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PHARMACOGNOSTIC AND HPTLC CHARACTERIZATION OF *KIRATATIKTA*, *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN

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Keywords:

Swertia chirayita (Roxb. ex Flem.) Karsten, Pharmacognostic standardization, *Kiratatikta*, Ayurveda

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ABSTRACT: Background: *Swertia chirayita* (Roxb. ex Flem.) Karsten known as *Kiratatikta* in Ayurveda, the Indian indigenous health system, is used for various ailments like liver disorders, diabetes, fever, worm infestation, and others. Due to huge demand and non-availability, other Swertia species and other herbs are used as substitutes/adulterants for *Swertia chirayita* (Roxb. ex Flem.) Karsten. Hence, the present study is carried out with the objective of characterizing the macroscopic, microscopic features, the physicochemical and chromatographic profile of the herb. **Material & Methods:** Pharmacognostical parameters like microscopy, physico-chemical, phytochemical and chromatographic investigation for the whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten was done using standard methodology. **Results:** The chief macroscopic features of the whole plant are wrinkled roots, quadrangular stem in the upper portion, leaves brittle, and presence of flowers and fruits. In powder microscopy, fragments of the epidermis with stomata, resin containing cells, pitted parenchyma, cells forming epidermis of fruit, parenchyma cells, pitted fibers, fragments of cotyledon, endosperm cells, sclereids were the key features. Phytochemical analysis revealed the presence of alkaloid, glycoside, tannin, and proteins. Chromatographic profile at 254 nm showed 6 spots with R_f values 0.01, 0.08, 0.33, 0.56, 0.65, 0.74 with maximum concentration of 40.68% at R_f value 0.33. **Conclusion:** This study will serve as a standard reference for the identification of *Swertia chirayita* (Roxb. ex Flem.) Karsten.

INTRODUCTION: *Swertia chirayita* (Roxb. ex Flem.) Karsten, an annual or biennial herb, belongs to the family of Gentianaceae. It is known as Chirata in Hindi, *Kiratatikta* in Sanskrit, and in a trade known as Chiretta. The plant occurs sporadically in subtropical and temperate forests, in open forest margins, cool and moist places or in shady, moist slopes among tall grasses. It is reported as endangered due to loss of habitat and over-exploitation for medicinal uses [1].

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This ethnomedicinal herb is known mostly for its bitter taste caused by the presence of different chemical constituents such as amarogentin, swerchirin, swertiamarin, and other bioactive compounds [2, 3].

Due to the huge demand for the herb, other species of Swertia and plants like *Andrographis paniculata* (green chirayita), *Exacum tetragonum*, *E. bicolor*, *E. pedunculatum* and *Slevolgia orientalis* are adulterated/substituted in the trade [4, 5].

Hence, the present study is carried out with the objective of characterizing the macroscopic, microscopic features, the physicochemical and chromatographic profile of the *Swertia chirayita* (Roxb. ex Flem.) Karsten.

MATERIALS AND METHODS:

Plant Material: The whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten was purchased from Khajrekhar, a raw drug distributor, Belgaum, Karnataka, India. The authentication was done at S.D.M Research Center for Ayurveda, and Allied Sciences, Udupi, and a voucher specimen (No-17111703-08) maintained in the same laboratory. A portion of the whole plant was kept in an airtight container for macroscopic and sensory evaluation while another portion was pulverized into a coarse powder. It was stored in a well-closed container, free from environmental, climatic changes, or any other contamination till usage for further studies.

Macroscopy: The external features of the whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten was documented using Canon IXUS digital camera. The macroscopic features were compared to Ayurvedic Pharmacopeia of India (API) for authentication.

Microscopy: *Swertia chirayita* (Roxb. ex Flem.) Karsten whole plant parts were preserved in a fixative solution. The fixative used was FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Ethyl alcohol-90ml). The materials were left in the FAA for more than 48 hours.

The preserved specimens were cut into a thin transverse section using a sharp blade, and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder Microscopy: A pinch of *Swertia chirayita* (Roxb. ex Flem.) Karsten powder previously sieved was put on the slide and mounted in glycerine and powder characters observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physicochemical Evaluation: Parameters studied under API norms included: foreign matter (%w/w), total ash (%w/w), acid insoluble ash (%w/w), alcohol soluble extractive (%w/w), and water-soluble extractive (% w/w). Analysis of the sample as per API norms [6] was conducted at the Department of Dravyaguna, Sri Dharmasthala Manjunateshwara College of Ayurveda & Hospital, Hassan, Karnataka, India. All the tests were performed in triplicates and data presented as Mean ± SD.

Total Ash Value: 1g of the sample was taken in a pre-weighed crucible and ignited at around 450 °C for 4 h in a muffle furnace. The crucible was later cooled down in a desiccator to room temperature and weighed. The ash value was calculated with reference to the sample weight taken.

Acid Insoluble Ash Value: The ash obtained from the sample was taken into a beaker, and 25 ml dilute HCl (10% v/v) was added to it. The solution was boiled for 5 min and cooled down to room temperature. The solution obtained was filtered through Whatman no. 41 filter paper. The residue was washed with 50 ml boiling distilled water, and the filter paper, along with the residue, then transferred into the crucible and ignited for 4 h at around 450 °C. The crucible then cooled down into a desiccator and weighed. The acid-insoluble ash value was calculated with reference to the sample taken.

Water & Alcohol Soluble Extractive Value: 5 g coarsely powdered sample with 100 ml of water/ alcohol (95% v/v) was shaken frequently during the first 6 h and kept for 18 h. The sample was filtered using normal filter paper, from the filtrate 25 ml solution evaporated in a dry tarred drying dish and dried to constant weight at 105 °C in a hot air oven. The water/alcohol soluble extractive value was calculated with respect to the sample weight taken.

Preliminary Phytochemical Evaluation: The aqueous and alcoholic extracts of the whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten was screened for phytoconstituents using standard reagents and methodology [7].

High-Performance Thin-Layer Chromatography: One gram of powdered *Swertia chirayita* (Roxb. ex Flem.) Karsten was suspended in 10 ml ethanol and kept for cold percolation for 24 h and filtered. 5 µl of the sample was applied on a pre-coated silica gel F254 on aluminum plates to a bandwidth of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plate was visualized in short UV, long UV and then

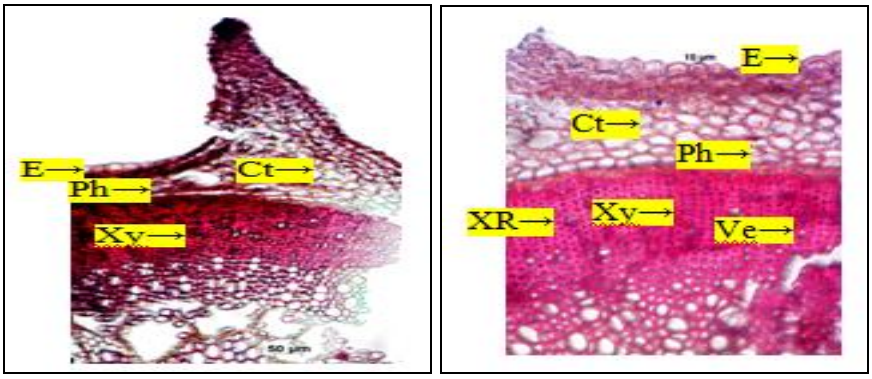
derivatized with vanillin sulphuric acid reagent and scanned under UV 254 nm, 366 nm, and 620 nm following derivatization. R_f, the colour of the spots, and the densitometric scan were recorded.

RESULTS:

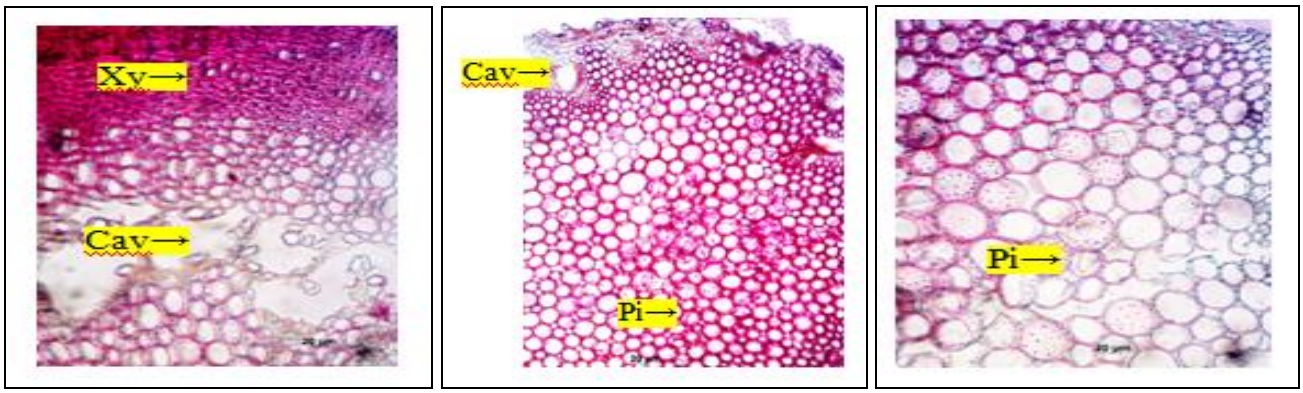
Macroscopic and Organoleptic Evaluation: The sample included the whole plant with roots, stem, leaves, flowers, and fruits. Roots were simple, tapering, faintly longitudinally wrinkled, and externally brownish. Stem almost 1m long, cylindrical in the lower part, quadrangular in the upper portion, brownish in color externally. Leaves shriveled, brittle, with flowers, fruits, and brown in color. All parts of the plant were bitter to taste **Fig. 1**.

Microscopic Features: The transverse section of the stem was circular in outline, showed a layer of the epidermis and a narrow cortex, parenchymatous. Cortex was many-layered in the winged region of the stem. Below the cortex, was a narrow phloem tissue followed by xylem vessels. Pith was parenchymatous, very large, and formed cavity due to the disintegration of its peripheral cells **Fig. 2**.

FIG. 1: *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN DRY PLANT



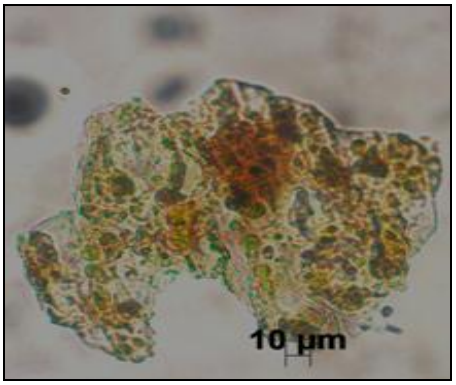
A PORTION OF STEM ENLARGED **CORTEX, PHLOEM AND XYLEM**



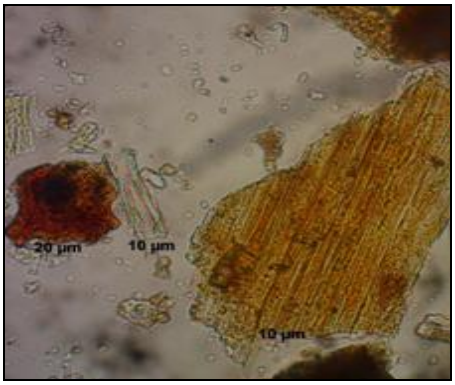
XYLEM REGION **CAVITY AND PITH** **PITH ENLARGED**

FIG. 2: MICROSCOPY OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN (STEM) ct – cortex; cav – cavity; e – epidermis; ph – phloem; pi – pith; ve – vessels; xr – xylem rays; xy – xylem

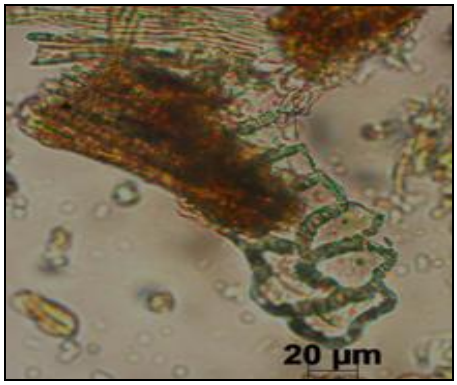
Powder Microscopy: Whole plant powder showed fragments of the epidermis with stomata, resin containing cells, pitted parenchyma, cells forming epidermis of fruit, parenchyma cells, pitted fibers, fragments of cotyledon, endosperm cells, sclereids, parenchyma with vessels, a cell with resinous mass, pitted tracheids **Fig. 3**.



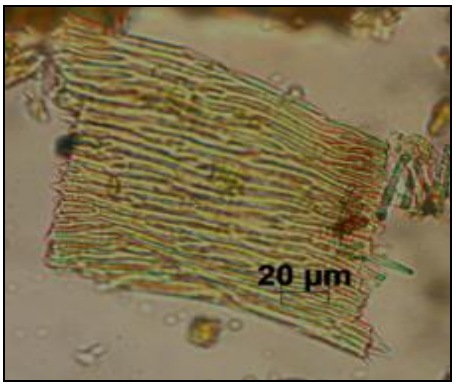
EPIDERMIS SHOWING STOMATA



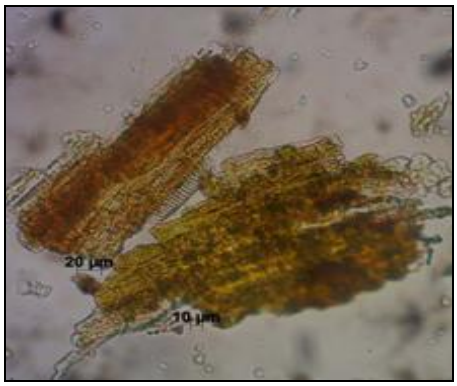
RESIN CONTAINING CELLS, PARENCHYMA



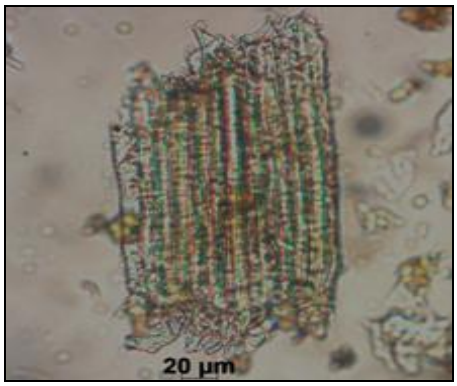
PITTED PARENCHYMA



CELLS FORMING EPIDERMIS OF FRUIT



PARENCHYMA WITH FIBRES



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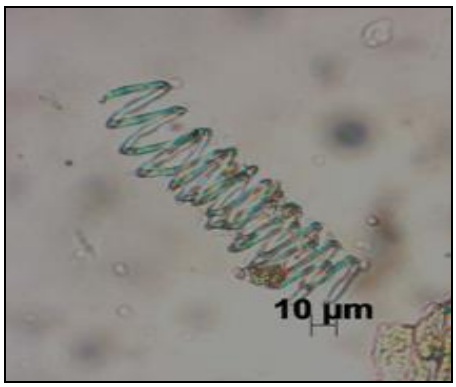
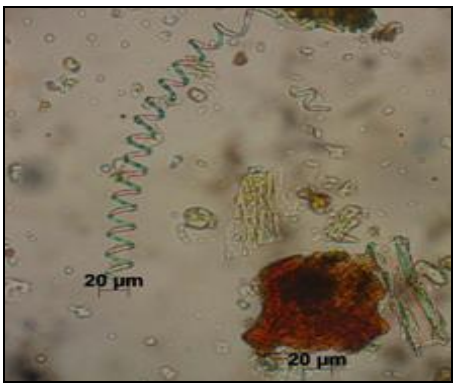
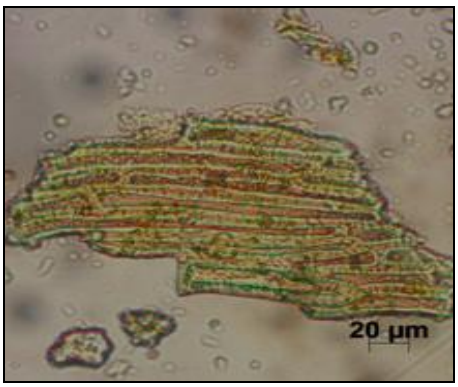


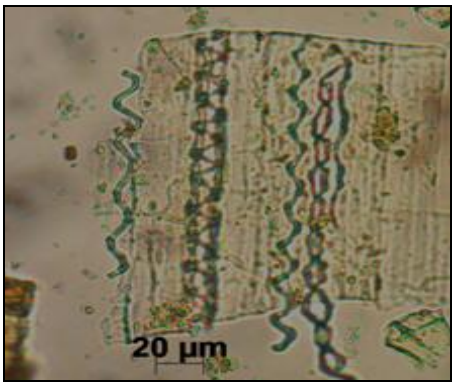
FRAGMENTS OF COTYLEDON



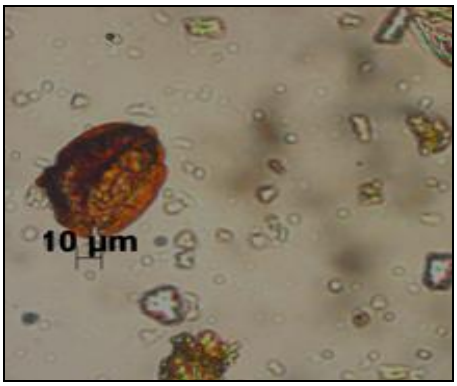
ENDOSPERM CELLS



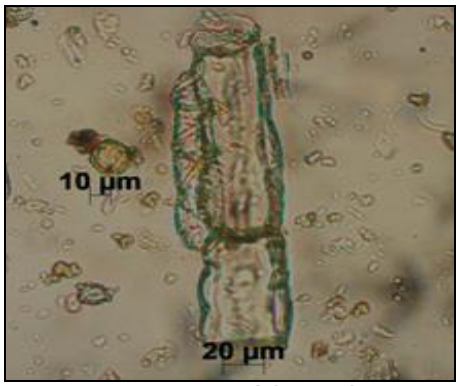
SCLEREIDS



PARENCHYMA WITH VESSELS



CELL WITH RESINOUS MASS



PITTED TRACHEIDS

FIG. 3: POWDER MICROSCOPY OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN

Physico Chemical Evaluation: The results of the physicochemical evaluation are tabulated and compared with the values of standards stated in Ayurvedic Pharmacopeia of India **Table 1**.

TABLE 1: PHYSICOCHEMICAL EVALUATION OF WHOLE PLANT OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN

Parameter	Result n = 3 (% w/w)	API- Part I, Vol-1:99-100
Foreign matter	0.003 ± 0.01	Not more than 2%
Loss on drying at 105 ºC	7.24 ± 0.60	-
Total ash	4.49 ± 0.39	Not more than 6%
Acid insoluble ash	1.53 ± 0.34	Not more than 1%
Water soluble extractive	9.67 ± 5.69	Not less than 10%
Alcohol soluble extractive	9 ± 0	Not less than 10%

Preliminary Phytochemical Evaluation: The preliminary phytochemical investigation of the aqueous and alcoholic extracts of the whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten revealed the presence of alkaloids, glycosides, tannins, and proteins in **Table 2**.

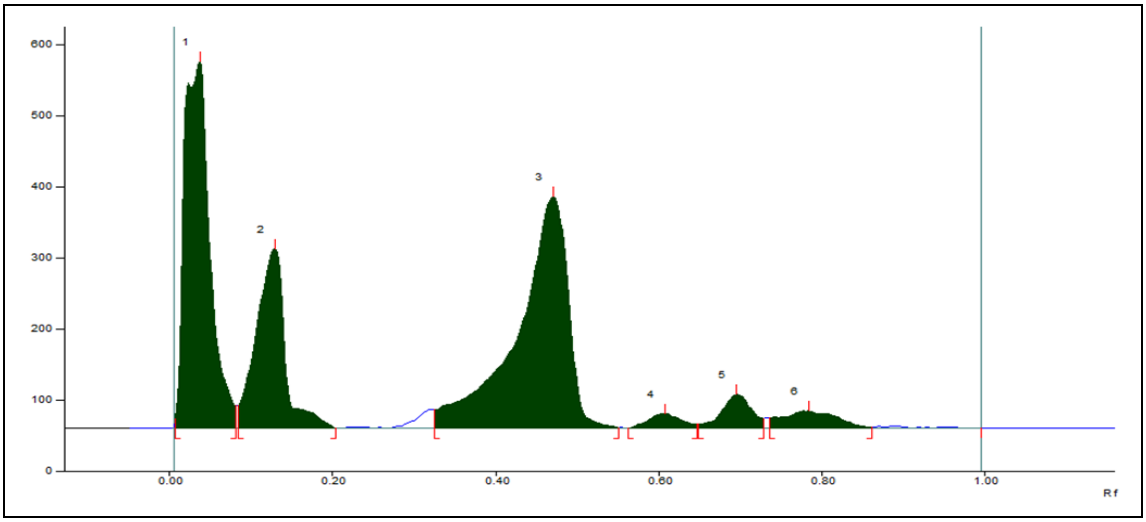
TABLE 2: PRELIMINARY PHYTOCHEMICAL EVALUATION OF WHOLE PLANT OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN

S. no.	Chemical	Test	Alcoholic extract	Aqueous extract
1	Alkaloid	Dragendroff's test	+ve	-ve
		Wagners's test	+ve	+ve
		Mayer's test	+ve	-ve
2	Test for Carbohydrates	Fehlings test	-ve	-ve
		Bendicts test	+ve	-ve
		Iodine test	-ve	-ve
3	Test for Proteins	Biuret test	-ve	-ve
		Precipitation test		
		Absolute alcohol	-ve	-ve
		5% Mercurric chloride	+ ve	- ve
		5% Copper sulphate	+ ve	+ ve
		5% Lead acetate	+ ve	+ ve
		5% Ammonium sulphate	+ ve	- ve
		Ninhydrin test	-ve	-ve
4	Test for Steroid	Salkowski reaction	-ve	-ve
		Libermann burchard reaction	-ve	-ve
		Libermann's reaction	-ve	-ve
5	Test for glycosides	Borntrager's test	+ve	+ve
		Libermann's reaction	-ve	-ve
		Foam test	-ve	-ve
6	Test for Flavonoids	Sulphuric acid test	-ve	-ve
7	Test for Tannins	5% Ferric chloride solution	+ve	+ve
		Lead acetate	+ve	+ve
		Bromine water	-ve	-ve
		Acetic acid solution	-ve	-ve

High-Performance Thin-Layer Chromatography: Results of the chromatography of alcoholic extract of the whole plant of *Swertia chirayita* (Roxb. ex Flem.) Karsten showed presence of various compounds as represented in the densitometric scan at various wavelengths. **Fig. 4**.

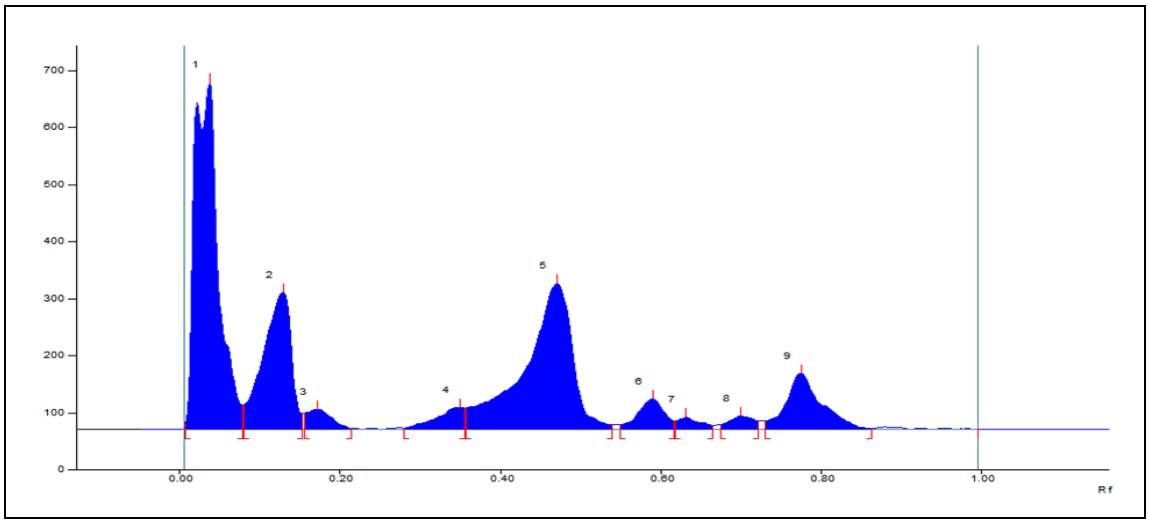
Densiometric scan of TLC at 254 nm revealed 6 spots with R_f values 0.01, 0.08, 0.33, 0.56, 0.65, 0.74 with maximum concentration of 40.68% at Rf value 0.33. In the densiometric scan at 366nm showed 9 spots with R_f values 0.01, 0.08, 0.16, 0.28, 0.36, 0.55, 0.62, 0.68, 0.73 with maximum concentration of 35.27% at R_f value 0.01.

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 R_f	12.9 AU	0.04 R_f	515.2 AU	43.55%	0.08 R_f	31.3 AU	11633.3 AU	32.85%
2	0.08 R_f	31.8 AU	0.13 R_f	251.2 AU	21.23%	0.20 R_f	0.2 AU	637.5.7 AU	18.00%
3	0.33 R_f	26.0 AU	0.47 R_f	324.9 AU	27.46%	0.55 R_f	0.6 AU	14405.8 AU	40.68%
4	0.56 R_f	0.0 AU	0.61 R_f	20.7 AU	1.75%	0.65 R_f	2.2 AU	596.8 AU	1.69%
5	0.65 R_f	5.3 AU	0.70 R_f	47.1 AU	3.98%	0.73 R_f	13.6 AU	1234.4 AU	3.49%
6	0.74 R_f	14.3 AU	0.79 R_f	24.0 AU	2.03%	0.86 R_f	0.8 AU	1165.3 AU	3.29%



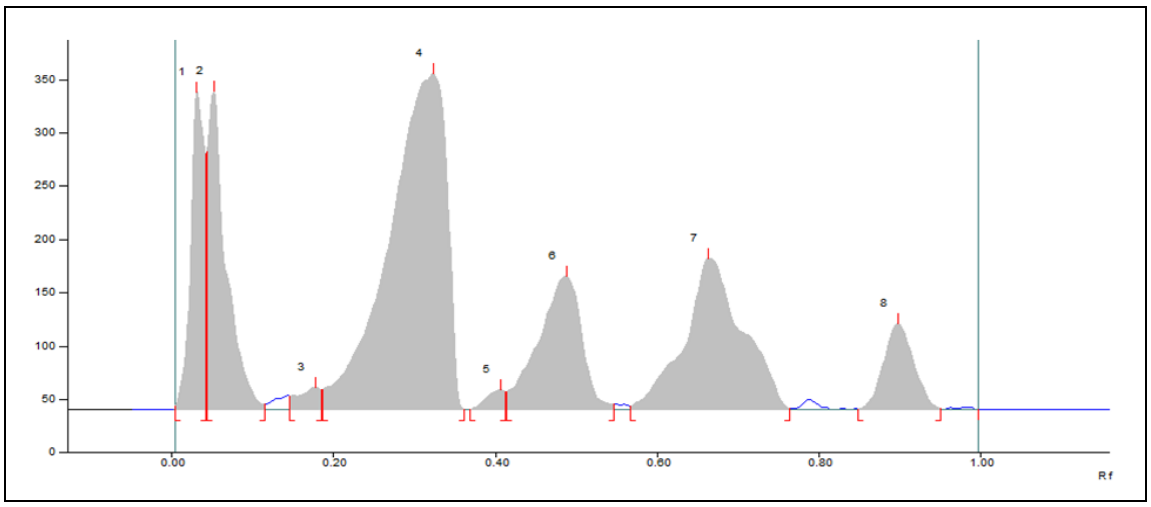
DENSITOMETRIC SCAN OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN AT 254 NM

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 R_f	8.5 AU	0.04 R_f	608.1 AU	44.34%	0.08 R_f	42.9 AU	13017.6 AU	35.27%
2	0.08 R_f	43.4 AU	0.13 R_f	239.2 AU	17.44%	0.15 R_f	27.8 AU	6038.1 AU	16.36%
3	0.16 R_f	28.8 AU	0.17 R_f	35.4 AU	2.58%	0.21 R_f	0.9 AU	774.0 AU	2.10%
4	0.28 R_f	2.3 AU	0.35 R_f	38.7 AU	2.82%	0.36 R_f	37.1 AU	984.3 AU	2.67%
5	0.36 R_f	37.4 AU	0.47 R_f	255.6 AU	18.64%	0.54 R_f	7.5 AU	10807.7 AU	29.28%
6	0.55 R_f	7.8 AU	0.59 R_f	53.2 AU	3.88%	0.62 R_f	14.7 AU	1254.7 AU	3.40%
7	0.62 R_f	15.0 AU	0.63 R_f	20.7 AU	1.51%	0.67 R_f	6.4 AU	426.8 AU	1.16%
8	0.68 R_f	8.1 AU	0.70 R_f	22.9 AU	1.67%	0.72 R_f	14.1 AU	516.4 AU	1.40%
9	0.73 R_f	14.9 AU	0.78 R_f	97.8 AU	7.13%	0.86 R_f	1.2 AU	3093.5 AU	8.38%



DENSITOMETRIC SCAN OF OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN AT 366NM

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.00 R_f	3.5 AU	0.03 R_f	298.0 AU	22.95%	0.04 R_f	40.0 AU	3630.6 AU	9.20%
2	0.04 R_f	241.6 AU	0.05 R_f	299.0 AU	23.02%	0.11 R_f	4.9 AU	4860.3 AU	12.32%
3	0.15 R_f	12.8 AU	0.18 R_f	20.9 AU	1.61%	0.19 R_f	18.7 AU	423.0 AU	1.07%
4	0.19 R_f	19.8 AU	0.32 R_f	315.2 AU	24.27%	0.36 R_f	0.2 AU	16008.0 AU	4057%
5	0.37 R_f	0.1 AU	0.41 R_f	18.3 AU	1.41%	0.41 R_f	16.9 AU	306.2 AU	0.78%
6	0.41 R_f	17.0 AU	0.49 R_f	125.2 AU	9.64%	0.55 R_f	4.8 AU	4778.8 AU	12.11%
7	0.57 R_f	3.5 AU	0.66 R_f	141.8 AU	10.92%	0.76 R_f	1.0 AU	7302.6 AU	18.51%
8	0.85 R_f	0.4 AU	0.90 R_f	80.2 AU	6.17%	0.95 R_f	0.7 AU	2146.5 AU	5.44%



DENSITOMETRIC SCAN OF OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN AT 620 NM

FIG. 4: DENSITOMETRIC SCAN OF OF *SWERTIA CHIRAYITA* (ROXB. EX FLEM.) KARSTEN AT VARIOUS WAVELENGTHS

DISCUSSION AND CONCLUSION: *Swertia chirayita* (Roxb. ex Flem.) Karsten is one of the high prioritized plants by the National Medicinal Plants board [8]. It is an official herb for *Kiratatikta* in Indian Ayurvedic pharmacopeia [6] and a reputed herb for its therapeutic potential in malaria, liver disorders, and diabetes [2]. Due to its high demand and paucity in trade, other Swertia species are used as an adulterant or its substitute [9]. Hence, a detailed systematic pharmacognostic evaluation of plant and plant material provides means of standardization of an herb. The morphological studies reported herein established the macroscopic and microscopic parameters of the plant, which corroborates with the previous study carried out to differentiate the species of Swertia [10].

These morphological characters may be utilized for quick identification of the drug. The microscopic characteristics are particularly useful in the case of the powdered drug. The physicochemical evaluation of an herb establishes its quality and purity. In the present study, Foreign matter, Total ash are within the stated standard limits, whereas Acid insoluble ash is more than the standard limit (1.53 ± 0.34) probably as the entire plant including roots was used for the study, which may relate to a mixture of the plant with sand and soil material. Similarly, water (9.67 ± 5.67) and alcohol extractive (9 ± 0) are below the standard limits, which relate to the purity of the herb. In the study, the preliminary phytochemical investigation revealed the presence of alkaloids, glycosides, tannins and proteins. Previous phytochemical studies have also reported the presence of these phytoconstituents and its role for its varied pharmacological activities [2, 11]. HPTLC fingerprinting for various phytoconstituents in the extract serve as a specific tool to differentiate various extracts from the raw material of different species of herbs [12]. The results obtained from HPTLC may serve as identification for *Swertia chirayita* (Roxb. ex Flem.) Karsten. Thus the microscopic, physicochemical, and chromatographic fingerprinting can be used to judge the adulteration and purity of the drug. The plant exhibits a set of diagnostic characteristics which will help for identification. HPTLC fingerprinting will help to supplement the information in regard to its identification and standardization.

ACKNOWLEDGEMENT: Authors acknowledge Rajiv Gandhi University of Health Sciences, Karnataka, India, for the financial assistance provided to carry out this research work.

CONFLICTS OF INTEREST: None

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