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PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF *CHLOROPHYTUM BREVISCAPUM* DALZ.

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ABSTRACT

Chlorophytum breviscapum Dalz. belongs to family Liliaceae and is being used in the indigenous systems of medicine as a galactogogue and aphrodisiac. The drug part is usually used as the white tuberous roots of this plant. The present study includes the macro and microscopic characters, histochemistry and phytochemistry. The phytochemical screening is also confirmed by HPTLC analysis for saponins and stegmasteroids

Keywords: *Chlorophytum breviscapum*, Pharmacognosy, phytochemical analysis, HPTLC.

INTRODUCTION

Chlorophytum breviscapum Dalz. belongs to family Liliaceae. In India, it is found to be growing in rain fed areas. The plant generally grows along the forest margins, grassy slopes and rocky places along valleys (between 1300-2800 m)[1]. The plant body is erect up to 1.5- 2 ft height with sheathing leaf base acute to acuminate with entire margin. Tuberous root are slightly broad at the base and gradually tapering at the end. Tubers are oblong, pendulous in the middle again it becomes fibrous and are measuring 8-12 cm long, 1-1.7 cm diameter [2]. There are 17 species of *Chlorophytum* recorded in India out of these 11 species of *Chlorophytum* are found to be growing in Maharashtra [(3)]. For the present investigation *Chlorophytum breviscapum* is selected as for their correct botanical identification and standardization of drug. The drug part is usually used as the white tuberous roots. The drug is used an aphrodisiac and galactogogue[4,5,6]. Review of literature revealed that it is also used as a nutritive and health promoting properties as well as an immunoenhancing, hepatoprotective and antioxidants [7,8,9,10,11]. The tubers are also used as a medicinal expectorant in fever, leucorrhoea and also as an aphrodisiac[12.]

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The plant materials were collected from in and around Pune district of Maharashtra during rainy season. Efforts were made to collect the plants in flowering and fruiting condition for the correct botanical identification. It was identified with help of Flora of The Presidency of Bombay[2.] The herbariums were prepared and finally authenticated from Botanical Survey of India, Western Circle, Pune (India). The voucher specimen number is PAVICH3/ 2009.

Microscopic and Macroscopic evaluation

Thin (25µ) hand cut sections were taken from the fresh tuberous roots, permanent double stained and finally mounted in Canada balsam as per the plant microtechniques method of Johansen[13.] The macroscopic evaluation was studied by the following method of Trease and Evans[14] and Wallis[15.]

Histochemical study

The thin transverse sections of fresh root were taken (about 25µ). It was treated with respective reagent for the detection and localization of chemicals in the tissues as per the method of Krishnamurthy[16.]

Phytochemical evaluation:

Some materials were dried under the shade so as to avoid the decomposition of chemical constituents, powdered in blender and finally stored in dry air tied containers for phytochemical screening. Ash and percentage extractives were accomplished by following standard pharmacopoeal techniques[17.] Fluorescence analysis was carried out as per Chase and Pratt[18]. Qualitative phytochemical test were carried out by standard methods of Harborne[19] and Trease and Evans[14]. Quantitative phytochemical analysis were determined for proteins, carbohydrates and saponins by the methods of Lowry *et al.*,[20;] Nelson[21] and Obadoni and Ochuko[22] respectively. The phytochemical screening was also detected by the High Performance-Thin Layer Chromatography (HPTLC). HPTLC study was carried out on instrument comprising of Linomat 5 for application using Densitometer- TLC Scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum fluorescent plates (E. Merck). For HPTLC studies, an extract of methanol (25% GR) solvent system was used and after development, plate was scanned at 254 and 366 nm [23, 24, 25.]

RESULTS AND DISCUSSIONS

Macroscopic evaluation

(Figure 1 and 2)

Herb: 1.5 - 2 ft. in height.

Roots: Tuberous root are slightly broad at the base and gradually tapering at the end. Tubers are oblong, pendulous in the middle again it becomes fibrous and are measuring 8-12 cm long, 1-1.7 cm diameter.

Leaves: Green, 6 – 9 (10-15 also) in number, slightly thick dark green, flat with undulate margin, apex acuminate, linear, oblong or lanceolate,

membranous, shining above with sheathing leaf base. 60 – 66 × 2.70 – 3.5 cm long.

Scape: Unbranched, Naked, 60 – 65 cm long.

Flower: White, racemose, clusters of 2 – 3 flowers, 2 – 4 cm long, erect.

Bract: Membranous, Ovate- lanceolate, lower bracts 0.5 – 1.5 cm and upper 0.8 cm long.

Pedicels: 0.5 – 0.8 cm long, jointed near the top.

Perianth: Segments, linear acute, 3 nerved, 0.9 × 0.3 cm in broad.

Stamen: 0.5 – 1 cm long, anther- 0.7 cm long, linear, oblong.

Style: 0.6 cm long, slender, stigma minute.

Capsule: Globose, 0.8 – 1.2 cm in diameter, 3 winged, emarginate, (0.7 – 0.8 cm in height and 0.8 cm in diameter)

Seeds: Black, globose, compressed 0.1 – 0.3 cm diameter, papillose.

Microscopic characters

The transverse section of root shows a circular in outline. The outermost layer is epidermis. Epidermis consists of uniseriate trichomes. This is followed by a very large zone of cortex. The outermost layer of the cortex just below the epidermis consists of cells which are mostly rectangular, appearing much longer than wide. The rest of the cortical cells are rounded to polygonal parenchymatous and have no intercellular spaces. The innermost layer of the cortex is single layered endodermis. The stellar structure shows that the endodermis is followed by the pericycle layer. Xylem is exarch type. The phloem is group in between the xylem along with the parenchyma. The centre region is occupied by large pith. These are mostly polygonal in shape (Figure 3).

Histochemical Screening

Histochemical screening showed the presence of starch, protein, fat, saponins, tannin, sugars and alkaloids (Table 1).

Phytochemical Study

It contains the total ash 12.8 % and acid insoluble ash is 5.1 % (Table 2). The values of percentage extractives were higher in chloroform and lower in benzene solvent (Table 3). Fluorescence analysis was carried out to check the purity of the drug. The powder drug was observed in visible light as Pale white in color. The powder was then observed in ultraviolet light. It was treated with reagent like nitrocellulose, 1 N sodium hydroxide, 1 N sodium hydroxide in nitrocellulose and dry for 30 minutes and then it was observed under ultraviolet light and it emits the color as shown in (Table 4). Qualitative analysis of the root drug indicated the presence of proteins, reducing and non-reducing sugars, saponins, fats, tannin, glycoside and alkaloids in the plant (Table 5). The quantity of proteins is higher than saponins and carbohydrates (Table 6). Saponins are the important chemical and justify the use of tubers of this plant and are used as a well known health tonic, aphrodisiac and galactogogue[3,4,6,26]. In HPTLC study,

the methanolic extract is ultrasonic for 15 minutes and filtered. The filtrate is used as an application for saponins and stegmasteroids. For each application 20 µl, 10µ and 5µl extracts were used and loaded on instrument comprising of Linomat 5 for application using Densitometer- TLC Scanner 3 with "WINCATS" software (Camag, Switzerland). These studies were carried out on pre-coated aluminum fluorescent plates (E. Merck). The plates were scanned at 254 and at 366 nm[23, 24.]

Analytical studies (Saponins)

The HPTLC analysis showed that, the saponins from the *C. breviscapum* samples gave light yellow bands in visible light and blue bands after derivatization in fluorescence light. The plates were scanned at 254 and 366 nm. When images were compared with the graph and table values. It showes maximum area 22.99 % at 366nm after derivatization. The table also indicates the starting Rf values and end Rf values (Figure 4; Graph 1; Table 7).

Analytical studies (Stegmasteroids)

In HPTLC analysis, stegmasteroids revealed white bands in visible light. After derivatization in fluorescence light it showed the dark blue bands. The plates were scanned at 254 and 366 nm. It was covered the area 29.31% at 366nm after derivatization. The tables also indicate the Rf values for all the peaks scanned by "WINCATS" software (Figure 5; Graph 2; Table 8).

CONCLUSION

The plant *C. breviscapum* showed the correct taxonomy. The morphological characters and histochemical study with double staining of the root, percentage extractives, fluorescence and ash analysis and the phytochemical screening of the plant is helpful for the standardization of drug. As in case of saponins and stegmasteroids, the peaks are denoted by the Rf values. Finding the over all results of the study of *C. breviscapum*, these investigations will be useful for the correct botanical identification and authentication of the drug.

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Table 1: Histochemical study of *C. breviscapum* Dalz.

Test	Reagents	Color	Tissue
Starch	I_2KI	Blue	Peri, Xy, Phlo.
Protein	Potassium Ferrocyanide + water + acetic acid + 60% alcohol + $FeCl_3$	Blue	Epi., Cort., Xy.
Tannin	Acidic $FeCl_3$	Light brown	Epi., Peri., Phlo., xy.
Saponin	Conc. H_2SO_4	Yellow	Epi., Peri., Phlo., xy.
Fat	Sudan III	Pink	Epi., phlo., xy.
Sugar	20% aq. NaOH	Yellow	Epi., Xy. Phlo.
Glycosides	Guignard's Test	Brown	Epi., Cort., Xy., Phlo.
Alkaloids:	Mayer's Reagent	Colorless	Hair, Epi., Cort xy. Phlo..
	Wagner's Reagent	Orange	Cort., Peri.
	Dragendorff's Reagent	Orange to dark brown	Epi., Cort., Phlo., xy.
	Tannic acid	Buff color	Epi., Endo., Peri., Phlo., xy, pith
	Hager's Reagent	Yellow	Cort.

Abbreviations: I_2KI: Potassium iodide, $FeCl_3$: Ferric chloride, Conc. H_2SO_4: Concentrated sulphuric acid, NaOH: Sodium hydroxide.
Epi: Epidermis, Endo: Endodermis, Peri: Pericycle, Cort: Cortex, Xy: Xylem, Phlo: Phloem
+ Sign indicates the addition of Potassium Ferrocyanide in water, then acetic acid , 60% alcohol and lastly $FeCl_3$

Table 2: Ash and Acid Insoluble Ash of *C. breviscapum* Dalz.

Parameter	Results
Total Ash	12.8 % dry wt.
Acid Insoluble Ash	5.1 % dry wt.

Table 3: Percentage extractives of *C. breviscapum* Dalz.

Solvent Used	Extract (%)
Distilled Water	2.95 %
Absolute Alcohol	0.175 %
Petroleum ether	0.185 %
Benzene	0. 115 %
Chloroform	8.89 %
Diethyl ether	0. 145 %
Acetone	0.165 %

Table 4: Fluorescence analysis of *C. breviscapum* Dalz. at 230 nm

Treatments	Color Emits
Powder as such	Pale white
Powder as such in UV-light	Pale yellow
Powder + Nitrocellulose	Whitish gray
Powder + 1 N NaOH in Methanol	Blackish gray
Powder + 1 N NaOH in Methanol dry for 30 min. + Nitrocellulose	Grayish white

Table 5: Phytochemical study of *C. breviscapum* Dalz.

Compound	Reagents	Results
Water Extracts		
Starch	I_2KI	+ ve
Protein	Millon's reagent	+ ve
Tannins	Acidic FeCl3	+ ve
Saponin	Distilled water	+ ve
Steroids	Liebermann – Burchard's Test	+ ve
	Salkowski Test	+ ve
Anthroquinone's	Benzene + 10% NH_4OH	- ve
Sugars	Benedict's reagent	+ ve
Fats	Sudan III	+ ve
Alcoholic extracts		
Alkaloids		
a	Mayer's Reagent	+ ve
b	Wagner's Reagent	+ ve
c	Dragendorff's Reagent	+ ve
d	Tannic acid	+ ve
e	Hager's Reagent	+ ve
f	Folin-Phenol Reagent	+ ve
Glycosides	Benzene	+ ve

Table 6: Quantitative estimation of *C. breviscapum* Dalz.

Quantitative estimation	(mg /g dry weight)
Protein	1.38496
Reducing Sugar	0.75942
Non - Reducing Sugar	0.01947
Starch	0.28419
saponins	1.11726

Graph 1 - Qualitative analysis of saponins in the root of *C. breviscapum*

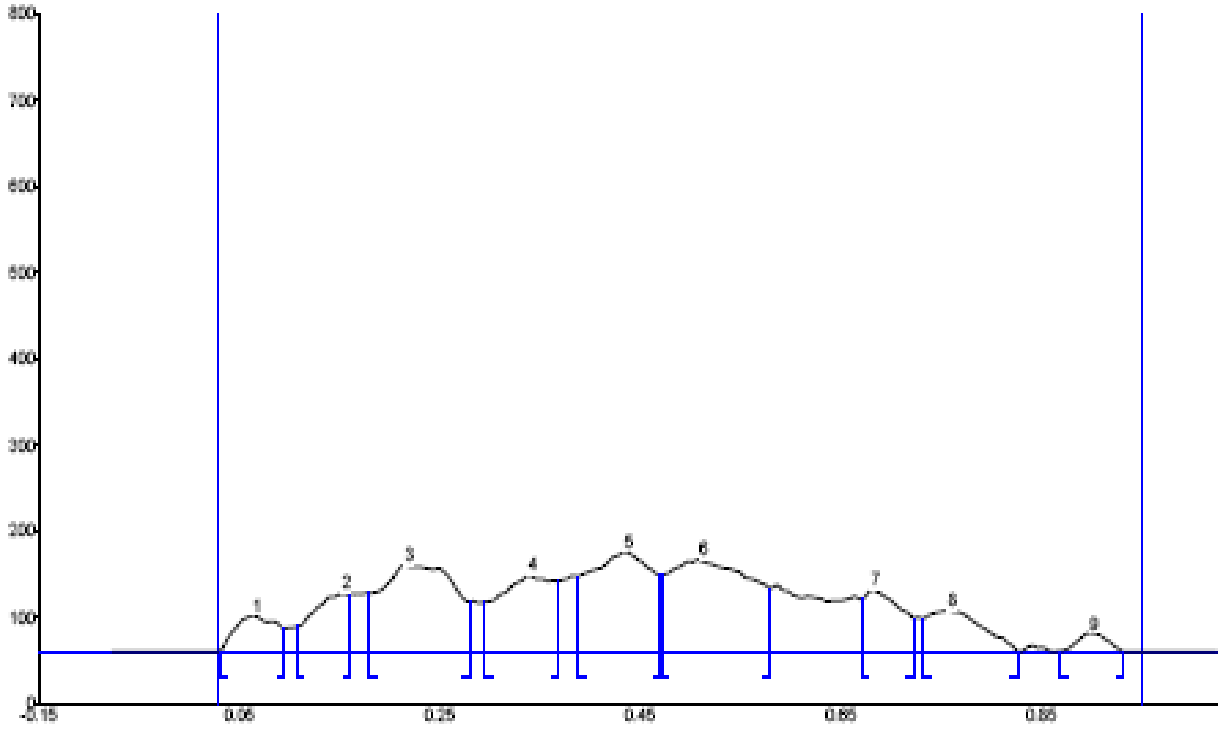


Table 7 - Showing the values of peak for saponins in *C. breviscapum*

Peak	Start Rf	Start Height	Max Rf	Mex Height	Max %	End Rf	End Height	Area	Area %
1	0.03	0.7	0.07	42.7	6.38	0.10	27.1	1371.8	4.42
2	0.11	29.4	0.16	68.1	10.18	0.16	67.5	2035.8	6.56
3	0.18	68.4	0.22	101.2	15.14	0.28	58.7	5919.1	19.08
4	0.30	28.3	0.34	89.6	13.39	0.37	82.9	3930.4	13.63
5	0.39	88.2	0.44	116.9	17.48	0.47	90.3	5816.7	18.75
6	0.47	90.4	0.51	107.6	19.09	0.58	74.5	7133.2	22.99
7	0.68	61.7	0.69	72.1	10.78	0.73	39.5	2181.8	7.03
8	0.74	39.0	0.76	48.6	7.27	0.83	0.3	2123.1	6.84
9	0.87	1.0	0.90	22.0	3.29	0.94	0.4	513.0	1.65

Graph 2 - Qualitative analysis of stegmasteroids in the root of *C. breviscapum*

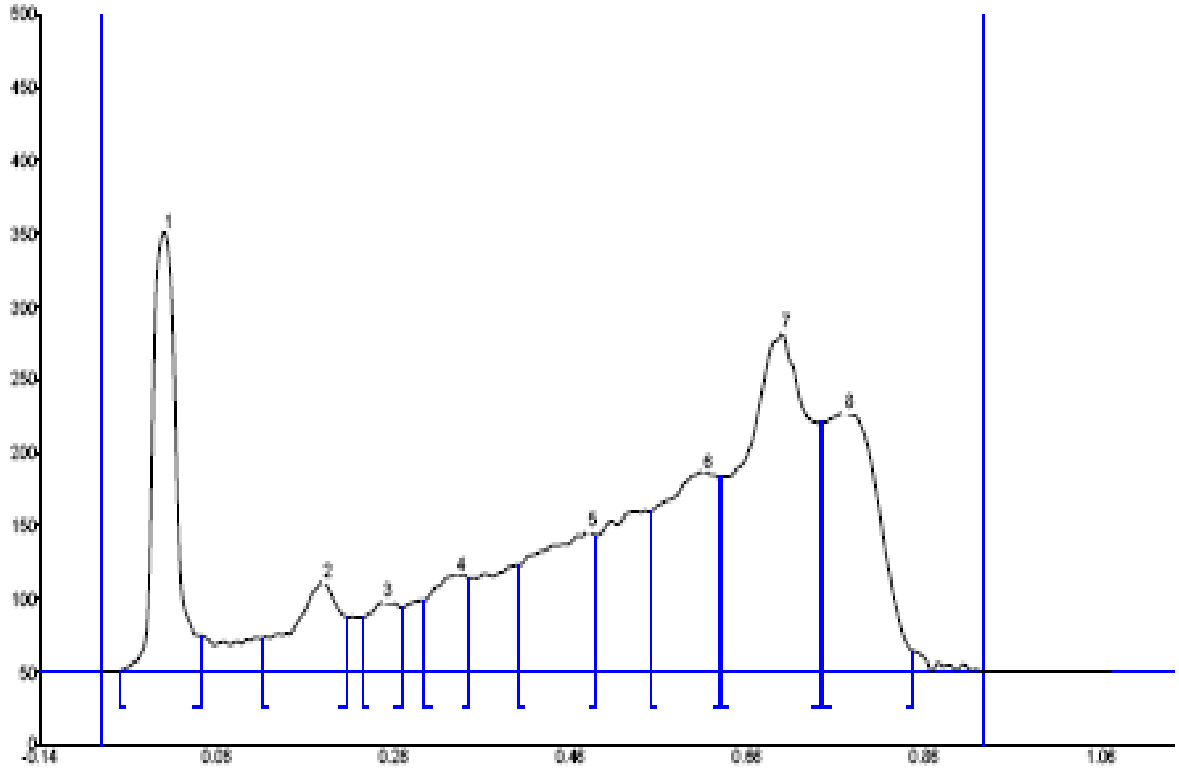


Table 8 - Showing the values of peak for stegmasteroids in *C. breviscapum*

Peak	Start Rf	Start Height	Max Rf	Mex Height	Max %	End Rf	End Height	Area	Area %
1	-0.05	0.3	-0.00	301.3	26.86	0.04	24.9	6434.9	13.32
2	0.11	23.1	0.18	61.6	5.49	0.21	37.7	2691.9	5.57
3	0.22	37.2	0.25	48.0	4.28	0.27	43.7	1407.6	2.91
4	0.29	48.9	0.33	67.2	5.99	0.34	63.8	2260.6	4.68
5	0.40	72.8	0.48	96.6	8.61	0.49	93.2	5433.5	11.25
6	0.55	109.6	0.61	137.2	12.23	0.63	132.9	7188.0	14.88
7	0.63	132.9	0.70	232.0	20.68	0.74	170.2	14156.3	29.31
8	0.75	170.9	0.77	177.9	15.86	0.85	14.1	8733.1	18.08

Figure 1: Habit of *C. breviscapum* Dalz.

Figure 2: Tuberous roots of *C. breviscapum* Dalz.

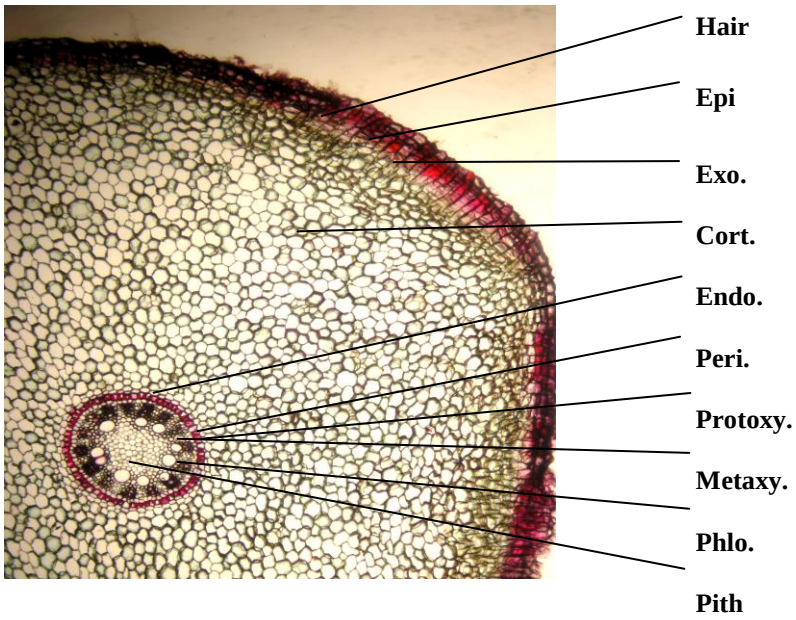
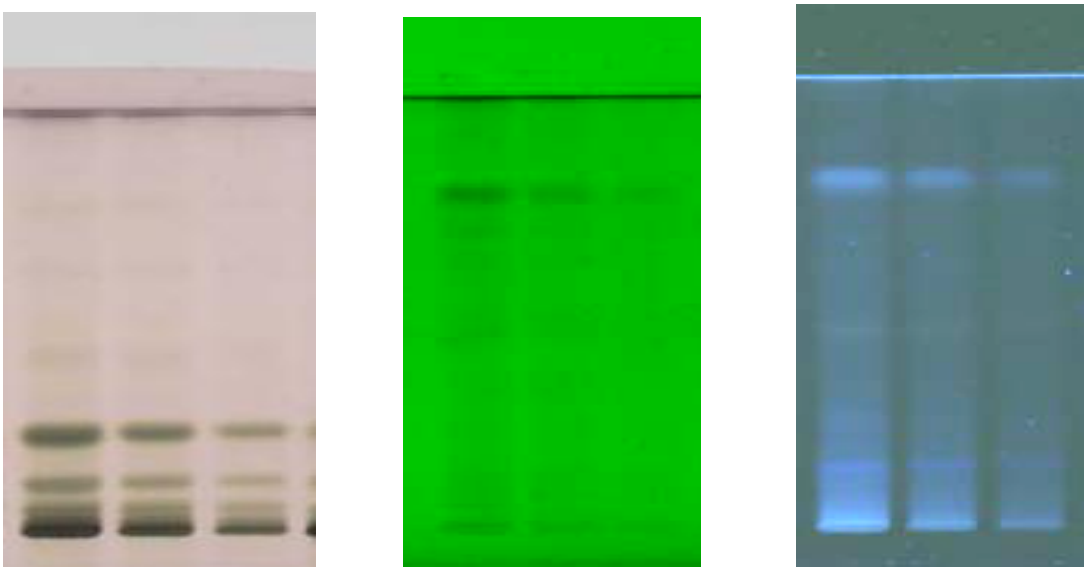


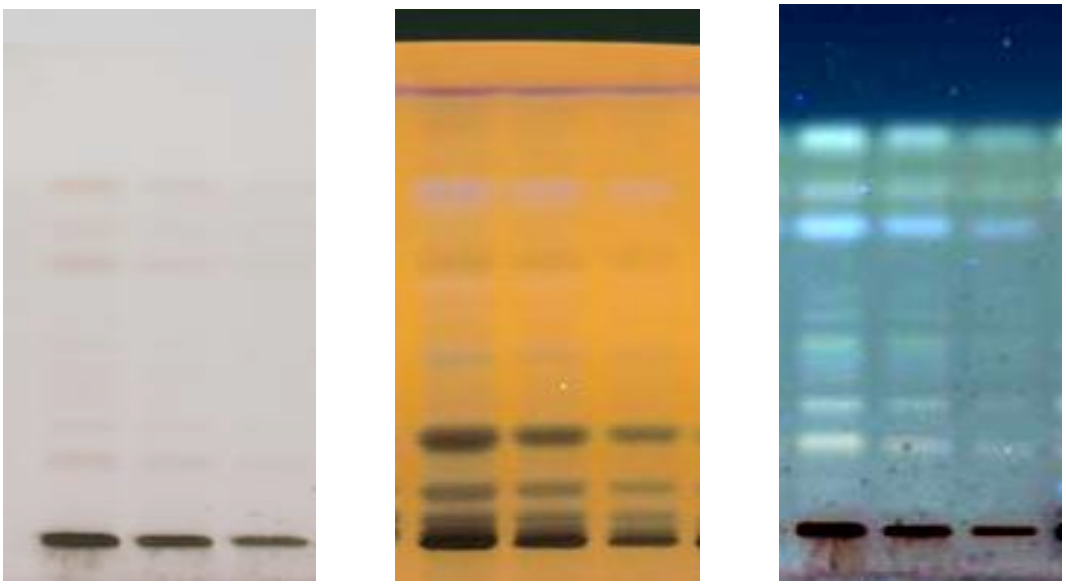
Figure 3: Transverse section of root of *C. breviscapum* Dalz. (10x X 3.3x)

Figure 4: Detection of saponins by HPTLC techniques



In Visible Light | Image at 254 nm (Before derivatization) | Image at 366 nm (After derivatization)

Figure – 5: Detection of stegmasteroids by HPTLC techniques



In Visible Light | Image at 254 nm (Before derivatization) | Image at 366 nm (After derivatization)