, China, Korea and Japan. The ripe seeds used as feed for cattle and chicken.[28] Indian indigenous system of medicine use the fruit in reducing *kapha, pitta* and burning sensation, as antiseptic, anti-allergic, insect repellent, diuretic, wound healer, against ulcers, acne, obesity, stomach-ache, dysentery, conjunctivitis, cough, asthma, headache, kidney problems general debility and the tender pod soup used by Tripura tribal people for curing jaundice.[6,29] As per a Siddha text there are different types of *Avarai* used in medicine, *Kozhiyavarai* is equated to *C. gladiata*, the unripe fruit causes hypercholia, vomiting, ageusia, pododynia and ptyalism.[6,30] Peoples use the tender pods and mature seeds of *C. gladiata* as a vegetable, the ripe seeds are said to be poisonous and reported to contain a sapotoxin which causes nausea and vomiting.[5]

C. gladiata (Jacq.) DC. is confused with another closely related species *C. ensiformis* (Linn.) DC (Jack bean), the tender pods of both species have a close similarity in morphology, shape and size and hence difficult to differentiate during earlier and fruiting stages.[28] *C. gladiata* seeds are mostly reddish brown but rarely white in colour and *C. ensiformis* seed colour also white in the mature stage but the hilum shape and size is one of the distinguishing characters.[31] *C. ensiformis* hilum is much shorter (up to 11 mm long) than the *C. gladiata* (up to 22 mm long) in dried form. *C. ensiformis* is mentioned as a synonym of *C. gladiata* in some of the earlier literatures.[32] Some published literature on *C. gladiata*,[33-36] revealed different colours of seeds but some seed picture resembles *C. ensiformis* and *C. rosea* (SW). DC.[33-37] It is doubtful if the seeds of *C. ensiformis* (Linn.) DC. or *C. gladiata* (Jacq.) DC was studied in those publications as seed colour should not be the character to decide species. As there are chances of wrong identity due to morphological differences there is a scope for a macro-microscopic study of the dried materials for obtaining key identifying characteristics.

The tender pods and seeds were underutilized for nutritional supplements, therefore it was thought essential to investigate and develop key distinguishing parameters for standardization of the raw material as per WHO guideline. This study is aimed at cost effective and simple standardization procedures like morphological, anatomical, powder microscopical and TLC/HPTLC analysis of *C. gladiata* dried fruit.

MATERIALS AND METHODS

Authentic fruit of *Canavalia gladiata* (Jacq.) DC. were collected from villages of Vellore district - 631102, Tamil Nadu and Annamayya district - 516115, Andhra Pradesh (India). The voucher specimens (J/Fr/056) were deposited in the Department of Pharmacognosy museum, SCRI, CCRS, Arumbakkam, Chennai, India for future reference.

Macro-microscopy

The fruits were submerged in water for 12 hr and free-hand transverse sections are taken following standard procedures.[38-40] Macro-morphological characters were examined under Zeiss stereo Discovery V.8 fitted with Axiocam ERc5s and micro-morphological characters were examined under Zeiss Axiolab 5 fitted with Axiocam 208 color camera. Powder characters were drawn under 200X magnifications with the help Olympus BX43 Trinocular microscope fitted with a drawing tube.

Instrument for HPTLC

Automatic sampler ATS4 was used for the application of extracts on the TLC plate; twin trough chamber (10×10 cm) was used for plate development; visualizer was used for photo documentation under UV-visible conditions; Scanner 4 with win CATS software was used for obtaining densitograms; TLC plate heater was used for derivatization (all from CAMAG, Switzerland).

HPTLC Procedure

Powdered plant samples (1 g of each) were sonicated with 10 ml of methanol for 15 min, filtered and transferred to a sample vial for application. 20 µl of each extract of the pericarp (1), testa (2), cotyledon (3), and seed (4) were applied on a silica-coated TLC plate (60 F_{254}) using CAMAG ATS4 applicator and developed in twin trough chamber (CAMAG) (10×10 cm) pre-saturated with the mobile phase ethyl acetate: methanol: ammonia (6:3:1, *v/v/v*). The plate was developed up to 80 mm from the bottom. After development, the plate was photo-documented using CAMAG TLC Visualizer under UV λ_{max}254 nm and UV λ_{max}366 nm. Then the plate was scanned using CAMAG Scanner 4 at λ_{max}254 nm (D2 lamp, Absorption mode) and λ_{max}366 nm (Hg lamp, Fluorescence mode) respectively and chromatographic fingerprint profiles of the extract were performed. Subsequently, the plate was dipped in vanillin sulphuric acid solution in ethanol followed by heating at 105°C till the development of the colored spots. The plate was then photo-documented in white light using CAMAG TLC Visualizer. For alkaloid detection, a separately developed plate was dipped in Dragendroff's reagent and photo documented under white light.[41-43]

RESULTS

Macroscopy

Fruits are sword or concavo-convex in shape, up to 40 cm long and up to 5.5 cm wide, surface flat shiny with slight ridges and grooves and irregular wrinkles, two minor clefts of longitudinal furrow lies on the concave marginal side and two major clefts of longitudinal furrow lie on the margin and two minor clefts at the center on the convex side; fresh fruits light green in color and dried fruit yellowish brown, each fruit contain 8 to 16 seeds (Figures 1 A and B).

Dried seeds are hard, surface glossy with slight ridges and grooves, reniform to ellipsoid or oval to oblong, 2 to 3.5 cm long, 1.5 to 2 cm wide and 0.4 to 0.8 cm thick; micropyle, raphe and hilum distinct, hilum located at the ventral flat edge of seed, dark reddish brown, up to 2 cm in length and up to 0.3 cm in width, well marked long oval rim-aril with centrally located vertical white line of hilar groove; seed coat thick and reddish brown or maroon color externally and dirty white internally; testa up to 0.6 mm in thickness, white coloured fleshy, embryo embedded in yellowish white cotyledon (Figure 1 C). Odour is not distinct; the taste is slightly mealy.

Figure 1: A. Fresh fruit; B. Dried fruit; C. Dried seeds.

Microscopy

Pericarp

Detailed TS passing through the center of the fruit epicarp consists of 3 to 5 cell layers of thick-walled, wide-lumened, irregularly shaped sclerenchymatous cells covered by a single layer of the epidermis with thick cuticle followed by thin-walled parenchymatous mesocarp embedded with starch grains, brownish content, vascular strands and underneath this a narrow band of endocarp consists thick walled narrow lumen sclerenchymatous cells; TS of pericarp passing through the ventral and dorsal suture region differ from other areas of the pericarp, its shows the plano-convex shape, layer of epicarp is followed by mesocarp, an upper elevated portion is occupied by an arc of vascular bundle capped by pericyclic fibre bundle and the remaining portion of mesocarp and endocarp are same as above (Figures 2 A and B).

Placentae (Funicle)

TS is plano-convex in outline with tangentially elongated rectangular shaped single layered epidermis covered by thick cuticle followed by two layers of sub-epidermal cells filled with reddish brown content on the upper side; underneath this, there is a wide zone of thick-walled, non-lignified, stellate parenchymatous cortex embedded with a few starch grains followed by a centrally located arc of open collateral vascular bundle; round to oval, non-lignified, collapsed parenchymatous cells with intercellular space present at the lower side (Figure 3).

Seeds

Detailed TS passing through the center of the seed shows the outer testa having a layer of lignified, thick-walled column-like palisade cells forming an epidermis covered by thick cuticles having a central narrow lumen embedded with reddish brown content, underneath this lies 3 or 4 cells rows of lignified spool-shaped cells of hypodermis followed by a wide zone of compressed, tangentially elongated thin-walled parenchymatous tissue embedded with vascular strands (Figure 4).

TS of testa passing through the hilum region differ from testa in other regions of the seed. It shows inwardly curved and horizontally running, thick-walled, non-lignified (funicular) parenchymatous remnants of adherent arillus followed by two layers of palisade and a centrally located narrow vertical passage (hilum) connecting the pear-shaped lignified tracheidal bar lying underneath it and also embedded in hypodermal tissue. The outer counter layer of palisade cells is extended to a short distance with a central narrow lumen embedded with reddish

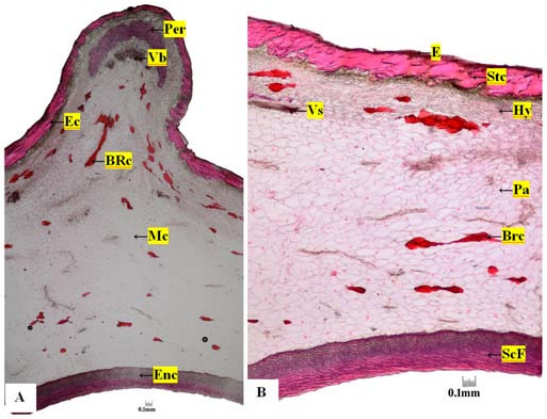

Figure 2: A. Detailed TS of pericarp passing through the ventral and dorsal suture region; B. Detailed TS of passing through the centre of the pericarp.

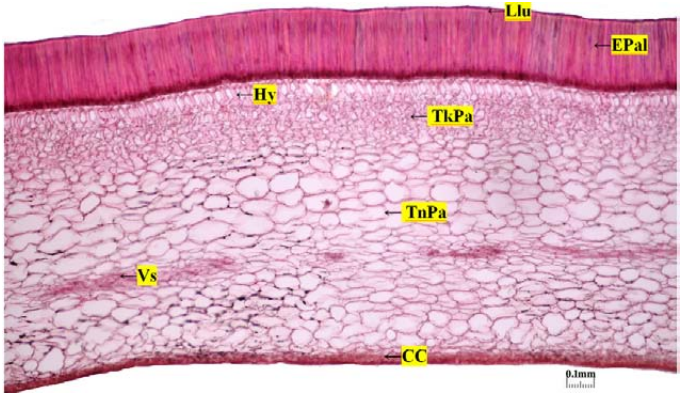

Figure 5: Detailed TS of testa passing through the centre of the seeds.

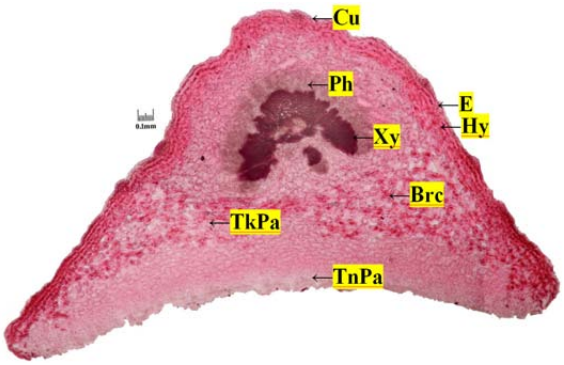

Figure 3: Detailed TS of Placentae (Funicle).

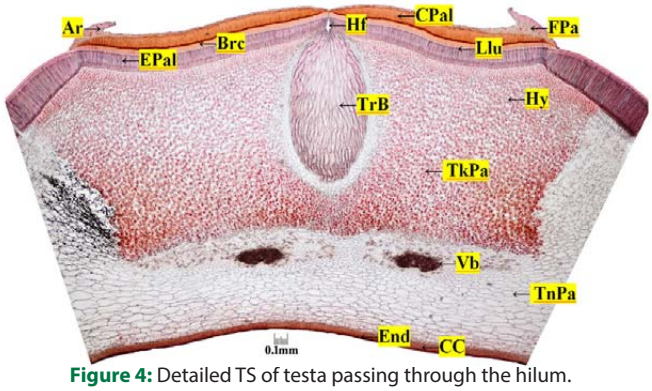

Figure 4: Detailed TS of testa passing through the hilum.

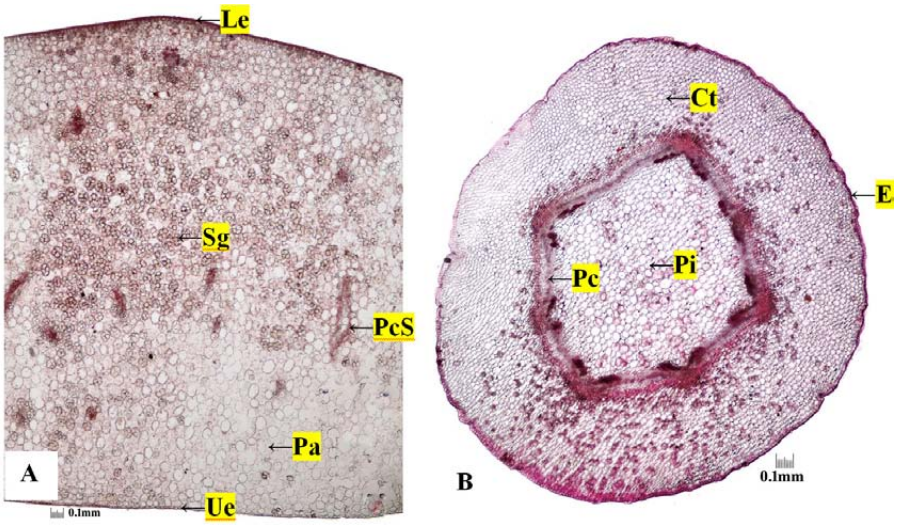

Figure 6: A. Detailed TS of cotyledon; B. Detailed TS of radical.

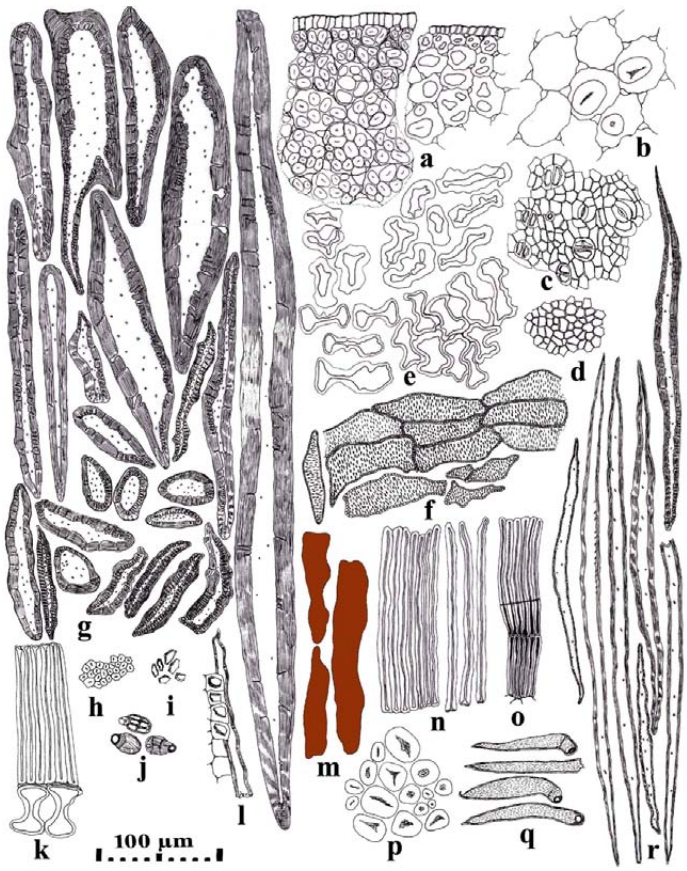

Figure 7: Powder microscopy of fruits of *C. gladiata.* a and b, fragment of cotyledon in sectional view with starch grains; c, pericarp epidermal cells in surface view with paracytic stomata and pair of prismatic crystals of calcium oxalate; d, cotyledon epidermal cells surface view; e, thick walled parenchyma cells from testa hypodermal region; f, tracheids from hilum region; g, stone cells, sclereids and fibre sclereids; h, testa epidermal cells in surface view; i, prismatic crystals of calcium oxalate; j, glandular trichome; k, testa in sectional view; l, crystal fibre; m, reddish brown content; n, testa palisade cells; o, testa palisade cells form hilar region; p, starch grains; q, warty covering trichome sharp hooked end; r, fibres from endocarp region.

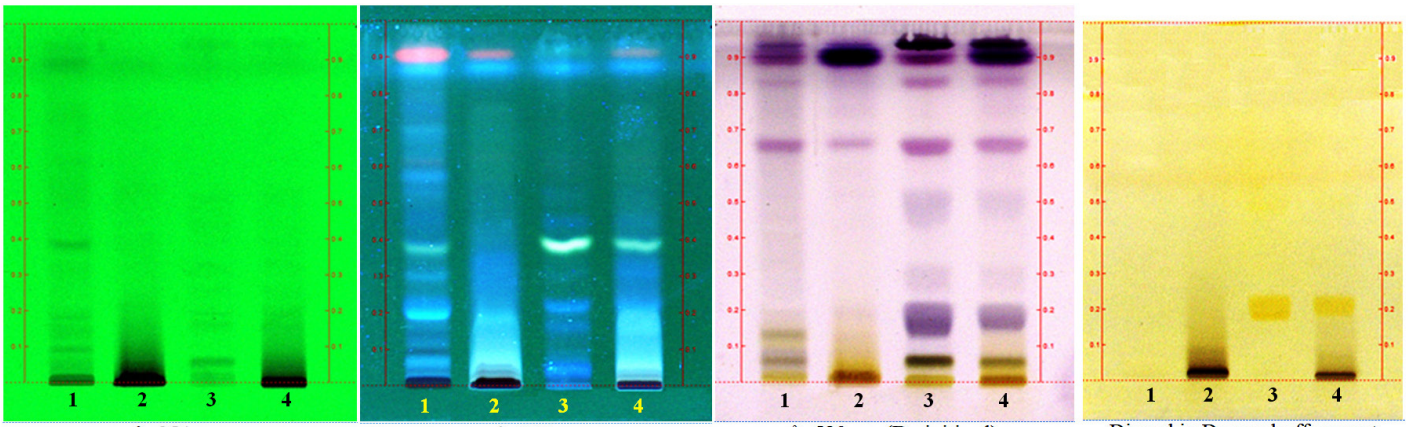

Figure 8: TLC plate at 254 nm, 366 nm, 520 nm and Dipped with Dragendorff's reagent.

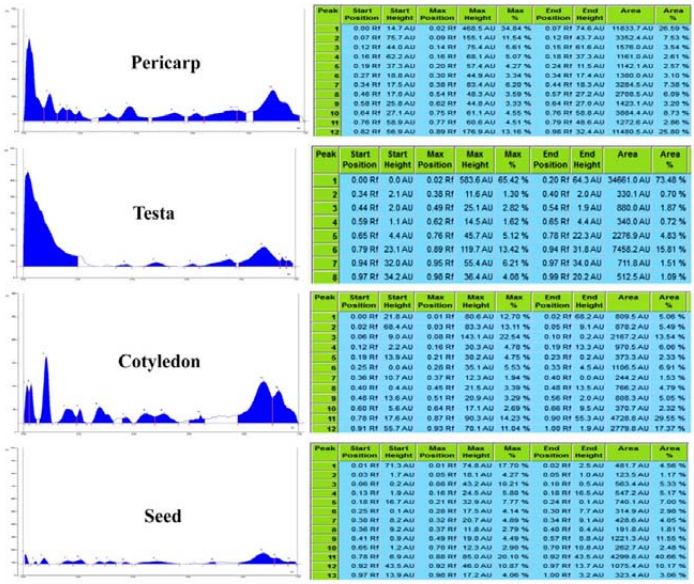

Figure 9: HPTLC densitogram at 254 nm.

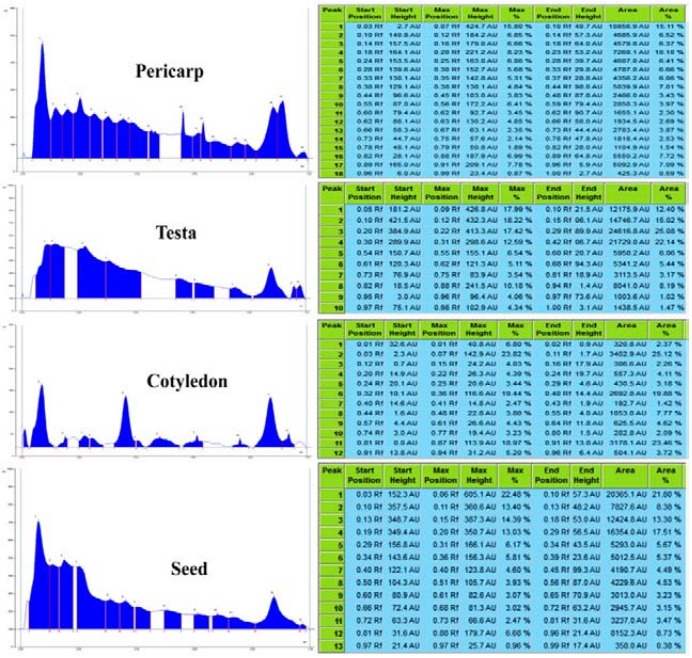

Figure 10: HPTLC densitogram at 366 nm.

brown content. The inner layer which is longer in height exhibits a line of linea lucida crossing across and extending throughout the section in the upper region. Underneath this lies a wide zone of hypodermis consisting of non-lignified, thick-walled spool-shaped cells with intercellular space embedded with brownish content. The remaining adjacent to it is thin-walled, compressed parenchymatous tissue embedded with few vascular tissues followed by the lower portion of the section made up of collapsed parenchymatous cells (Figure 5).

Cotyledon consists of thick-walled parenchymatous cells with intercellular space, embedded with starch grains, enclosed by the radially elongated outer epidermis and tangentially elongated inner epidermis embedded with aleurone grains (Figure 6A). The embryo is differentiated into radicle and plumule, the latter being very short; TS of radicle consists almost circular in outline with a single layer of epidermis covered by thin cuticle followed by round to oval, thin-walled parenchymatous cortex with intercellular spaces and centrally located thin-wall parenchymatous pith encircled by a ring of pro-cambium tissue (Figure 6B).

Powder Microscopy

The powder shows cotyledon in sectional view embedded with starch grains; cotyledon parenchyma cells with starch grains; pericarp epidermal cells in surface view embedded with paracytic

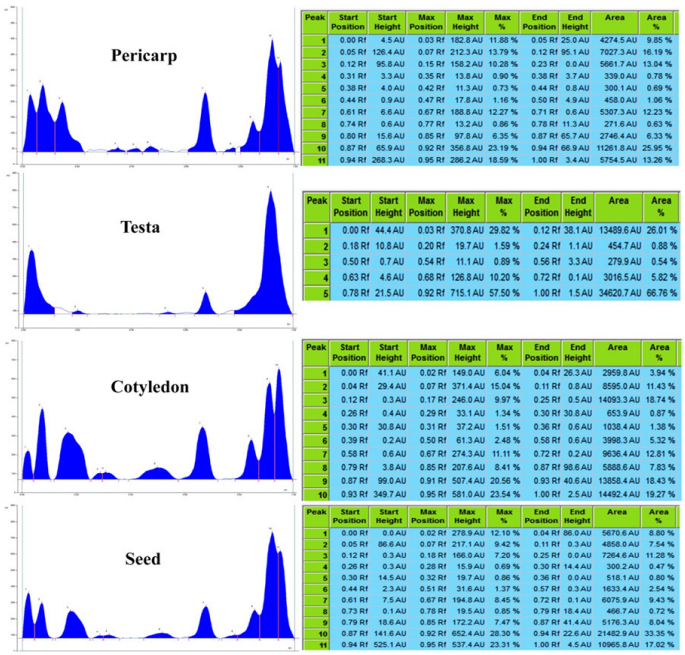

Figure 11: HPTLC densitogram at 520 nm (Derivatized with VSR).

Table 1: R_f values of TLC fingerprint profiling methanol extracts of fruits of *C. gladiata* at λ_{max}254 nm.

Pericarp		Testa		Cotyledon		Seed	
R_f	Color	R_f	Color	R_f	Color	R_f	Color
0.04	green	0.04	green	0.04	green	0.04	green
-	-	0.06	green	0.06	green	-	-
0.09	green	-	-	-	-	-	-
-	-	-	-	-	-	0.15	green
-	-	-	-	0.17	green	-	-
0.19	green	-	-	-	-	-	-
-	-	-	-	0.20	green	-	-
-	-	-	-	-	-	0.32	green
0.39	green	-	-	-	-	0.39	green
-	-	-		0.52	green	0.52	green
0.62	green	-	-	-	-	-	-
0.75	green	-	-	-	-	-	-
0.89	green	0.89	green	0.89	green	0.89	green
0.95	green	-	-	0.95	green	0.95	green

stomata and pair of prismatic crystals of calcium oxalate; cotyledon epidermal cells surface view; thick walled parenchyma cells from testa hypodermal region; tracheids from hilum region; different shape and size of sclerenchymatous cells such as stone cells, sclereids and fibre sclereids; testa epidermal cells in surface view; prismatic crystals of calcium oxalate; glandular trichome; testa in sectional view; crystal fibre; reddish brown content; testa palisade cells; testa palisade cells form hilar region; starch grains; warty covering trichome with hooked and sharp end; and fibres from endocarp region (Figure 7).

HPTLC fingerprint of Methanol extract of *C. gladiata*

The TLC profile of methanol extracts of fruits of *C. gladiata* was developed in the solvent system ethyl acetate: methanol: ammonia (6:3:1, *v/v/v*). The solvent system ratio was chosen by trial and error method to obtain distinguishable band separation. TLC photo documentation (Figure 8) showed numerous phytochemicals as bands at 254 nm, 366 nm and vanillin-sulphuric acid reagent. At 254 nm 8 bands appeared in pericarp, 3 bands in testa, 7 bands in cotyledon and 7 bands in seeds (Table 1), at 366 nm 9 bands appeared in pericarp, 5 bands

Table 2: R_f values of TLC profiling of methanol extract of fruits of *C. gladiata* at λ_{max} 366 nm.

Pericarp		Testa		Cotyledon		Seed	
R_f	Color	R_f	Color	R_f	Color	R_f	Color
-	-	-	-	-	-	0.03	blue
-	-	0.04	blue	0.04	blue	-	-
-	-	-	-	-	-	0.05	blue
0.06	blue	0.06	blue	-	-	-	-
-	-	-	-	-	-	0.15	blue
-	-	-	-	0.16	blue	-	-
-	-	0.19	blue	-	-	-	-
0.20	F blue	-	-	-	-	-	-
-	-	-	-	0.22	blue	0.22	blue
0.30	F green	-	-	0.30	blue		-
-	-	-	-			0.31	blue
0.37	F green	-	-	0.37	F green	-	-
-	-	-	-	-	-	0.38	F green
-	-	-	-	0.46	blue	-	-
0.52	blue	-	-	-	-	-	-
0.58	blue	-	-	-	-	-	-
0.71	blue	-	-	-	-	-	-
0.88	blue	0.88	blue	0.88	blue	0.88	blue
0.92	red	0.92	red	-	-	0.92	red

Table 3: R_f values of TLC profiling of methanol extract of fruits of *C. gladiata* Derivatized with Vanillin Sulphuric acid reagent in white light.

Pericarp		Testa		Cotyledon		Seed	
R_f	Color	R_f	Color	R_f	Color	R_f	Color
-	-	0.02	yellow	-	-	-	-
0.06	grey	-	-	0.06	gray	0.06	grey
0.13	grey	-	-	-	-	-	-
-	-	-	-	0.17	violet	0.17	violet
-	-	-	-	0.20	violet	0.20	violet
-	-	-	-	0.29	violet	0.29	violet
-	-	-	-	0.51	violet	0.51	violet
0.66	pink	0.66	pink	0.66	pink	0.66	pink
0.83	pink	-	-	0.83	pink	0.83	pink
0.91	violet	0.91	violet	0.91	violet	0.91	violet
0.94	violet	0.94	violet	0.94	violet	0.94	violet

Table 4: R_f values of TLC profiling of methanol extract of fruits of *C. gladiata* Derivatized with Dragendorff's reagent in white light.

Pericarp		Testa		Cotyledon		Seed	
R_f	Color	R_f	Color	R_f	Color	R_f	Color
-	-	-	-	0.18	yellow	0.18	yellow
-	-	-	-	0.22	yellow	0.22	yellow

in testa, 7 bands in cotyledon and 8 bands in seeds (Table 2), the plate derivatized with vanillin-sulphuric acid showed 6 bands in pericarp, 4 bands in testa, 9 bands in cotyledon and 9 bands in seeds (Table 3); plate dipped with Dragendorff's reagent 2 orange bands appeared showing the presence of alkaloid in cotyledon and seeds but absent in pericarp and testa (Table 4). The HPTLC densitometric scan profiles of methanol extract of fruits of *C. gladiata* are presented in Figures 9 to 11.

DISCUSSION

Plant anatomy is an important basic tool for authentication. Microscopic characters could be significantly used for authentication at various levels. Though researcher feel anatomical features are difficult to differentiate in close genera in a certain family, thorough analysis at the cellular level or by powder microscopy, solving the authentication issues is not that difficult. Quantitative and qualitative macro and micro-morphological characters can be strengthening the taxonomic decisions within the marketed crud drug. TLC/HPTLC studies are crucial for identification of any herbal drug in addition to microscopic identification. Pharmacopoeias on herbal drugs emphasis the use of TLC for the identification of raw drugs procured from market before using for formulations.[44,45]

In the present study on fruits of *C. gladiata* size and shape of fruit and internal anatomical characters of pericarp, plecenta, testa, cotyledon and radicle were systematically recorded. Powder showed epidermal cells with stomata and pair of prismatic crystal cystolith, trichomes, testa palisade cells, sclereids of different shape and size, brownish content, starch grains, tracheids, fibres and prismatic crystals of calcium oxalate.

Canavalia gladiata L. contains toxic antimetabolites like Canavanine and its primary metabolite Canaline, which are non-protein amino acids and the content of Canavanine has been quantitatively estimated and reported to vary from 2.8 to 4.1% in different processed seeds.[46] In the present study, the two Dragendorff's positive spots present in cotyledon and seed may be Canaline and Canavanine. Their combined content in cotyledon is 18.74% and in seed is 11.28% which are calculated based on the peak area of the respective peaks in the vanillin sulphuric acid derivatized plate in comparison to other separated peaks. Only these two peaks were Dragendorff's reagent positive showing feasibility of the above two compounds.

CONCLUSION

This study sets specific macro-microscopic protocol on both seeds and also differentiates it from closely related species which will help detect adulteration practices in the herbal market. The pericarp and testa do not contain any alkaloid; cotyledons only contain alkaloids. The TLC spots and HPTLC densitograms of methanol extract can be used as botanical reference standard for the identification and differentiation the authentic seed from the related species. The results will be helpful in diagnosing the identity and purity to focus on quality control and standardization of this herbal drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Ar: Arillus; **Brc:** Brownish content; **CC:** Collapsed cells; **CPal:** Counter palisade; **Ct:** Cortex; **Cu:** Cuticle; **E:** Epidermis; **Ec:** Epicarp; **Enc:** Endocarp; **End:** Endosperm; **EPal:** Epidermal palisade; **F:** Fluorescent; **Fpa:** Funicular parenchyma; **Hf:** Hilar fissure; **HPTLC:** High-performance thin-layer chromatography; **Hy:** Hypodermis; **Le:** Lower epidermis; **Llu:** Linia lusida; **Mc:** mucilage cells; **Pa:** Parenchyma; **Pc:** Pro-cambium; **PcS:** Pro-cambium strand; **Per:** Pericycle; **Ph:** Phloem; **Pi:** Pith; **RBc:** Reddish brown content; **ScF:** Sclerenchymatous fibre; **Sg:** Starch grains; **Stc:** Stone cells; **TLC:** Thin layer chromatography; **TkPa:** Thick-walled parenchyma; **TnPa:** Thin-walled parenchyma; **TrB:** Tracheial bar; **Ue:** Upper epidermis; **Vb:** Vascular bundle; **Vs:** Vascular strand; **Xy:** Xylem.

SUMMARY

The present study on fruits of *C. gladiata* surface characters, size, shape, colour, odour, taste and internal anatomical characters of pericarp, testa, plecenta, cotyledon, radicle were systematically recorded; powder microscopic characters epidermal cells with stomata and pair of prismatic crystal cystolith, trichomes, testa palisade cells, sclereids of different shape and size, brownish content, starch grains, tracheids, fibres and prismatic crystals of calcium oxalate were documented. The difference in TLC spot and HPTLC densitograms in the methanol extract can be used as Botanical Reference Standard (BRS) for the identification and differentiation of the authentic seed from the related species. HPTLC with different spots having unique R_f value for pericarp, testa, cotyledon and seeds will be helpful for critical identification of the species.

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