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Original Article

Morpho-anatomy and HPTLC Profiling of *Senna* Mill. Seeds Used in Traditional System of Indian Medicine

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ABSTRACT

Background: The present study was carried out to compare macro-microscopy, powder microscopy and HPTLC analysis of six species such as *Senna auriculata* (L.) Roxb., *Senna alata* (L.) Roxb., *Senna alexandrina* Mill., *Senna occidentalis* (L.) Link., *S. uniflora* (Mill.) Irwin and Barneby, *S. tora* (L.) Roxb. of the genus *Senna* Mill. belongs to the family Caesalpiniaceae. **Materials and Methods:** Six selected *Senna* species were collected, shade dried and pharmacognostic study were performed used techniques such as microscopy and HPTLC analysis. **Results:** Seed macro-morphology of the selected six species shows significant variations in size, shape, colour, ornamentation and number of seeds per pod. The basic cellular structure of six *Senna* species was anatomically similar but variation was observed in number of layers. Microscopic characters of the selected species revealed key diagnostic features that can help to determine the relationship between the closely related species; macrosclerieds and osteosclerdies were observed in *Senna auriculata* and *S. occidentalis*. Powder microscopy of selected six *Senna* species varied in colour, texture and odour. Numerous rosette crystals in cotyledonary cells were found only in *Senna tora* which is a significant variation from other five *Senna* species. HPTLC finger print profiling was performed with ethanol extract at 254 nm, 366 nm and 575 nm derivatization using vannilin-sulphuric acid and the results were documented. In 254 nm 14 bands were separated but the major peak at R_f 0.31 (22.24%) appeared in *S. alexandrina*. **Conclusion:** A detailed morphological and pharmacognostical evaluations play an important role to avoid misidentification and delimitation of selected six *Senna* species and HPTLC profiling is an additional analytical tool for species identification.

Keywords: Senna, *S. auriculata*, *S. alata*, *S. alexandrina*, *S. occidentalis*, *S. uniflora*, *S. tora*, anatomy.

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INTRODUCTION

Caesalpiniaceae is a sub-family of Leguminosae and well known for their showy and attractive flowers. About 600 species are reported in this family. The genus *Senna* Mill. which previously treated under the generic name of "*Cassia* L." is one of the medicinally important genera of Caesalpiniaceae and it consists of about 350 species distributed in the tropical and subtropical regions of the World.[1] In India the genus *Cassia* L. is represented by more than 18 species;[2] whereas, 23 taxa from the erstwhile Presidency of Madras,[3] 29 taxa from the state of Tamil Nadu.[4] However, the herbaceous/shrubs of this genus are separately treated under the Generic name *Senna* Mill. at present. The taxonomic position of the genus *Senna* Mill was delimited from the genus *Cassia* L. and *Chamaecrista* Moench by its characteristic features of extrafloral nectaries, ebracteolate pedicels, anthers characters and areolate seeds.[5] The herbaceous *Senna* species are *Senna absus, S. occidentalis, S. alexandrina, S. tora, S. auriculata, S. alata* and *S. italica.*

Several studies have been made based on the foliar anatomy, taxonomy, phytochemistry, pharmacology of this genus with reference to delimitation of the species since almost all of them are herbaceous and sharing uniform characters. Cuticular anatomy of leaves have been utilised as a key in the discrimination of ten species where types of stomata and trichomes were used to delimit the species.[6] A number of phytochemical and pharmacological studies have been done in the species of *Senna* Mill. to explore the bioactive compounds and clinical activities[7-12] since the seeds of many species of *Senna* Mill. are used as important raw drug

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for many traditional formulations and having a high chance of adulteration and substitution.

Seed coat characters have been widely used in the identification of taxa, particularly in the Leguminaceae family[13-15] and it is highly diverse and can be used as an important diagnostic character including surface patterns, colour and texture. These can be used to distinguish between different genera and species.

The seeds, flowers, stem bark and leaves of different members of the family Leguminosae are distinguishingly used for treating various ailments in Indian traditional system of medicine, especially in Siddha, a remarked traditional Medicinal System of Tamil Nadu, India. Among them the genus *Senna* and its species are being used mainly for skin disorders. In Siddha, leaves, root and flowers of *S. alata* are used to treat skin disorders and as purgative.[16] *S. auriculata* seeds are used in the preparation of Pooranadhi Ilagam used for curing different types of fevers and gastric disorders.[17] Leaf, flower, fruit, root and root bark were used in the treatment of various diseases such as wounds, body heat, body ache, diarrhoea, fever, thirst, diabetes, leucorrhoea etc. Seed powder used to treat eye diseases with the adjuvants of water/coconut oil.[18] In Ayurveda, bark, flower and seeds are used to treat Polyuria, leprosy, worm infestation, diarrhoea, eye diseases and haemorrhage.[16] Roasted seed powder of *Senna occidentalis* is used as a substitute for coffee powder.[18] The root, leaf, flower and seeds are used in Ayurveda for the treatment of eczema, filariasis, cough, dyspnoea and polyuria and the leaf and fruits of *Senna alexandrina* are used for the treatment of skin diseases, constipation, fever, abdominal disorders, gout, blood disorders and worm infestation.[16] The leaf, root and seeds of *Senna tora* are used to treat ringworm infestation, psoriasis, laziness and fever due to tuberculosis. Seeds used to treat various diseases such as urticarial caused by beetle sting, abscess, chronic itching and pitta. Seeds soaked and ground with cow urine (*pasuneer*) are applied externally for ringworm and boils.[18] In Ayurveda, leaf and seeds are used for skin diseases, hemiplegia, constipation, abdominal disorders, obesity, dyspnoea, cough and blood disorders.[16]

Thus, identification of these seeds used in various Siddha and Ayurvedic formulations for curing various diseases are hereby considered as an important issue in the scrutiny of adulterants in the market sample. The present study aims to provide a comprehensive description on anatomical characters and HPTLC profiling of seeds of six *Senna* species.

MATERIALS AND METHODS

Macro-microscopic analysis

Six species of *Senna* Mill. seeds used in the present study are *S. auriculata* (L.) Roxb., *S. alata* (L.) Roxb., *S. alexandrina* Mill., *S. occidentalis* (L.) Link., *S. uniflora* (Mill.) Irwin and Barneby and *S. tora* (L.) Roxb (Figure 1). The selected plants were collected from Tiruppur and Tirunelveli Districts of Tamil Nadu. The vegetative and floral characters and identifying features of the collected plants were confirmed with description given in regional flora.[3] Seeds were shade dried and powdered after 10 to 14 days and

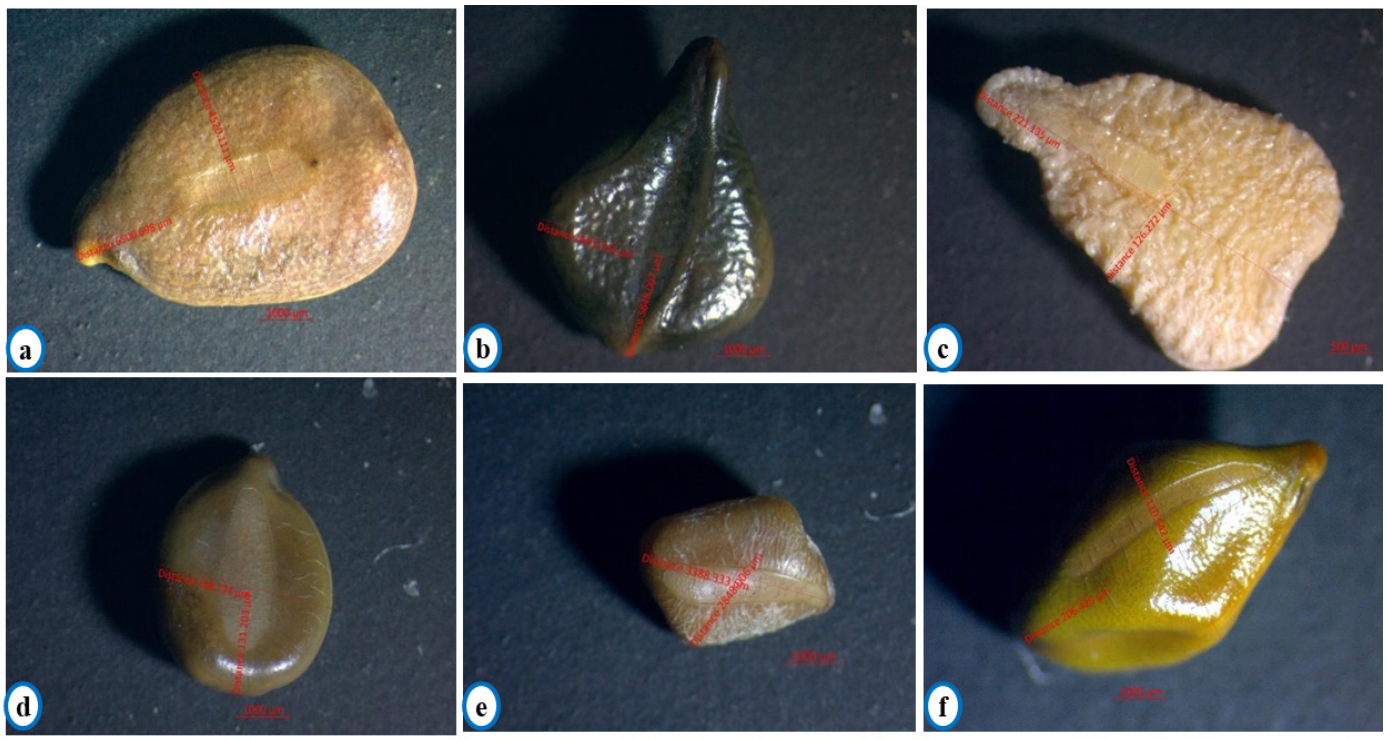


Figure 1 a-f: Macroscopy of the seeds of *Senna* species studied a) *Senna auriculata*; b) *Senna alata*; c) *Senna alexandrina*; d) *Senna occidentalis*; e) *Senna uniflora*; f) *Senna tora*

subjected to powder microscopic and HPTLC analysis. For microscopic studies, seeds were processed by traditional fixation and preservation methods. Transverse section of seeds and cotyledons were performed by hand sectioning followed by staining with safranin. For powder studies, the samples were mounted on glass slides with 50% glycerine for clear observation. Selected views of anatomy and powder studies were captured with pre-calibrated scale bars using Nikon ECLIPSE E200 trinocular microscope with Zeiss Axio Cam Erc5s digital camera under bright field light.

Preparation of the powder

Selected six species of *Senna* seeds each separately powdered and stored in airtight container for further studies.

Preparation of extracts

Each *Senna* species sample one gram was added into 25 ml of water, boiled until it reduces to one fourth of the original volume, filtered and freed from moisture. Extracted the sample with ethanol and made up to 10 ml in a standard flask for quantitative TLC and finger printing.

Preparation of standards

Chrysophanol (10 mg) was dissolved in ethanol and made up to 10 ml in standard flask.

Derivatizing agent

One gram of Vanillin is dissolved in ethanol sulphuric acid mixture (95:5 *v/v*) was used as derivatizing agent.

Instrumentation

A CAMAG'S HPTLC system equipped with Linomat IV (CAMAG, Muttenz, Switzerland) applicator was used for application of extract. An aluminium plate pre-coated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used as the TLC plate. To perform qualitative and quantitative scanning, CAMAG's scanner 030618, equipped with WINCATS software was used. The CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm, and in visible lights after dipping in vanillin-sulphuric acid reagent and heated in an air circulated oven until coloured spots appeared.

TLC Identification

To perform the TLC, of chrysophanol (2 µl) and ethanol extracts from *Senna* seeds (25 µl each) were applied on a TLC aluminium plate (10 cm × 10 cm) were precoated with Silica gel $60F_{254}$ of 0.2 mm thickness as 10 mm bands. Developed the plate in toluene:ethyl acetate (9.5:0.5) solvent system up to a height of 8 cm from the point of application using a pre-saturated twin through chamber. The plate is dried and viewed under UV light at two different wavelengths, 254 nm and 366 nm Capture images of the plate using a visualizer. The plate was dipped in vanillin sulphuric acid reagent, heated at 105°C until coloured spots appear, and capture and documents the resulting image.[19]

HPTLC finger printing

Both the above TLC plates were scanned under UV 254 nm using deuterium lamp in the absorbance/reflectance mode. The finger print profile of successive ethanol extracts of *Senna* species and the 3D chromatogram was documented.

Quantitative estimation

1 to 6 µl of each sample extracts were applied on a TLC aluminium plate (20 cm × 10 cm) precoated with Silica gel $60F_{254}$ of 0.2 mm thickness as 8 mm bands in the tracks 1, 2,3,5,6,7. The Crysophanol standard was applied on the track 4 with the volume of 2 µl. The plate was developed using the mobile phase, toluene:ethyl acetate (9.5:0.5). The developed plate was scanned denistometrically under UV 254 nm in quantitative mode using deuterium lamp.

RESULTS

Seed morphology of *Senna* sp.

The seeds of six *Senna* species were taken for the present study and their colour, size, shape and seed coat ornamentations were recorded (Table 1). Colour of the seed varied from brown (*S. auriculata, S. occidentalis, S. uniflora* and *S. tora*), dark brown to black (*S. alata*) and creamish yellow (*S. alexandrina*). Among all the six species, seed size varied from 0.3 cm to 0.7cm in length and 0.5 to 0.2 cm width (Table 1). *S. auriculata* seed is bigger in size in length (0.7cm) and *S. uniflora* is very small size in length as well as width (± 0.3cm length and ± 0.2cm width; Figure 1). All the species showed variation in shape. Winged pods with maximum number of seeds were observed only in *S. alata*. Sometimes, the size and shape of the seeds may be useful for the delimitation in the species level.

Microscopy and powder microscopy

Selected six *Senna* species shows significant variations in morphology, anatomy and powder microscopy (Figure 2 to 4; Table 1a). Seed coat of selected species varied from smooth (lavigate) to rough wrinkle (sub striate). The cross section of seeds shows distinguishing parts; exotesta mesotesta, endotesta and cotyledons. The thick walled epidermal layer is covered externally by waxy cuticle. In exotesta, epidermis followed by the hypodermis which is made up of Macrosclerieds (thick walled parenchyma cells). In *Senna auriculata, S. occidentalis* the mesotesta is formed of Osteosclerdies (hourglass cells) which is separated from macroscleried by air spaces. The above feature varies in the hilum region as the epidermis is two to multilayers, macrosclerieds are absent sometimes. The Endotestal layer is made of small sized one to three layered hourglass cells. The shape of the cotyledon is varied from species to species based on the size

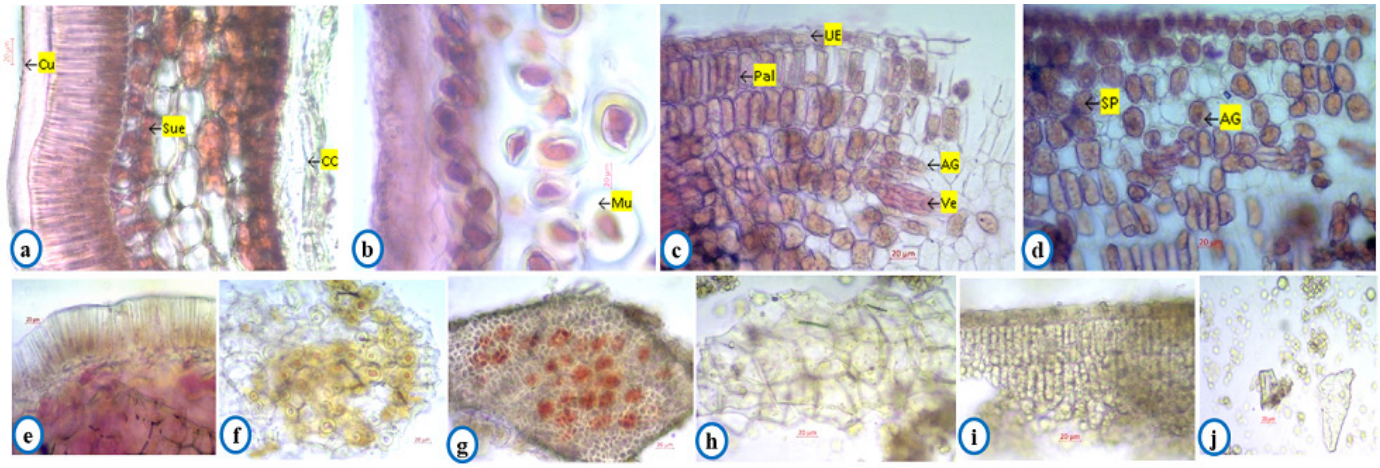


Figure 2A. Microscopy and powder microscopy of the seeds of *Senna auriculata* Roxb. a-d: Microscopic study; a & b) T.S of Testa; c & d) T. S of cotyledon; e-j: powder microscopy - e) Testa is sectional view f & g) Testa in surface view; h) Endosperm cells; i) cotyledon cells j) Oil droplets

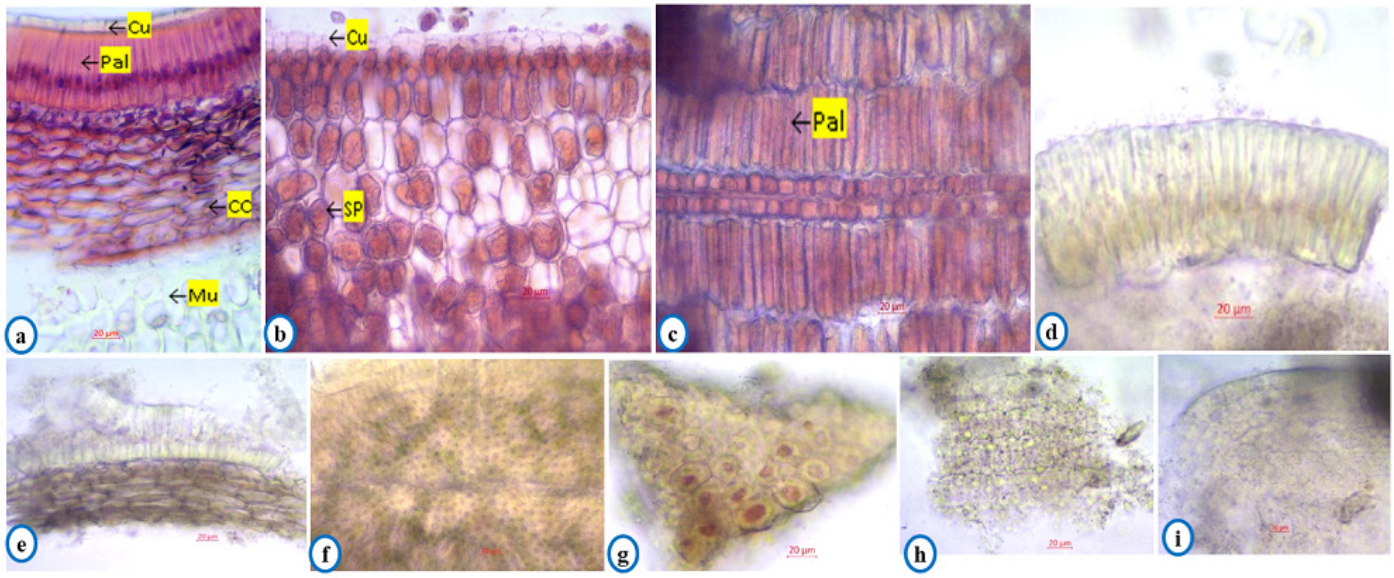


Figure 2B. Microscopy and powder microscopy of the seeds of *Senna alata* (L.) Roxb. a-c: Microscopic study; a) T.S of Seed; c & c) T. S of cotyledon; d-i: powder microscopy – d & e) Testa is sectional view f & g) Testa in surface view; h & i) cotyledon cells

Figure 2: Microscopy and powder microscopy of the seeds of *Senna auriculata* Roxb. & B. Microscopy and powder microscopy of the seeds of *Senna alata* (L.) Roxb.

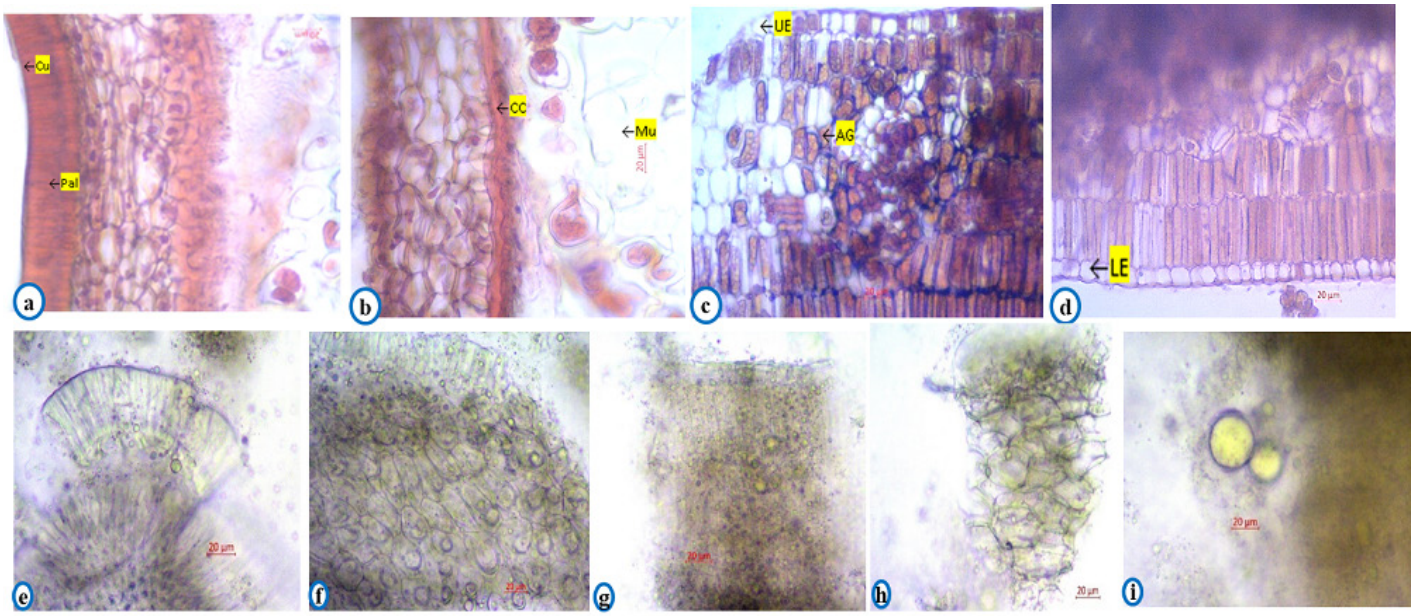


Figure 3A. Microscopy and powder microscopy of the seeds of *Senna alexandria* Mill. A-d: Microcopis study a & b) T.S of Seed; c & d) T. S of Cotyledon; e) Testa is sectional view; f) Testa in surface view; g) Cotyledon cells; h) Endosperm cells; i) Oil globules

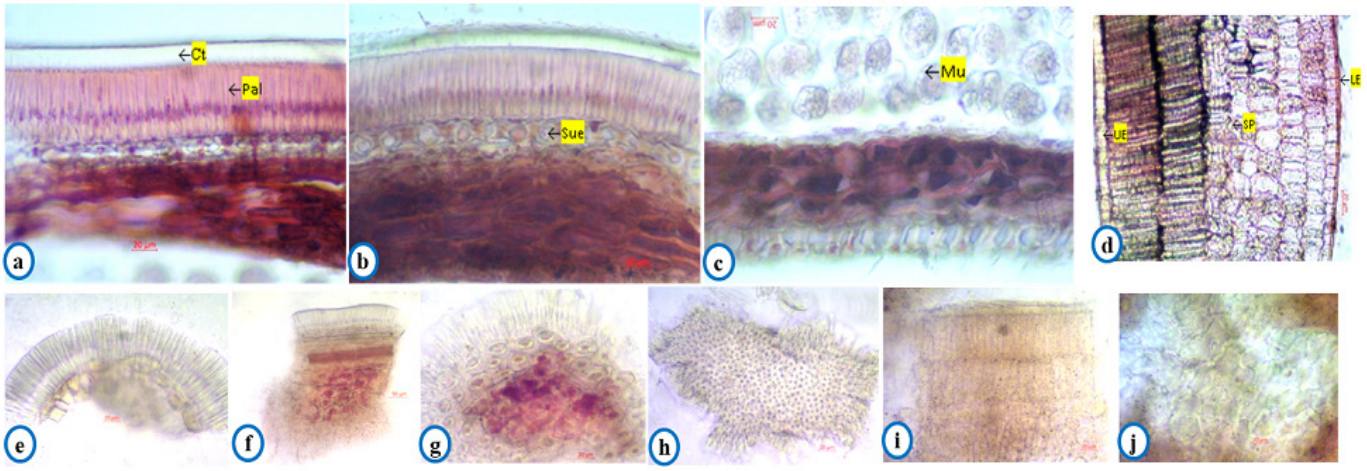


Figure 3B: a-j: Microscopy and powder microscopy of the seeds of *Senna occidentalis* (L.) Link. a-d: Microscopy a&b) TS of Seed outer part; c) T.S.of seed inner part; d) cotyledon; e-j) Powder microscopy: e & f) Testa in sctional view g & h) Testa in surface view; i) endosperm cells; j) Cotyledon cells

Figure 3: Microscopy and powder microscopy of the seeds of *Senna alexandria* Mill. & B. Microscopy and powder microscopy of the seeds of *Senna occidentalis* (L.) Link.

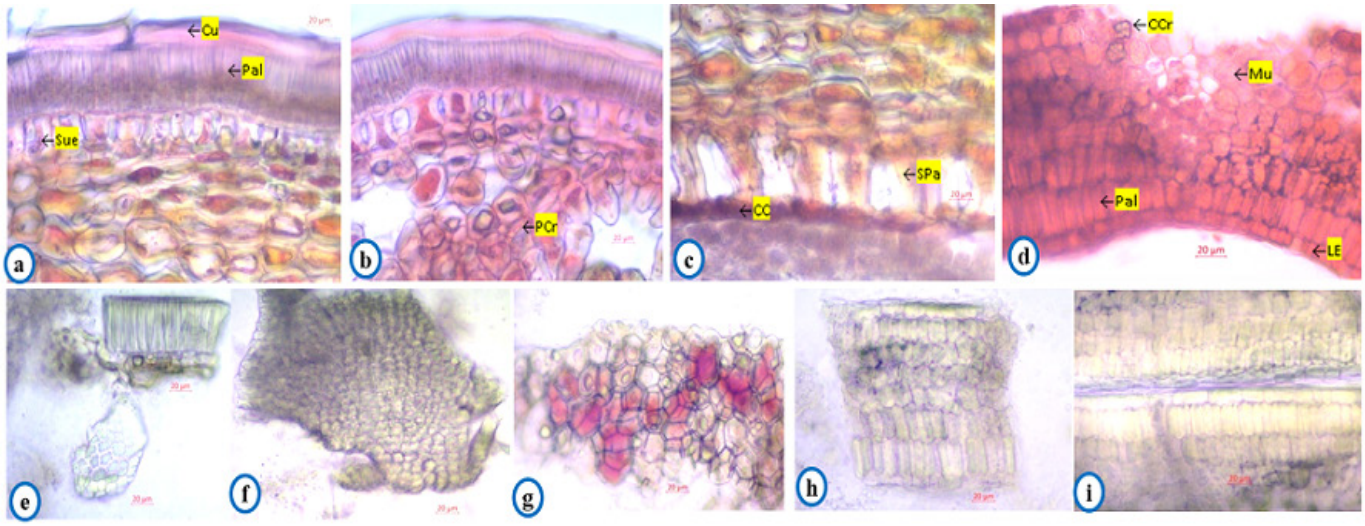


Figure 4A. Microscopy and powder microscopy of the seeds of *Senna uniflora* (Mill.) H.S.Irwin & Barneby; a-d represents Microscopy; a & b) T.S.of Outer region; c) T.S. of inner region; d) T.S of cotyledon; e-i: Powder Microscopy – e) Testa in sectional view f) Testa in surface view g) Sclerenchymatous mesocarp cells; h & i) Cotyledon cells

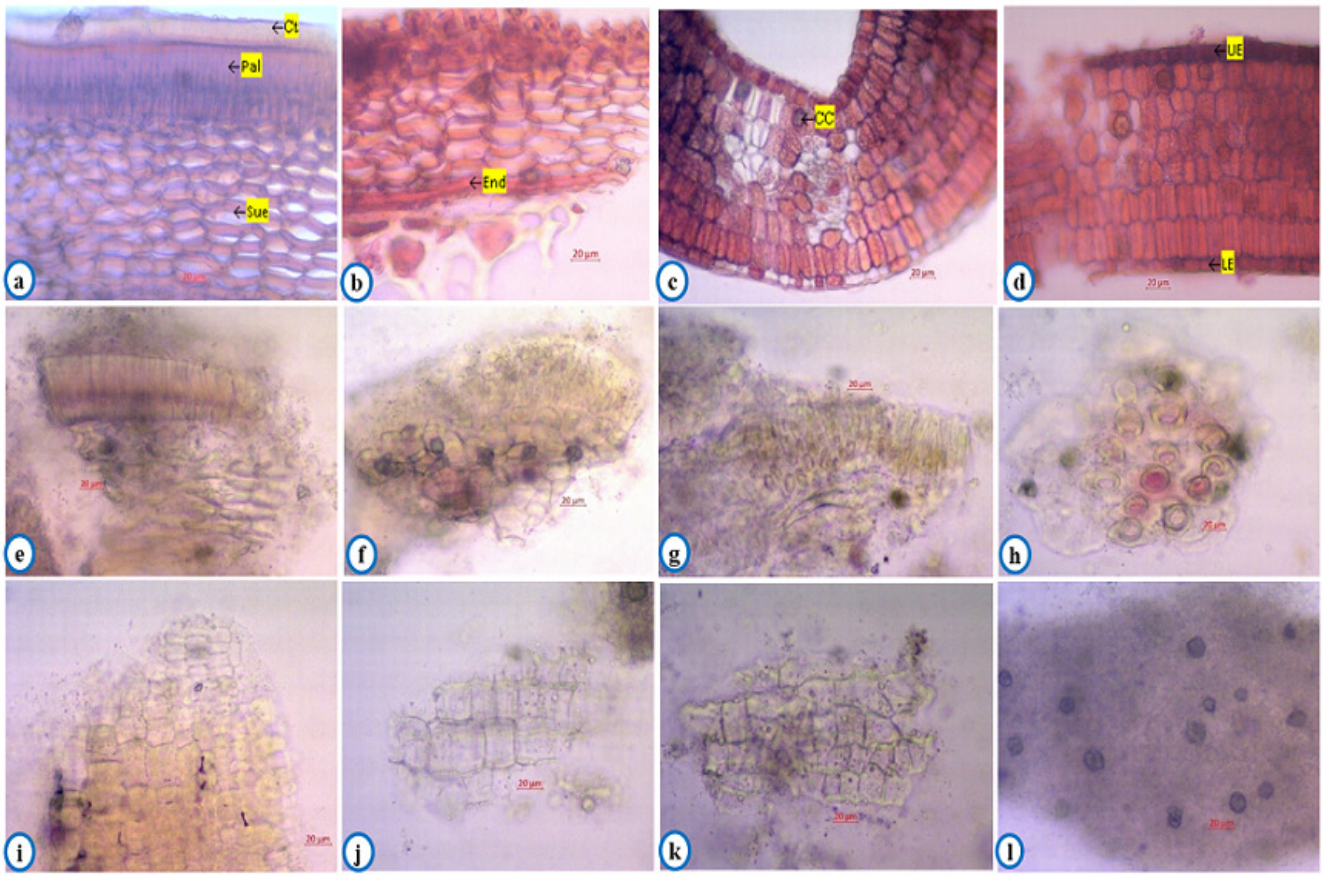


Figure 4B: a-l - Microscopy and powder microscopy of the seeds of *Senna tora* (L.) Roxb.; a-d) Microscopy; a & b) T.S. of seed; c & d) T.S. of cotyledon; e-l- Powder Microscopy: e-g) Testa cells; h) Mesocarp cells; i & j) Cotyledonary cells; k) Endosperm cells ; l) Cells with rosette crystals

Figure 4: Microscopy and powder microscopy of the seeds of *Senna uniflora* (Mill.) H. S. Irwin and Barneby.& B. Microscopy and powder microscopy of the seeds of *Senna tora* (L.) Roxb.

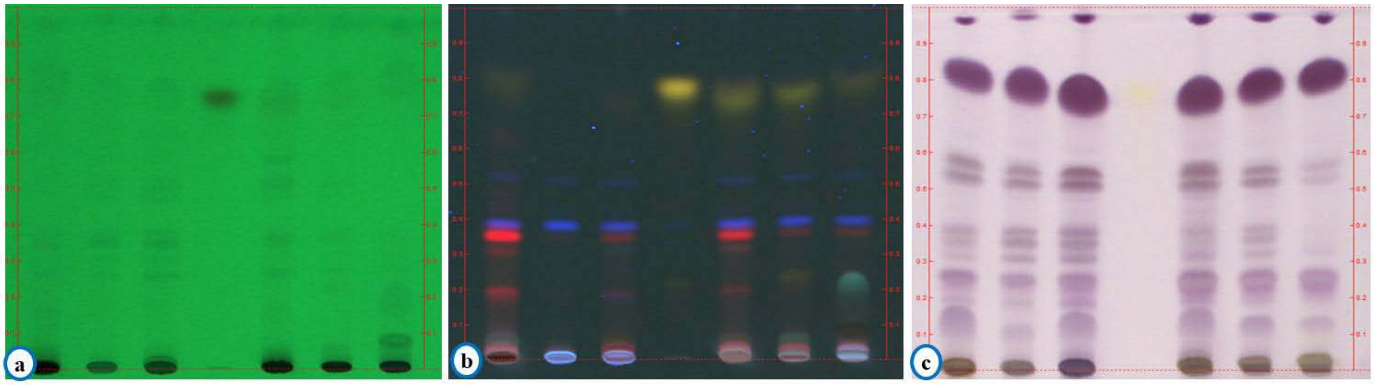
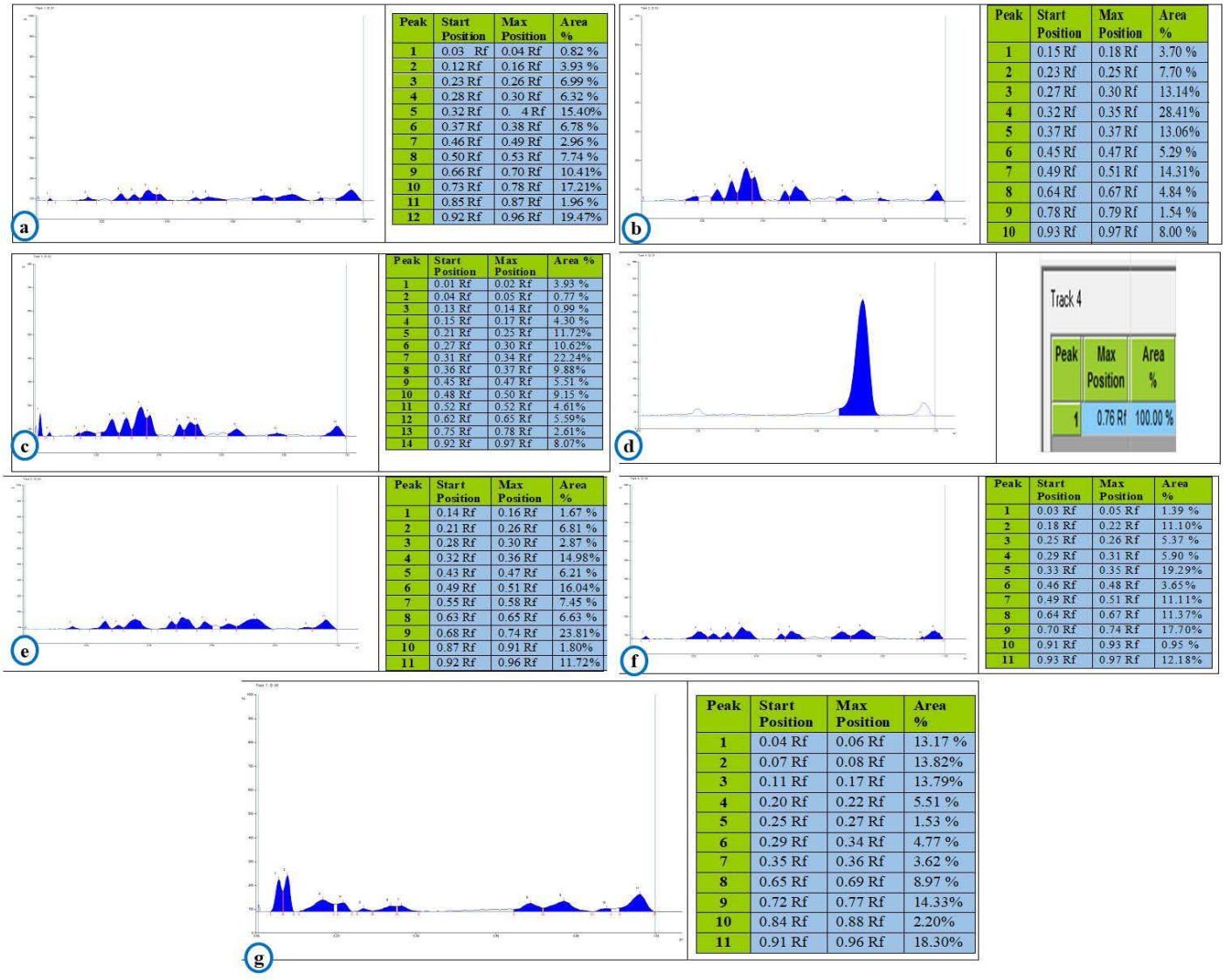


Figure 5A: a-c: TLC Photo documentation of ethanolic extract of *Senna* species and chrysophenol marker; a) Under Short UV at 254nm b) Under long UV at 366nm; c) under white light after derivatization using Vanillin Sulphuric acid reagent at 575 nm



a

Peak	Start Position	Max Position	Area %
1	0.03 Rf	0.04 Rf	0.82 %
2	0.12 Rf	0.16 Rf	3.93 %
3	0.23 Rf	0.26 Rf	6.99 %
4	0.28 Rf	0.30 Rf	6.32 %
5	0.32 Rf	0. 4 Rf	15.40%
6	0.37 Rf	0.38 Rf	6.78 %
7	0.46 Rf	0.49 Rf	2.96 %
8	0.50 Rf	0.53 Rf	7.74 %
9	0.66 Rf	0.70 Rf	10.41%
10	0.73 Rf	0.78 Rf	17.21%
11	0.85 Rf	0.87 Rf	1.96 %
12	0.92 Rf	0.96 Rf	19.47%

b

Peak	Start Position	Max Position	Area %
1	0.15 Rf	0.18 Rf	3.70 %
2	0.23 Rf	0.25 Rf	7.70 %
3	0.27 Rf	0.30 Rf	13.14%
4	0.32 Rf	0.35 Rf	28.41%
5	0.37 Rf	0.37 Rf	13.06%
6	0.45 Rf	0.47 Rf	5.29 %
7	0.49 Rf	0.51 Rf	14.31%
8	0.64 Rf	0.67 Rf	4.84 %
9	0.78 Rf	0.79 Rf	1.54 %
10	0.93 Rf	0.97 Rf	8.00 %

c

Peak	Start Position	Max Position	Area %
1	0.01 Rf	0.02 Rf	3.93 %
2	0.04 Rf	0.05 Rf	0.77 %
3	0.13 Rf	0.14 Rf	0.99 %
4	0.15 Rf	0.17 Rf	4.30 %
5	0.21 Rf	0.25 Rf	11.72%
6	0.27 Rf	0.30 Rf	10.62%
7	0.31 Rf	0.34 Rf	22.24%
8	0.36 Rf	0.37 Rf	9.88%
9	0.45 Rf	0.47 Rf	5.51 %
10	0.48 Rf	0.50 Rf	9.15 %
11	0.52 Rf	0.52 Rf	4.61%
12	0.62 Rf	0.65 Rf	5.59%
13	0.75 Rf	0.78 Rf	2.61%
14	0.92 Rf	0.97 Rf	8.07%

d

Track 4

Peak	Max Position	Area %
1	0.76 Rf	100.00 %

e

Peak	Start Position	Max Position	Area %
1	0.14 Rf	0.16 Rf	1.67 %
2	0.21 Rf	0.26 Rf	6.81 %
3	0.28 Rf	0.30 Rf	2.87 %
4	0.32 Rf	0.36 Rf	14.98%
5	0.43 Rf	0.47 Rf	6.21 %
6	0.49 Rf	0.51 Rf	16.04%
7	0.55 Rf	0.58 Rf	7.45 %
8	0.63 Rf	0.65 Rf	6.63 %
9	0.68 Rf	0.74 Rf	23.81%
10	0.87 Rf	0.91 Rf	1.80%
11	0.92 Rf	0.96 Rf	11.72%

f

Peak	Start Position	Max Position	Area %
1	0.03 Rf	0.05 Rf	1.39 %
2	0.18 Rf	0.22 Rf	11.10%
3	0.25 Rf	0.26 Rf	5.37 %
4	0.29 Rf	0.31 Rf	5.90 %
5	0.33 Rf	0.35 Rf	19.29%
6	0.46 Rf	0.48 Rf	3.65%
7	0.49 Rf	0.51 Rf	11.11%
8	0.64 Rf	0.67 Rf	11.37%
9	0.70 Rf	0.74 Rf	17.70%
10	0.91 Rf	0.93 Rf	0.95 %
11	0.93 Rf	0.97 Rf	12.18%

g

Peak	Start Position	Max Position	Area %
1	0.04 Rf	0.06 Rf	13.17 %
2	0.07 Rf	0.08 Rf	13.82%
3	0.11 Rf	0.17 Rf	13.79%
4	0.20 Rf	0.22 Rf	5.51 %
5	0.25 Rf	0.27 Rf	1.53 %
6	0.29 Rf	0.34 Rf	4.77 %
7	0.35 Rf	0.36 Rf	3.62 %
8	0.65 Rf	0.69 Rf	8.97 %
9	0.72 Rf	0.77 Rf	14.33%
10	0.84 Rf	0.88 Rf	2.20%
11	0.91 Rf	0.96 Rf	18.30%

Figure 5B: HPTLC finger print profile of 25 µlofethanolic extract at 254 nm: a) *Senna auriculata* b) *Senna alata* c) *Senna alexandrina* d) Chrysophanol e) *Senna occidentalis* f) *Senna uniflora* g) *Senna tora*

Figure 5: A. TLC Photo documentation of ethanolic extract of *Senna* species and chrysophanol marker. & B. HPTLC finger print profile of 25µl of ethanolic extract at 254 nm.

Table 1: Morphological characters of seeds of the taxa studied.

Taxa	Pod			Seed			
	No. of seeds/ pod	Size		Shape	Colour	Size (Average)	
		Length (cm)	Width (cm)			Length (cm)	Width (cm)
Senna auriculata	8-17	11.1-15.2	1.6-2.1	Ovate	Brown	0.6	0.4
Senna alata	35-57	14.9-18.5	1.5-2.4	Rhomboid	Dark brown	0.6	0.5
Senna alexandrina	5-9	4.5-6.2	1.2-1.8	Obovate	Creamish yellow	0.6	0.4
Senna occidentalis	30-56	11-12.6	0.6-0.7	Depressed ovate	Greyish brown	0.4	0.3
Senna uniflora	4-10	3.7-5.5	0.3-0.4	Oblong	Brown	0.3	0.2
Senna tora	8-26	8.8-18.7	0.3	Oblong	Shiny brown	0.5	0.3

of the seeds. A more detailed study is required to describe the precise structure of the cotyledons. However, embryo of the seeds having all the above-mentioned structures with central mass of parenchyma cells rich in starch and oil droplets.

Powder Microscopy

Powder of the *Senna* seeds are varied in color, texture and odour. Powder contains sclerenchymatous fibre with pitted walls and narrow lumen, collenchyma cells, aleurone grains of embryos, oil globules, parenchymatous cells with starch. However, the hourglasses cells of cotyledons are present rather the hourglass cells of seed coats are not able to find in the powder. Some cell of cotyledons, testa, endosperms, mesocarp were observed and documented.

HPTLC finger printing

TLC plates were scanned under UV 254 nm using deuterium lamp in the absorbance/reflectance mode. The finger print profiles of successive ethanol extracts of six *Senna* species and the 3D chromatogram was documented.TLC photodocumentation of 25µl of ethanol extract under UV 254nm, 366nm are shown in Figure 5A. Under UV 254nm all spots were observed in green colour and under UV 366nm red, blue, yellow and green colour bands were observed Figure 5 A-B. After derivatization with Vanillin – Sulphuric acid all bands are appeared in violet colour bands Post-derivatized with the vanillin sulphuric acids shown in Figure 5A-c. The R_f value and colour of spots under UV 254nm, 366 nm and white light after derivatization are presented in Table 2. The HPTLC finger print profile of ethanol extract at 254nm, 12 number of peaks separated but the major peak appeared in *S. auriculata* sample at R_f 0.92 (19.47%), 0.73 (17.21%), 0.32 (15.40%), 0.66 (10.41%) (Figure 5B-a). In *S. alata*, totally 10 peaks were recorded and the major peaks appeared at R_f 0.32 (28.41%), 0.49 (14.31%), 0.27 (13.14%), 0.37 (13.06%) (Figure 5B-b). Fourteen bands in *S. alexandrina* with observed major peaks at 0.31 (22.24%), 0.21 (11.72%), 0.27 (10.62%), 0.36 (9.88%), 0.48 (9.15%) are shown in Figure 5B-c. The chrysophanol marker was appearing at R_f 0.76 (100% showing purity) (Figure 5B-d). There are 11 bands detected in *S. occidentalis*, but the major peaks appeared at R_f 0.68 (23.81%), 0.49 (16.04%), 0.32 (14.98%), 0.92 (11.72%) (Figure 5B-e). In *S. uniflora* sample, 11 peaks were observed and the major peaks were at R_f 0.33 (19.29%), 0.70 (17.70%), 0.93 (12.18%), 0.64 (11.37%), 0.49 (11.11%) and at 0.18 (11.10%) (Figure 5B-f). In *S. tora* species, there are 11 peaks were appeared and the major peaks were at R_f of 0.91 (18.30%), 0.72 (14.33%), 0.07 (13.82%), 0.11 (13.79%), 0.04 (13.17%) and the other peaks are observed at minor peaks (Figure 5B-g).

DISCUSSION AND CONCLUSION

Seed morphology and anatomical structure of the Leguminosae has been reported by the many researchers [13,20-22]. Macro and micro-morphological characters of 21 species of the genus *Cassia* showed larger variation in seed morphology.[23] The size of the seed varied from 3.7 cm to 18.5 cm (length) 0.3cm to 2.4 cm (width) among all the six selected *Senna* species in this study. The large size of the seed is observed in *Senna alata* while seeds of *S. tora* is observed with small size. Seed size may be useful for the delimitation of the species. The variability of the shape and surface pattern of the seeds is superficially very useful for the recognition of the studied six species, which is accordance with the reported results.[13,21] The microscopic characters of the studied six species of *Senna* seeds are in agreement with the other reported findings.[9,24,25]

The six species taken up for this study have wide use in traditional medicine. Morphologically, *S. alata* have the biggest seed and *S. uniflora* has the smallest one. The seed coat texture differed markedly in the samples studied. The shapes of seeds varied from ovate to obovate to oblong. The basic cellular structure of the seed was anatomically similar but variation was observed in the number of layers which makes the anatomical study relevant to species identification. However, the Seed coat sculpture of *Senna* species was well studied,[23] it emphasized the Palisade and

Table 1a: Anatomical characters of selected six *Senna* species.

Characters	*S. auriculata*	*S. alata*	*S. alexandrina*	*S. occidentalis*	*S. uniflora*	*S.tora*
Palisade cells	Slight bulbous enlargement at lower end.	Slight bulbous enlargement at middle portion.	-	Slight bulbous enlargement at lower end.	Flat at both ends	Slight bulbous enlargement at lower end.
Linea lucida	Distinct	Not distinct	Absent	Absent	Not distinct	Distinct
Cuticle	Distinct and thick	Distinct and thick	Distinct and thin	Distinct and thick	Distinct and thick	Distinct and thick
Parenchymatous cells	Four to five layers of enlarged parenchymatous cells filled with brownish content	Six to seven layers of enlarged parenchymatous cells filled with brownish content	Five to six layers of enlarged parenchymatous cells.	Four to five layers of enlarged parenchymatous cells filled with brownish content	Six to seven layers of enlarged parenchymatous cells filled with brownish content and few prismatic crystals	Six to eight layers of enlarged parenchymatous cells filled with brownish content and few prismatic crystals
Cotyledons	Mesophyll cells differentiated into seven layers of spongy and two layers of palisade.	Mesophyll cells differentiated into six layers of spongy and two layers of palisade cells.	Mesophyll cells differentiated into seven layers of spongy and two layers of palisade cells.	Mesophyll cells differentiated into five layers of spongy and two layers of palisade cells.	Mesophyll cells differentiated into five layers of spongy and two layers of palisade cells.	Mesophyll cells differentiated into four layers of spongy and two layers of palisade cells.
Crystals	Found absent	Found absent	Present	Found absent	Found absent	Present (rosette crystals of calcium oxalate)

Table 2: R_f values and colours observed after derivatization

S. auriculata		*S. alata*		*S. alexandrina*		*Chryso-phanol*		*S. occidentalis*		*S. uniflora*		*S. tora*	
R_f values and colours observed at 254nm													
R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color
0.26	Green	0.27	Green	0.27	Green	0.76	Green	0.26	Green	0.23	Green	0.08	Green
0.30	Green	0.30	Green	0.30	Green	-	-	0.31	Green	0.28	Green	0.20	Green
0.33	Green	0.37	Green	0.38	Green	-	-	0.35	Green	0.38	Green	0.37	Green
0.78	Green	0.77	Green	0.48	Green	-	-	0.48	Green	0.53	Green	0.76	Green
-	-	-	-	0.50	Green	-	-	0.52	Green	0.77	Green	0.80	Green
-	-	-	-	0.77	Green	-	-	0.59	Green	-	-	-	-
-	-	-	-	-	-	-	-	0.76	Green	-	-	-	-
R_f values and colours observed at 366nm													
0.19	Red	0.36	Red	0.19	Red	0.76	Yellow	0.19	Red	0.22	Yellow	0.22	Green
0.31	Red	0.40	Blue	0.36	Red	-	-	0.36	Red	0.36	Red	0.36	Red
0.36	Red	0.52	Blue	0.40	Blue	-	-	0.40	Blue	0.40	Blue	0.40	Blue
0.40	Blue			0.52	Blue	-	-	0.52	Blue	0.75	Yellow	0.52	Blue
0.52	Blue	-	-	-	-	-	-	0.76	Yellow	-	-	0.77	Light Yellow
0.78	Yellow	-	-	-	-	-	-	-	-	-	-	-	-
R_f values and colours observed in white light after derivatization using Vanillin sulphuric acid													
0.12	Violet	0.12	Violet	0.12	Violet	0.78	yellow	0.12	Violet	0.12	Violet	0.12	Violet
0.25	Violet	0.25	Violet	0.20	Violet	-	-	0.25	Violet	0.25	Violet	0.25	Violet
0.32	Violet	0.32	Violet	0.25	Violet	-	-	0.32	Violet	0.32	Violet	0.32	Violet
0.35	Violet	0.35	Violet	0.32	Violet	-	-	0.35	Violet	0.35	Violet	0.35	Violet
0.39	Violet	0.39	Violet	0.35	Violet	-	-	0.39	Violet	0.39	Violet	0.39	Violet
0.54	Violet	0.54	Violet	0.39	Grey	-	-	0.54	Violet	0.54	Violet	0.54	Violet
0.59	Violet	0.59	Violet	0.54	Violet	-	-	0.59	Violet	0.59	Violet	0.59	Violet
0.82	Violet	0.82	Violet	0.59	Violet	-	-	0.82	Violet	0.82	Violet	0.82	Violet
-	-	-	-	0.82	Violet	-	-	-	-	-	-	-	-

hourglass cells' size and shapes are considered a key character in the delimitation of *Senna* species.

In the TLC photodocumentation, necklace effect was observed as depicted in Figure 5A-c. The only spot at R_f 0.78 in Track 4 was yellow in colour which is chrysophanol marker. Whereas the same spot was observed at varied R_f from 0.78 to 0.82 due to the necklace effect. From Figure 5A-a, the spot corresponding to chrysophanol was slightly observed in all *Senna* species. But from Figure 5A-b, it was observed that *S. alata* was showing the absence of chrysophanol; *S. auriculata* was showing the least content of chrysophanol among all other *Senna* species; and *S. occidentalis* was exhibiting the maximum content among all *Senna* species. In Figure 5B-g, it was observed that violet spot was predominant hiding the yellow colour of chrysophanol. Hence, the content of chrysophanol was not quantified with this solvent successfully.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPTLC: High-Performance Thin Layer Chromatography; **TLC:** Thin Layer Chromatography; **Cu:** Cuticle; **CC:** Cotyledonary Cells; **Mu:** Mucilage; **UE:** Upper Epidermis; **LE:** Lower Epidermis; **Pal:** Palisade; **AG:** Aleurone grains; **Ve:** Vascular elements; **SP:** Sieve Plate; **Ct:** Cortex; **End:** Endodermis; **Sue:** Subepidermis;

Pcr: Prismatic crystal; **Spa:** Spongy parenchyma; **Ccr**: Calcium oxalate crystal.

SUMMARY

The present study is on six species of *Senna* Mill. (*Senna auriculata* (L.) Roxb., *Senna alata* (L.) Roxb., *Senna alexandrina* Mill., *Senna occidentalis* (L.) Link., *S. uniflora* (Mill.) Irwin and Barneby, *S. tora* (L.) Roxb.), which are commonly used in traditional system of medicine, examined various morphological and anatomical characteristics of the seeds, including their size, shape, and cellular structure. It is found that the basic structure of the seeds were similar across all species, there were variations in the number of layers and texture of the seed coat, which could be used to identify different species. In the performance of Thin-Layer Chromatography (TLC) to analyze the chemical composition of the selected six *Senna* species, the content of chrysophanol varied among the species, and it shows absence in *S. alata* and *S. occidentalis* exhibiting the maximum content. The violet spot was observed to be predominant, making the quantification of chrysophanol content difficult with this solvent.

REFERENCES

1. Mabberley DJ. The plant book. A portable Dictionary of Vascular Plants. Cambridge: Cambridge University Press; 2005.
2. Hooker JD. Flora of British India. L. Reeve and Co. London; 1878.
3. Gamble JS. Flora of the presidency of madras. London: Adlard and Son, Ltd.; 1919.
4. Nair NC, Henry AN. Flora of Tamil Nadu, India. Series I: Analysis. Vol. 1. Coimbatore: Botanical Survey of India; 1983.
5. Irwin HS, Turner BL. Chromosomal relationships and taxonomic considerations in the genus *Cassia*. Am J Bot. 1960;47(4):309-18. doi: 10.1002/j.1537-2197.1960.tb07130.x.
6. Begum A, Rahman MO, Begum M. Stomatal and trichome diversity in *Senna* Mill. from Bangladesh. Bangladesh J Plant Taxon. 2014;21(1):43-51. doi: 10.3329/bjpt.v21i1.19264.
7. Das C, Sahoo CD, Dash S, Sahu KA, Hota R. Pharmacognostical and phytochemical investigation of the stem bark of *Cassia tora* Linn. Caesalpiniaceae. J Pharm Pharm Sci. 2011;3(5):84-8.
8. Dubey RB, Sawant BS. Pharmacognostic study of *Cassia tora* L.: a review. J Pharm Sci Innov. 2015;4(4):208-11. doi: 10.7897/2277-4572.04446.
9. Nassar MAAA, Ramadan HRH, Ibrahim HMS. Anatomical structures of vegetative and reproductive organs of *Senna occidentalis* (Caesalpiniaceae). Turk J Bot. 2013;37(3):542-52. doi: 10.3906/bot-1111-26.
10. Kothapalli PL, Papule PP, Thomas BA, Bhujbal SS. Evaluation of *Senna uniflora* (Mill.) leaf extract and its topical gel formulation for biological activity. Int. J Pharm Drug Anal. 2017;5(8):354-65.
11. Nille GC, Reddy KRC. A phytopharmacological review of plant – *Cassia auriculata*. Int J Pharm Biol Arch. 2015;6(6):1-9.
12. Jain S, Patil UK. Phytochemical and pharmacological profile of *Cassia tora* Linn. – an Overview. Indian J Nat Prod Resour. 2010;1:430-7.
13. Fawzi NM. Macro-and micromorphological seed characteristics of some selected species of Caesalpinioideae-Leguminosae. Res J Bot. 2011;6(2):68-77. doi: 10.3923/rjb.2011.68.77.
14. Gunn CR. Fruits and seeds of genera in the subfamily Caesalpinioideae (Fabaceae). Bull. - U.S. Dep Agric. 1991;1755:1-408.
15. Sahai K. Structural diversity in the lens of the seeds of some *Cassia* L. (Caesalpinaceae) species and its taxonomic significance. Phytomorphology Int J Plant Morphol. 1999;49(2):203-8.
16. Yoganarasimhan SN. Medicinal Plants of India. Vol. 2. Tamil Nadu. Bangalore: Cyber Media, 2002.
17. The Siddha formulary of India. Part-II. 1st ed (Tamil version). Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy (AYUSH).New Delhi, 2011.
18. Murugesamudaliyar KS. Gunapadam- Siddha materia medica (Medicinal Plants Division), Indian medicine and homeopathy. Chennai; 1936.
19. Elankani P E, Murugammal S M, Shakila R S, Pitchiahkumar M P, Kabilan N. Chemical standardization pf Thetran Vithai Kutinir Choornanam – an antidiabetic siddha polyherbal formulation. Pharmacogn J. 2019;11(5):1106-13. doi: 10.5530/pj.2019.11.173.
20. Lu-Ayaz SH, Lu OBl. Seed anatomy of five *Vicia* L. (Leguminosae) species. Pak J Biol Sci. 2000;3(9):1440-2. doi: 10.3923/pjbs.2000.1440.1442.
21. Hussein AH, Ghareeb A, Khalifa SF, Fawzi NM. Taxonomic importance of seed characteristics of some species of *Cassia* and *Senna* (subfamily: Caesalpinioideae-Leguminosae). Egypt J Biotechnol. 2002;12:248-65.
22. Salimpour F, Mostafavi G, Sharifnia F. Micromorphologic study of the seed of the *genus Trifolium*, section Lotoidea, in Iran. Pak J Biol Sci. 2007;10(3):378-82. doi: 10.3923/pjbs.2007.378.382, PMID 19069504.
23. Sahai K, Kaur H, Pal A. Macro and micromorphological seed characteristics of some *Cassia* species and their taxonomic significance. Phytomorphology Int J Plant Morphol. 1997;47(3):273-9.
24. Srivastava M, Srivastava S, Khatoon S, Rawat AKS, Mehrotra S, Pushpangadan P. Pharmacognostical evaluation of *Cassia angustifolia* seeds. Pharm Biol. 2006;44(3):202-7. doi: 10.1080/13880200600686442.
25. Büyükkartal HN, Çölgeçen H, Pınar NM, Erdoğan N. Seed coat ultrastructure of hard-seeded and soft-seeded varieties of *Vicia sativa*. Turk J Bot. 2013;37(2):270-5. doi:10.3906/bot-1111-6.

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