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RESEARCH ARTICLE

PHARMACOGNOSTIC STANDARDIZATION OF *CHLOROPHYTUM BHARUCHAE* ANSARI, RAGH., & HEM.

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ABSTRACT

Chlorophytum borivilianum is an important medicinal plant known as 'Safed musli', used in many Ayurvedic vital tonics and aphrodisiac formulations. The species was first described from India in 1954 and reached rare status in nature due to overexploitation. Owing to its increased demand, the species has attracted the attention of farmers as well as researchers in several institutions. The present paper deals with to remove the controversy and for botanical standardization the detailed pharmacognostic study on the tuberous roots of *Chlorophytum bharuchae*, macroscopic and microscopic characters, histochemistry and phytochemistry. The Phytochemical and histochemical test includes starch, protein, saponins, sugar, tannins, glycosides and alkaloids. Percentage extractives, ash and acid insoluble ash and fluorescence analysis. The phytochemical screening is also conformed by HPTLC analysis for saponins and steroids.

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INTRODUCTION

Chlorophytum bharuchae is found to be growing in rain fed areas. Hara (1966) reported that the plant generally grows along the forest margins, grassy slopes and rocky places along valleys (between 1300-2800 m). The plant body is erect up to 2-2.5 ft height with sheathing leaf base acute to acuminate with entire margin. The tuberous roots are cylindrical and measuring 15 - 34 cm long, 5 - 15 numbers, 1-1.8 cm diameter. (Cooke, 1958). The tuberous roots are medicinally important known as safed musali in trade. Literature survey indicated that the species *Asparagus*, *Bombax* and Orchids are also known as safed musali (Nadkarni, 1927; Chopra et al., 1956; Murthy, 2004). There are 17 species of *Chlorophytum* recorded in India out of these 11 species of *Chlorophytum* are found to be growing in Maharashtra (The Wealth of India, 1992). For the present investigation *Chlorophytum bharuchae* is selected as, it is being sold in the market under the common name safed musali because of its white tuberous roots. The drug part is usually used as the white tuberous roots. The drug safed musali is useful as an aphrodisiac and galactagogue (Nadkarni, 1927; Chopra et al., 1956; Marais and Reilly, 1978). Review of literature revealed that the safed musali is used as a nutritive and health promoting properties as well as an immunoenhancing, hepatoprotective and antioxidants (Govindarajan et al., 2005; Anonymous, 2001; Dhuley, 1997; Nergard et al., 2004; Kirtikar and Basu, 1975).

The tubers are also used as a medicinal expectorant in fever, leucorrhoea and also as an aphrodisiac (Sreevidya et al., 2003). The plant has been widely used in the Indian system of medicines for rejuvenation and as instant energy as a 'Rasayana' drug (Puri, 2003). This aim was present study Pharmacognostic Standardization of *Chlorophytum bharuchae*.

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 (Cooke, 1958). The herbariums were prepared and finally authenticated from Botanical Survey of India, Western Circle, Pune (India). The voucher specimen number is PAVICH-1/ 2009.

Microscopic and Macroscopic evaluation

Thin (25 μ) hand cut sections were taken from the fresh tuberous roots, permanent double stained and finally mounted in Canada balsam as per the plant micro techniques method of Johansen (1940). The macroscopic evaluation was studied by the following method of Trease and Evans (2002) and Wallis (1967).

Histochemical study

The thin transverse sections of fresh root were taken (about 25 μ). It was treated with respective reagent for the detection and localization of chemicals in the tissues as per the method of Krishnamurthy (1988).

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Table 1: Histochemical study of *C. bharuchae*

Test	Reagents	Results	Tissue
Starch	Starch K ₂ KI	++	Epi, Peri, Xy, Phlo.
Protein	Potassium Ferrocyanide + water + acetic acid + 60% alcohol + FeCl ₃	++	Epi, Peri, Xy, Phlo.
Tannin	Acidic FeCl ₃	++	Xy, Phlo. Cort.
Saponin	Conc. H ₂ SO ₄	++	Epi., endo., Peri., Phlo., xy.
Fat	Sudan III	++	Epi., hairs, endo., Peri., Phlo., xy.
Sugar	20% aq. NaOH	++	Epi., xy. Phlo.
Glycosides	Guignard's Test	++	Epi., Cort
Alkaloids	Mayer's Reagent	++	Hair, Epi., Cort xy. Phlo..
	Wagner's Reagent	++	Epi., Peri., Phlo., xy.
	Dragendorff's Reagent	++	Epi., Peri., Phlo., xy., Pith
	Tannic acid	++	Cort. Xy., Phlo.
	Hager's Reagent	++	Cort.

+ Sign indicates the addition of Potassium Ferrocyanide in water, then acetic acid 60% alcohol and lastly FeCl₃

++ (Positive sign)= denotes the presence of chemicals.

Table 2. Ash and Acid Insoluble Ash of *C. bharuchae*

Parameter	Dr. wt.
Total Ash	12.6 %
Acid Insoluble Ash	5.6 %

Table 3. Percentage extractives of *C. bharuchae*

Solvent Used	(%) dry wt.
Distilled Water	2.815 %
Absolute Alcohol	0.26 %
Petroleum ether	0.25 %
Benzene	0.19 %
Chloroform	9.905 %
Diethyl ether	0.32 %
Acetone	0.395 %

Table 4: Fluorescence analysis of *C. bharuchae*

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

Table 6. Quantitative estimation of *C. bharuchae*

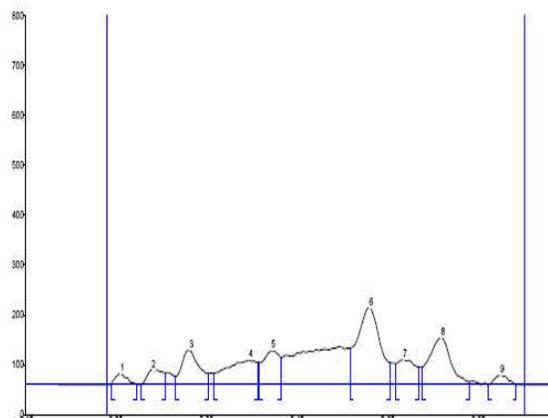
Quantitative estimation	(mg / gm)
Protein	3.29650
Reducing Sugar	1.60016
Non - Reducing Sugar	2.00849
Starch	3.43128
saponins	2.09846

Table 7. Showing the peak values for saponins for 20 µl plant extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	1.0	0.06	23.3	4.16	0.10	0.1	458.3	2.32
2	0.11	0.3	0.13	30.1	5.37	0.16	23.7	823.6	4.17
3	0.18	16.2	0.22	68.9	12.28	0.26	22.0	2112.2	10.70
4	0.27	22.3	0.35	49.7	8.86	0.37	44.2	2636.9	13.35
5	0.37	43.8	0.40	68.9	12.28	0.42	54.0	1986.7	10.06
6	0.57	73.4	0.61	154.7	27.59	0.66	44.0	6159.3	31.19
7	0.67	42.6	0.69	51.5	9.19	0.72	35.2	1608.3	8.14
8	0.73	34.4	0.77	94.3	16.82	0.83	5.9	3519.4	17.82
9	0.88	0.0	0.90	19.3	3.45	0.94	0.1	441.9	2.24

Table 5: Phytochemical study of *C. bharuchae*

Compound	Reagents	Results
Water Extracts		
Starch	Starch K ₂ KI	+ ve
Protein	Millon's reagent	+ ve
Tannins	Acidic FeCl ₃	+ ve
Saponin	Distilled water	+ ve
Steroids	Liebermann – Burchard's Test	+ ve
	Salkowski Test	+ ve
Anthroquinone's	Benzene + 10% NH ₄ OH	- 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



Graph 1. Showing the peak for saponins for 20 µl plant extract

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 of Anonymous (1955). Fluorescence analysis was carried out as per Chase and Pratt (1949). Qualitative phytochemical test were carried out by standard methods of Harborne (1973) and Trease and Evans (2002). Quantitative phytochemical analysis were determined for proteins, carbohydrates and saponins by the methods of Lowry et al., (1951); Nelson (1944) and Obadoni and Ochuko (2001) respectively. The phytochemical screening is 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 (Wagner and Bladt, 1996; Reich and Schibii, 2007).

RESULTS AND DISCUSSION

Macroscopic evaluation

Herb: 2 - 2.5 ft. in height.

Roots: Tuberos roots are cylindrical and are measuring 15 - 34 cm long, 5 - 15 numbers, 1-1.8 cm diameter.

Leaves: 5 - 8 in number, thick, acute to acuminate, 15 - 20 × 1.5 - 3 cm, margin wavy, hyaline, green at the base.

Scape: Branched, 1 - 2 ft. height.

Flower: White, yellow brown spot at the tip, racemose, alternate or sub-opposite, cluster of 4-8 flowers.

Bract: Ovate lanceolate.

Pedicels: Ascending, geminate, 0.5 - 1 cm long.

Perianth: white with green blotch at the apex, 3 nerved, lanceolate.

Stamen: 6 in number, 0.5 - 0.8 cm long, anther dorsifixed - 0.6 cm long

Style: 1, 0.5 - 0.8 cm long

Capsule: 3 lobed, 0.6 - 1 cm long, greenish, obchordate.

Seeds: Black, solitary flattened. (Figure 1 and 2)

Microscopic characters

The transverse section of root shows circular in outline. The outermost is a single layer epidermal cell. Epidermis consists of uniseriate trichomes which is having bulbous base and pointed end. Epidermis followed by exodermis. The rest of the cortex is composed of parenchymatous cells. The cortex is followed by a single layered parenchymatous cell of endodermis. Beside the endodermis one to two layer of parenchymatous pericycle cells are present. The stele represents the closely arranged vascular bundles. The phloem region is characterized by the presence of phloem parenchyma. The thin parenchymatous cell of xylem is surrounded by a phloem. The xylem shows a small and big cell. The small one is directed towards the periphery of pericycle region called protoxylem and big one is directed towards the centre of pith called as metaxylem. On the basis of localization of protoxylem and metaxylem, it shows the

exarch type of xylem. The centre region is occupied by large parenchymatous cells called pith. (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 12.6% and acid insoluble ash is 5.6% (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 and potency of the drug. The powder drug was observed in visible light as pale yellow. 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 galactagogue (Marais and Reilly, 1978; Oudhia, 2001; Nadkarni, 1927; Chopra et al., 1956). 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 (Wagner and Bladt, 1996; Reich and Schibii, 2007).

Analytical studies (Saponins)

The HPTLC analysis showed that, the saponins from the *C. bharuchae* root 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 showed maximum area 31.38 % at 366nm after derivatization. The table also indicates the starting Rf values and end Rf values (Figure 4; Graph 1-3; Table 7-9).

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 31.27% at 254 nm. The tables also indicate the Rf values for all the peaks scanned by "WINCATS" software (Figure 5; Graph 4-6; Table 10-12).

CONCLUSIONS

The plant *C. bharuchae* showed the correct taxonomy which is helpful for the standardization of drug. Findings of the present investigation will be useful for the correct botanical identification and authentication of the drug. After getting the overall results of *C. bharuchae* and if,

Table 8. Showing the peak values for saponins for 10 µl plant extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.3	0.06	12.9	2.99	0.09	2.9	259.9	1.77
2	0.11	0.6	0.13	18.2	4.22	0.14	12.5	287.9	1.97
3	0.18	15.0	0.22	52.2	12.10	0.26	21.8	1650.1	11.27
4	0.30	32.3	0.35	46.9	10.88	0.35	44.6	1404.1	9.59
5	0.37	45.6	0.41	65.2	15.10	0.43	58.9	2365.5	16.15
6	0.58	60.1	0.62	106.6	24.69	0.66	36.6	4322.0	29.51
7	0.69	37.0	0.70	40.1	9.28	0.72	27.3	957.2	6.63
8	0.72	27.5	0.77	67.1	15.56	0.87	0.0	2848.6	19.45
9	0.88	2.1	0.91	22.4	5.19	0.94	3.3	552.5	3.77

Table 9. Showing the peak values for saponins for 5 µl plant extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.19	6.5	0.22	35.7	12.09	0.25	21.5	988.8	9.22
2	0.30	31.9	0.36	44.6	15.08	0.36	43.1	1613.2	15.04
3	0.36	43.3	0.41	58.8	19.88	0.42	57.6	1975.5	18.41
4	0.58	54.9	0.62	78.4	26.50	0.66	39.3	3366.1	31.38
5	0.74	29.5	0.77	49.8	16.84	0.83	9.7	2119.6	19.76
6	0.87	0.3	0.91	28.4	9.60	0.94	1.7	664.7	6.20

Table 10. Showing the peak values for stegmasteroids for 20 µl plant extract

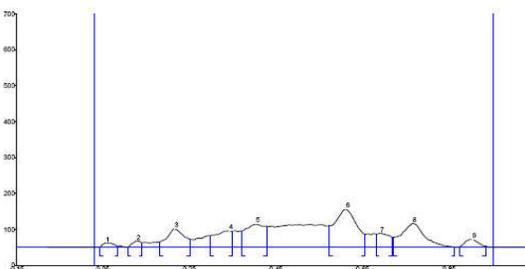
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	1.5	-0.00	350.7	27.82	0.05	31.4	8519.0	16.28
2	0.15	29.6	0.18	64.5	5.11	0.20	50.7	1946.7	3.72
3	0.28	53.8	0.33	70.8	5.61	0.33	69.4	2512.0	4.80
4	0.36	72.7	0.44	93.9	7.45	0.44	89.6	4816.0	9.21
5	0.49	104.8	0.54	119.8	9.50	0.55	115.5	4963.5	9.49
6	0.57	122.7	0.61	146.8	11.64	0.63	141.8	6633.6	12.68
7	0.64	143.0	0.70	221.0	17.52	0.74	177.4	13161.1	25.16
8	0.74	177.0	0.77	182.3	14.46	0.88	8.8	9679.1	18.50
9	0.88	9.3	0.88	11.2	0.89	0.89	0.7	88.4	0.17

Table 11. Showing the peak values for stegmasteroids for 10 µl plant extract

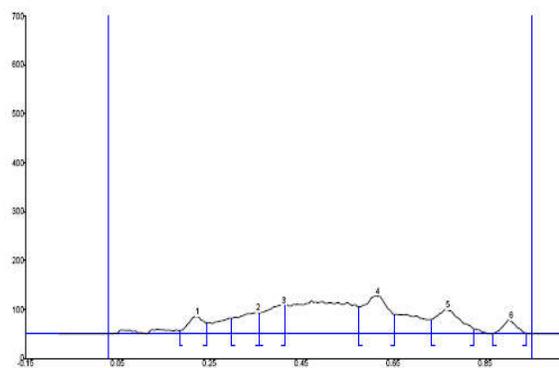
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	1.4	0.00	309.3	33.49	0.05	18.6	6302.9	14.20
2	0.12	24.0	0.17	45.7	4.95	0.19	38.1	1929.0	4.35
3	0.32	59.2	0.36	70.0	7.58	0.37	67.7	2387.0	5.38
4	0.46	87.2	0.61	136.8	14.81	0.63	135.0	13877.3	31.27
5	0.63	134.4	0.69	188.2	20.38	0.72	168.3	10506.3	23.67
6	0.74	166.7	0.77	173.5	18.79	0.86	7.3	9382.3	21.14

Table 12. Showing the peak values for stegmasteroids for 5 µl plant extract

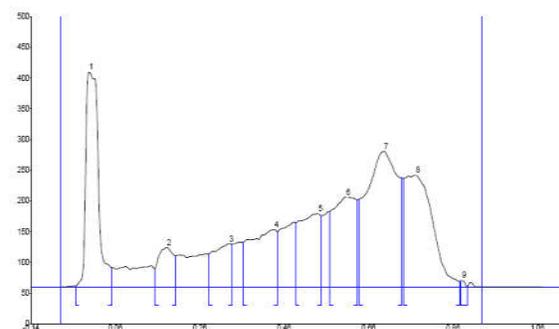
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	1.4	0.00	266.4	29.71	0.02	13.1	4057.1	10.40
2	0.07	18.0	0.11	24.7	2.75	0.12	23.9	714.6	1.83
3	0.13	26.2	0.16	37.6	4.19	0.18	33.7	1078.8	2.77
4	0.37	64.6	0.52	102.2	11.39	0.53	101.0	9473.8	24.29
5	0.55	108.2	0.61	132.8	14.81	0.63	130.5	6429.2	16.48
6	0.64	133.7	0.70	168.7	18.82	0.72	160.5	9077.2	23.27
7	0.74	160.8	0.76	164.3	18.32	0.85	7.9	8173.2	20.95



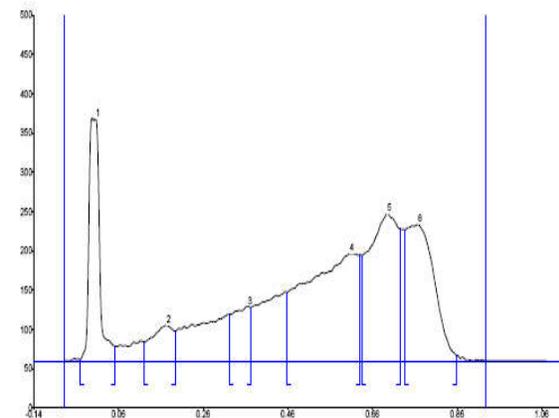
Graph 2. Showing the peak for saponins for 10 µl plant extract



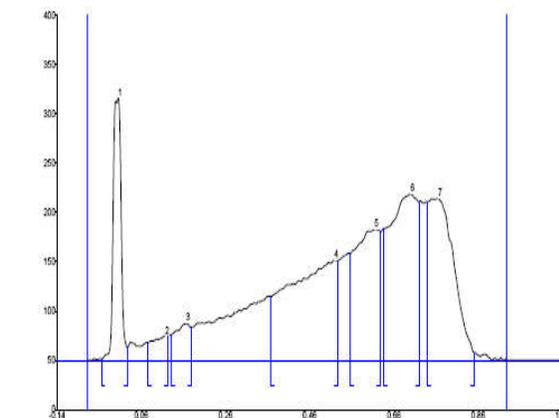
Graph 3. Showing the peak for saponins for 5 µl plant extract



Graph 4. Showing the peak for stegmasteroids for 20 µl plant extract



Graph 5. Showing the peak for stegmasteroids for 10 µl plant extract



Graph 6. Showing the peak for stegmasteroids for 5 µl plant extract



Figure 1 – Habit of *Chlorophytum bharuchae*

Figure 2: Tuberous roots of *Chlorophytum bharuchae*

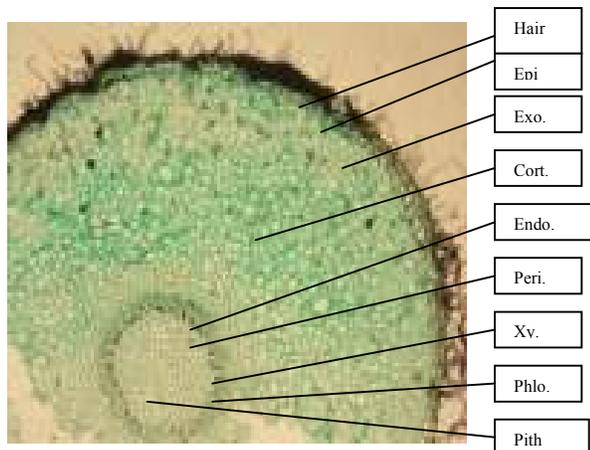


Figure 3: Transverse section of root of *Chlorophytum bharuchae* (10x X 33x)

Epi= Epidermis; Exo.= Exodermis; Cort.= Cortex; Endo.= Endodermis; Peri.= Pericycle; xv.= Xylem; Phlo.= Phloem

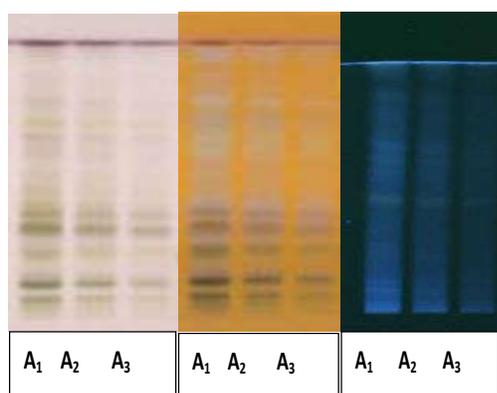


Figure 4 – Confirmation of saponins by HPTLC

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

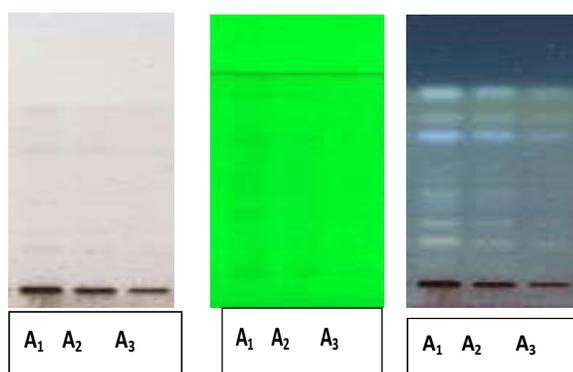


Figure 4 – Confirmation of stegmasteroids by HPTLC.

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

A₁= 20μl, A₂= 10μl and A₃= 5μl : Volume of Application of sample extract

these are comparable with other species of safed musali, it will be used as a substitute for them.

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