Pharmacogn J. 2018; 10(5):963-968
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Pharmacognostic Evaluation and HPTLC Finger Printing of Rhizome of *Chlorophytum borivilianum* Sant. and F. from Nepal

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ABSTRACT

Introduction: *Chlorophytum borivilianum* Sant. and F.; commonly known as Shveta Musali from the family Liliaceae is a perennial herb. It is used in Ayurveda, Traditional Chinese Medicine, Unani and in folklore practice as an aphrodisiac herb. Present study depicts pharmacognostic features of *Chlorophytum borivilianum* Sant. and F. collected from Nepal. **Methods:** Macro-microscopic analyses, physico-chemical studies and HPTLC finger printing of rhizomes of *Chlorophytum borivilianum* Sant. and F. were carried out according to pharmacopoeial procedures. **Results:** Microscopic analysis has shown presence of epidermis, cork, cortex, collenchymatous cells, starch grains, cluster crystals of calcium oxalate, idioblast, phloem, vascular bundles, pitted xylem parenchyma, sclereids, stone cells, fragment of epiblema, and acicular needles. Preliminary phytochemical analysis revealed presence of alkaloid, carbohydrate, carboxylic acid, resins and saponins. TLC photo-documentation revealed presence of many phyto-constituents with different Rf values and HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation). **Conclusion:** *Chlorophytum borivilianum* Sant. and F. was evaluated for its pharmacognostic features and HPTLC. These specific identities will be useful in identification and authentication of the raw drug.

Key words: *Chlorophytum borivilianum*, Pharmacognostic, Phytochemical, Shveta Musali, Quality Control.

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History
- Submission Date: 10-04-2018;
- Review completed: 22-05-2018;
- Accepted Date: 11-07-2018

DOI : 10.5530/pj.2018.5.163

Article Available online
http://www.phcogj.com/v10/i5

INTRODUCTION

Chlorophytum includes nearly 300 species which are distributed throughout tropical and subtropical parts of the world. Seventeen species of *Chlorophytum* is recorded in India.[1] Among them *Chlorophytum borivilianum* Sant. and F. is having highest saponin content which is responsible for its therapeutic utilities.[2] It was first reported in India in 1954 and reached rare status in nature due to over exploitation.[3] *C. arundinaceum* Baker, *C. laxum* R.Br., *C. tuberosum* Baker, *C. orchidastrum* Hook.f., p.p. non Lindl. are used as adulterant and substitutes.[4] *C. borivilianum* is commercially cultivated and commonly used by pharmaceuticals.[5] *Chlorophytum borivilianum* Sant. and F., also known as Shveta Musali, from the family Liliaceae, is a perennial herb, 10-35 cm in height; rhizome elongated, cylindrical, fleshy. Leaves are basal, linear-lanceolate and membranous with short petioles. White flowers with six petals, small, black seeds enclosed in flowering boles.[6–8] It is used in Ayurveda, Traditional Chinese Medicine, Unani and in folklore practice as an aphrodisiac herb. It's rhizome is *Shukrala, Rasayana, Vrisya, Balya, Brimhana, Madhura, Tikta, Snigdha, Sheeta,* and *Laghu.*[4,9,10] It has shown spermatogenic, aphrodisiac, immune-modulatory, anti-diabetic, anti-oxidant, anti-stress, anti-microbial, anti-aging, anti-tumor and anti-inflammatory activities.[11–15] Saponins (borivilianosides E-H), flavonoids, proteins, carbohydrate, phenolics, triterpenoids, tannis, sucrose, glucose, fructose, galactose, mannose and xylose have been reported from *C. borivilianum*.[2-3,15–16]

MATERIALS AND METHODS

Plant material

Fresh rhizomes were collected from Chitwan District, Nepal in the month of November - December. The plant material was authenticated at Department of Dravyaguna, SDM College of Ayurveda Hassan, Karnataka and the voucher specimen was deposited in the respective herbarium for future reference (SDMCAH-DG/2017/14). The rhizomes were cleaned and shade dried. The dried rhizomes were coarsely powdered and used for macroscopic, microscopic characterization, phyto-chemical analysis and HPTLC.

Cite this article: Kopila A, Anuradha KN, Prabhu Suchitra N. Pharmacognostic Evaluation and HPTLC Finger Printing of Rhizome of *Chlorophytum borivilianum* Sant. and F. from Nepal. Pharmacogn J. 2018;10(5):963-8.

Organoleptic and Macroscopic Evaluation

Fresh and dried rhizomes along with the powder were evaluated for their organoleptic and macroscopic features i.e. size, shape, color, odor, taste, texture and specific botanical characters were evaluated as per the standard procedure.[17] The external features of the test samples were documented using Canon IXUS digital camera.

Microscopic evaluation

Transverse section of rhizome: Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 h.[18–19] The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. The slides were also stained with iodine in potassium iodide for detection of starch. 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

Pinch of powder of rhizome previously sieved was put on the slide and mounted in glycerin. Powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physico-chemical analysis

Physico-chemical parameters viz. loss on drying at 105°C, total ash, acid insoluble ash, water soluble ash, ethanol and water soluble extractive values were evaluated using standard methods.[20]

Preliminary phyto-chemical screening

Ethanolic extract of *C. borivilianum* Sant. and F. was subjected to qualitative evaluation for the presence or absence different groups of phyto-constituents such as alkaloids, flavonoids, saponins, carbohydrates, carboxylic acid, coumarins, phenol, quinine, resins, steroid, tannin, terpenoid, and amino acids.[20] Detail of phyto-chemical evaluation is illustrated in Table 3.

HPTLC finger printing

One gm of powdered sample of *Chlorophytum borivilianum* Sant. and F. was suspended in 10.0ml of alcohol (99.9%) with intermittent shaking for the first few hours and kept for 24 h at room temperature followed by filtration, made up to 10.0ml with ethanol; 8µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 3.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. Retention factor (*Rf*), color of the spots and densitometric scan were recorded using CAMAG Scanner 4.[21–22]

RESULTS

Organoleptic and macroscopic observations

Results obtained from organoleptic and macroscopic observations of fresh rhizome and powder are illustrated in the Table 1.

Table 1: Organoleptic and Macroscopic Evaluation of *Chlorophytum borivilianum* Sant. and F.

Observations	Rhizome	Powder
Size	5-12cm long, 1.5-2cm diameter	NA
Shape	Elongated	NA
Color	Whitish	Brownish
Odor	Not characteristics	Not characteristics
Taste	Madhura, Tikta, Mucilagenous	Madhura, Tikta, Mucilagenous
Texture	Smooth and having horizontal wrinkles on drying	Smooth

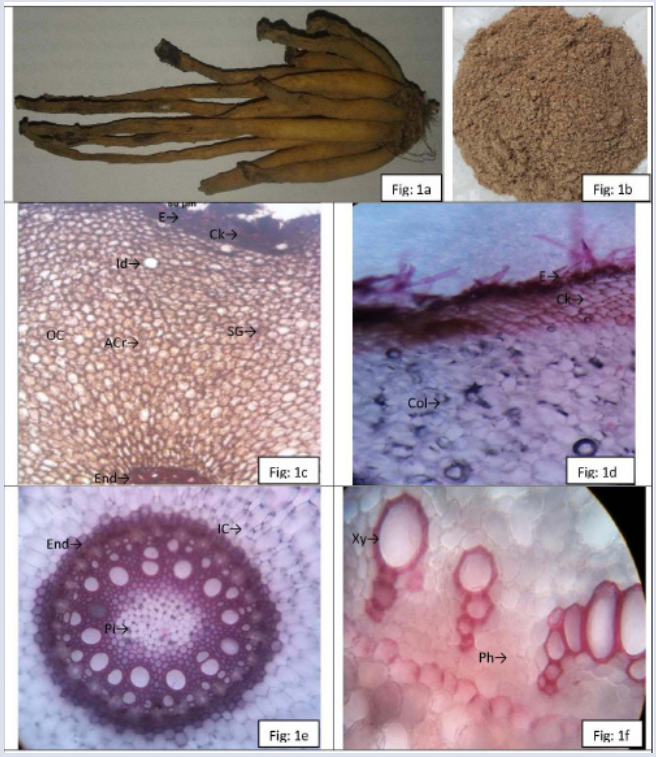

Figure 1: Macro-microscopic features of Rhizome of *Chlorophytum borivilianum* Sant. and F.
Fig. Ia - Rhi/ome; Fig. Ib - Powder; Fig. Ic - TS through outer region; Fig. Id - Enlarged TS through outer region; Fig. Ie - TS through inner region; Fig. If - Enlarged xylem and phloem; E - Epidermis; Ck - Cork; Id - Idioblast; OC - Outer cortex; ACr - Acicular crystal; SG - Starch grain: Col - Collenchyma: End - Endodermis; IC - Inner cortex; Pi - Pith; Xy - Xylem; Ph - Phloem.

Microscopic evaluation

Microscopic evaluation of transverse section of rhizome of *Chlorophytum borivilianum* Sant. and F. revealed the presence of epidermis, cork, starch grains, idioblast cells, outer cortex, xylem, phloem, radial vascular bundle, collenchyma cells, single layered endodermis, stellar region, pith, and numerous cluster crystals. The details of the microscopic evaluation of transverse section of rhizome are presented in Figure 1. Microscopic evaluation of powder of rhizome of *Chlorophytum borivilianum* Sant. and F. revealed the presence of starch grains, pitted xylem parenchyma, vessels, cork, sclereids, stone cells, fragment of epiblema, acicular needles. The details of the powder microscopy of rhizome are presented in Figure 2.

Physico-chemical analysis

Physico-chemical characters were evaluated and the results obtained are illustrated in Table 2.

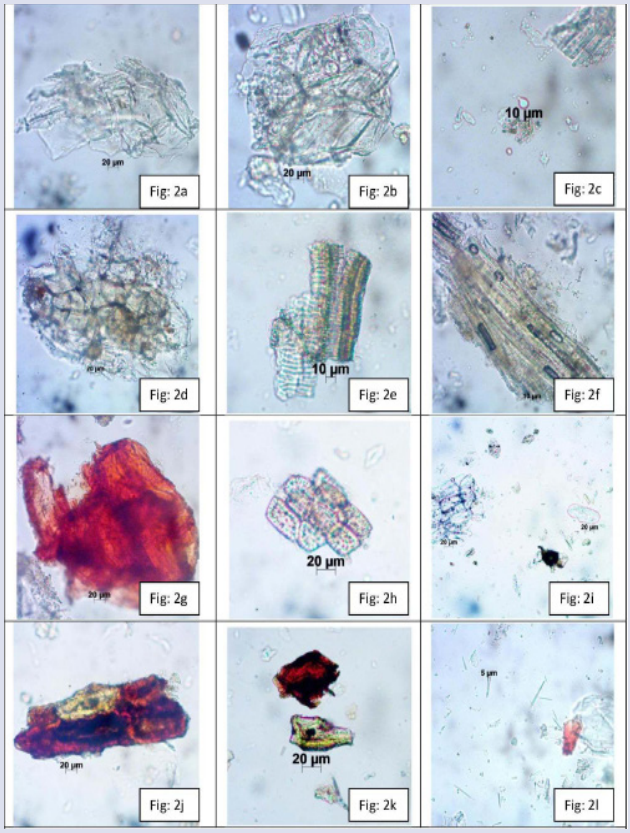

Figure 2: Powder Microscopy of Rhizome of *Chlorophytum borivilianum* Sant. and F.

Fig: 2a - Parenchyma with starch grains; Fig: 2b - Parenchyma with bunch of starch grains; Fig: 2c - Isolated starch: Fig: 2d - Fragments of Epiblema: Fig: 2e - Vessels; Fig: 2f - Bundle of fibres; Fig: 2g - Cork cells; Fig: 2h - Group of Stone cells: Fig: 2i - Pitted xylem parenchyma; Fig: 2j - Group of Sclereids; Fig: 2k - Isolated sclereids; Fig: 21 - Aciculur needles,

Table 2: Physico-chemical Evaluation of Rhizome of *Chlorophytum borivilianum* Sant. and F.

Observations	Observed values
Foreign matter	7.93±0.06%
Loss on drying	8.70±0.02%
Total ash	8.91±0.05%
Acid insoluble ash	4.51±0.01%
Water soluble ash value	06.45±0.02%
Alcohol soluble extractive	3.97±0.04%
Water soluble extractive	24.78±0.11%

Phytochemical Evaluation

Phytochemical evaluation of *Chlorophytum borivilianum* Sant. and F. revealed the presence of alkaloid, carbohydrate, carboxylic acid, resins and saponins in the rhizome. Detail of phyto-chemical evaluation is illustrated in Table 3.

HPTLC Finger printing

R_f values and color of the spots in chromatogram developed in toluene: ethyl acetate (7.0:3.0) for ethanolic extract of rhizomes of *Chlorophytum borivilianum* Sant. and F. were recorded. The details of *Rf* value is given in Table 4. TLC photo-documentation revealed presence of many phytoconstituents with different *Rf* values and HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation).

PHOTO DOCUMENTATION (SOLVENT SYSTEM PET ETHER: ETHER ACETATE)

Two spots were detected under short UV - 254 nm in *Chlorophytum borivilianum* Sant. and F. (*Rf* 0.70, 0.77). All of them were having light green color. Four spots were detected under long UV - 366 nm (*Rf* 0.04, 0.38 – fluorescent light blue color, *Rf* 0.72, 0.84 – fluorescent dark blue color). Three spots were detected after derivatisation - 620 nm (*Rf* 0.24, 0.54, 0.77 – light purple color). Detail of HPTLC photo documentation are illustrated in Figure 3.

HPTLC DENSITOMETRIC SCAN

Total 17 numbers of active components were detected in *Chlorophytum borivilianum* Sant. and F. having *Rf* value (0.01, 0.04, 0.25, 0.27, 0.33, 0.39, 0.60, 0.79, 0.85, 0.05, 0.42, 0.79, 0.03, 0.21, 0.28, 0.56, 0.68, 0.86). The detail of HPTLC photo documentation are illustrated in Figure 4. Nine peaks were detected under short UV - 254 nm; among them maximum percentage of area were occupied by *Rf* 0.01 (35.26%), 0.04 (12.94%), 0.33 (11.11%). Four peaks were detected under long UV - 366nm; among them maximum percentage of area were occupied by *Rf* 0.01 (67.65%), 0.05 (21.20%), 0.79 (7.54%). Six peaks were detected after derivatization - 620nm; among them maximum percentage of area were occupied by *Rf* 0.03 (65.06%), 0.68 (13.38%), 0.86 (9.51%).

DISCUSSION

Macroscopic characters

Rhizome of *C. borivilianum* was elongated, with whitish external surface. It was smooth and having horizontal wrinkles on drying. It was not having any characteristics odor. It was having *Madhura (Sweet), Tikta (Bitter)* and mucilaginous taste. Powder of *C. borivilianum* was brownish in color.

Microscopic characters

Transverse section of rhizome of *C. borivilianum* was having epidermis, cork, starch grains, idioblast cells, outer cortex, xylem, phloem, radial vascular bundle, collenchyma cells, single layered endodermis, stellar region, pith, and numerous cluster crystals. Powder microscopy of the given sample had pitted xylem parenchyma, starch grains, cork cells, sclereids, bundle of fibers, vessels, acicular needles, fragments of epiblema and stone cells.

Relevance of finding from current study related to microscopic evaluation can be also substantiated from the earlier works.[23]

Phytochemical Analysis

Phytochemical analysis of *Chlorophytum borivilianum* Sant. and F. had shown presence of alkaloid, carbohydrate, carboxylic acid, resins and saponins. Previous studies had shown presence of steroid, glycosides, saponins, and triterpenoid starch in *Chlorophytum borivilianum* Sant. and F.[23–24]

TLC AND HPTLC

R_f values and color of the spots in chromatogram developed in toluene: ethyl acetate (7.0:3.0) for ethanolic extract of rhizomes of *Chlorophytum borivilianum* Sant. and F. were recorded. TLC photo-documentation revealed presence of many phytoconstituents with different *Rf* values and HPTLC densitometric scan of the plates showed numerous bands under short UV, long UV and 620 nm (after derivatisation).

Table 3: Phytochemical evaluation of *Chlorophytum borivilianum* Sant. and F.

	Test	Color if positive	Result	Remarks
1.	Alkaloids			
	Dragendrof's test	Orange precipitate	Orange precipitate	Present
	Wagners test	Red precipitate	Red precipitate	Present
	Mayers test	Dull white precipitate	Dull white precipitate	Present
2.	Steroids			
	Liebermann-buchard test	Bluish green	No Bluish green	Absent
	Salkowski test	Bluish red to cherry red	Red color in the chloroform	Absent
3.	Carbohydrate			
	Molish test	Violet ring	Violet ring	Present
	Fehlings test	Brick red precipitate	Bluish color	Absent
	Benedicts test	Red precipitate	Bluish color	Absent
4.	Tannin			
	With $FeCl_3$	Dark blue or green or brown	Golden Yellow Color	Absent
5.	Flavanoids			
	Shinoda's test	Red to pink	Golden Yellow Color	Absent
6.	Saponins			
	With $NaHCO_3$	Stable froth	Stable froth	Present
7.	Triterpenoids			
	Tin and thionyl chloride test	Red	Black color	Absent
8.	Coumarins			
	With 2 N NaOH	Yellow	Colorless solution	Absent
9.	Phenols			
	With alcoholic ferric chloride	Blue to blue black, brown	Golden Color solution	Absent
10.	Carboxylic acid			
	With water and $NaHCO_3$	Brisk effervescence	Brisk Effervescence	Present
11.	Resin			
	With aqueous acetone	Turbidity	Turbidity	Present
12.	Quinone			
	5% NaOH	Pink/purple/red	Color less solution	Absent
13.	Amino acids			
	Ninhydrine reagent	Purple color	Color less solution	Absent

Photo documentation (solvent system pet ether: ether acetate)

Total eight spots detected in *Chlorophytum borivilianum* Sant. and F in different Rf value. Number of spots indicates the total number of active chemical components present in the given sample.

HPTLC Densitometric scan

Total 17 numbers of active components were detected in *Chlorophytum borivilianum* Sant. and F. having Rf value (0.01, 0.04, 0.25, 0.27, 0.33, 0.39, 0.60, 0.79, 0.85, 0.05, 0.42, 0.79, 0.03, 0.21, 0.28, 0.56, 0.68, 0.86) among them maximum percentage of area were occupied by Rf 0.01 (35.26% - 254nm); 0.01 (67.65% - 366nm), 0.03 (65.06% - 620nm). Relevance of finding from current study related to HPTLC fingerprinting can be also substantiated from the earlier works.[23]

Table 4: *Rf* values of Ethanolic Extract of Rhizome of *Chlorophytum borivilianum* Sant. and F.

Short UV	Long UV	After derivatisation
-	0.04 (FL. blue)	-
-	-	0.24 (L. purple)
-	-	-
-	-	-
-	0.38 (FL. blue)	-
-	-	-
-	-	-
-	-	0.59 (L. purple)
-	-	-
0.70 (L. green)	-	-
-	0.72 (FD. blue)	-
0.77 (L. green)	-	0.77 (L. purple)
-	0.84 (FD. blue)	-
D-Dark; L-Light; F-Fluorescent		

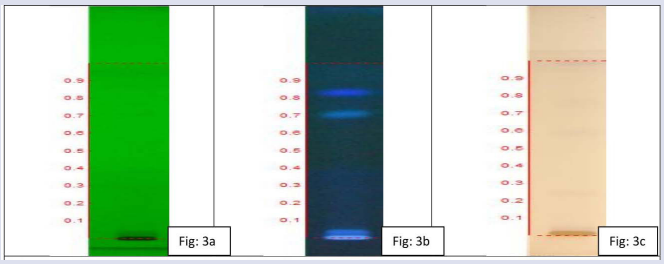

Figure 3: HPTLC Photo Documentation of Rhizome of *Chlorophytum borivilianum* Sant. and F.

CONCLUSION

Macro-microscopic, physico-chemical, preliminary phytochemical and HPTLC finger printing of *Chlorophytum borivilianum* Sant. and F. has been carried out as per pharmacopoeial methodology. The detail presented in the study shows the qualitative presence of various secondary metabolites and in the rhizome of *Chlorophytum borivilianum* Sant. and F. Thus, the study was helpful in the qualitative analysis of genuinity of the given drug.

ACKNOWLEDGEMENT

Authors are highly grateful to Department of Dravyaguna, SDM College of Ayurveda, Hassan; SDM Center for Research in Ayurveda and Allied science, Udupi for providing the lab facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TLC: Thin layer chromatography; **HPTLC:** High Performance Thin layer chromatography; ***Rf*:** Retention factor; **UV:** Ultra violet; **nm:** Nano meter; **ml:** Mili-liter; **µl:** Micro-liter; **viz:** Namely.

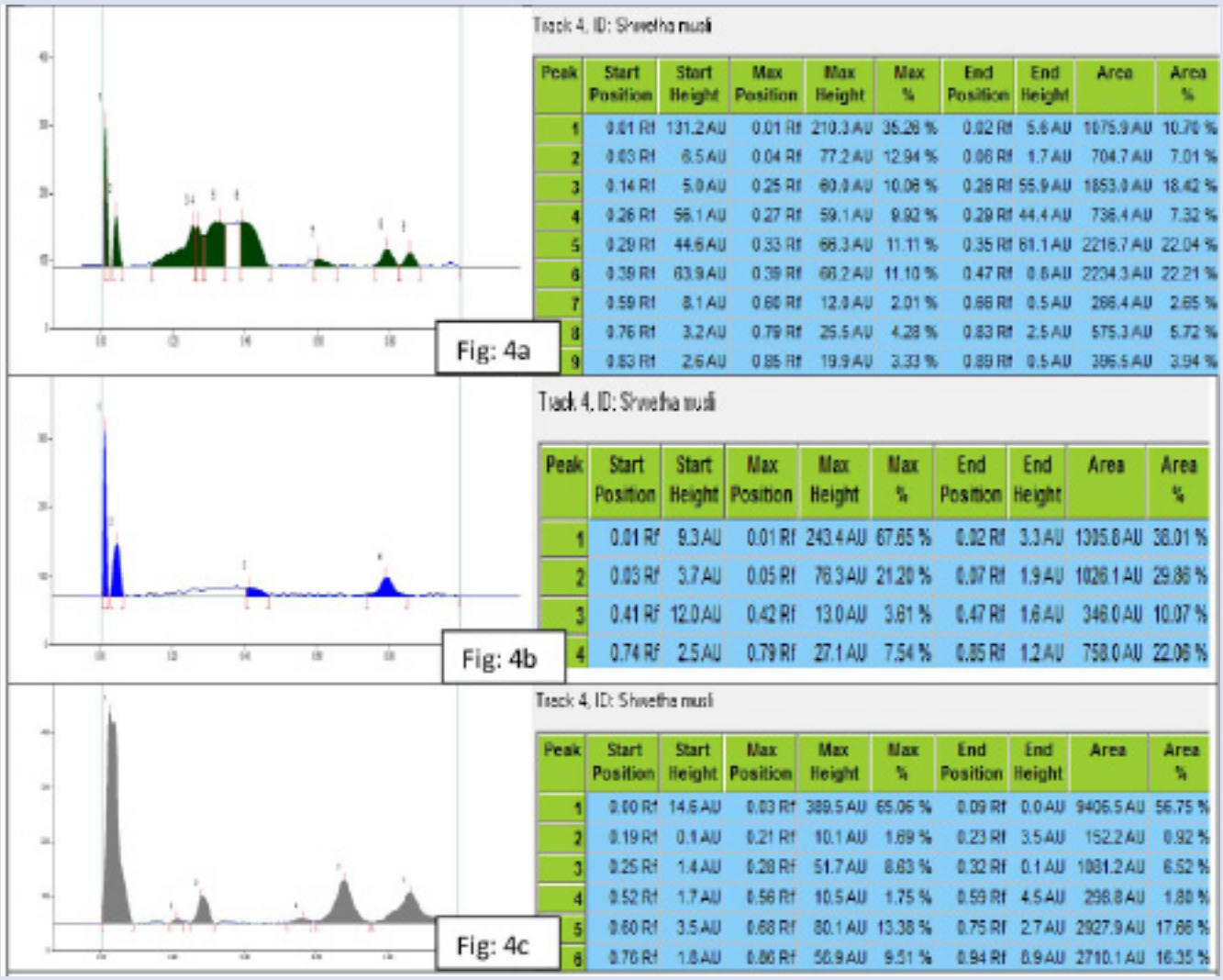

Figure 4: Densitometric scan of Rhizome of *Chlorophytum borivilianum* Sant. and F.

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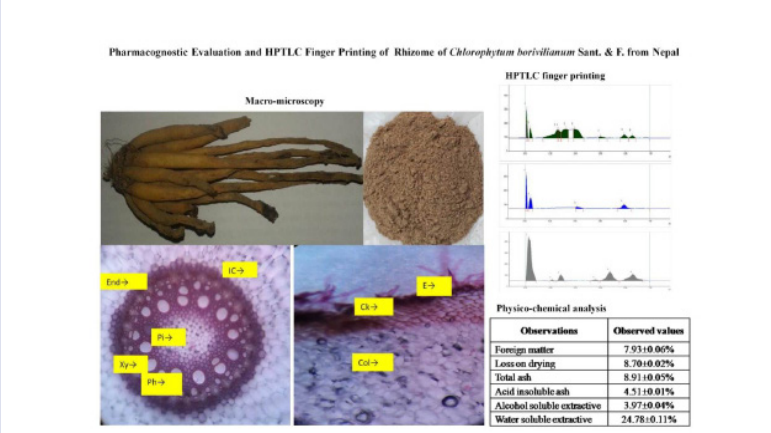
PICTORIAL ABSTRACT



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