Research Article

COMPARATIVE PHARMACOGNOSTIC STUDY OF TWO SPECIES OF *CHLOROPHYTUM* KER-GAWL

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ABSTRACT

Chlorophytum tuberosum Baker and *Chlorophytum laxum* R. Br. belong to family Liliaceae and is being used in the indigenous systems of medicine as a galactogogue and aphrodisiac. These species are commonly known as safed musali. The drug part is usually used as the white tuberous roots. The present studies include the macroscopic, microscopic characters, histochemistry and phytochemistry. The phytochemical screening is also confirmed by HPTLC analysis for saponins and stegmasteroids.

Keywords: *Chlorophytum*, Pharmacognosy, Phytochemical analysis, HPTLC.

INTRODUCTION

Chlorophytum tuberosum Baker and *Chlorophytum laxum* R. Br. belongs to family Liliaceae. In India, it is found in rainfed areas. The plant generally grows along the forest margins, grassy slopes and rocky places along valleys (between 1300 and 2800m)[1]. *C. tuberosum* is an erect plant growing up to a height of 1.5–2ft with sheathing leaf base acute to acuminate with entire margin. The roots are tuberous with ellipsoid tubers hanging from them, 10–12 cm long and 1–1.9 cm in diameter and *C. laxum* is also the erect plant growing up to a height of 1ft with sheathing leaf base acute to acuminate with entire margin. Tuberous roots are cylindrical and are measuring 10 -14 cm long and 1–1.4 cm diameter[2]. The tuberous roots of both the species are medicinally important and are commonly known as safed musali in indigenous system of medicine. It is used as an aphrodisiac and galactogogue[3-5] as well as for its nutritive, health promoting properties and immunoenhancing, hepatoprotective and antioxidants activities[6-10]. The tubers are also used in fever, leucorrhoea and also as an aphrodisiac (Kirtikar and Basu, 1975). The species *Asparagus, Bombax* and Orchids are also known as safed musali in the literature[3,4]. Therefore, it is important to define specifications that will allow the correct identification of the plant which is being sold as safed musali. In addition, there are 17 species of *Chlorophytum* recorded in India of which 11 species of *Chlorophytum* are found to be growing in Maharashtra[11]. Hence, *C. tuberosum* Baker and *C. laxum* R. Br. choose for the present investigation as it is being sold widely in the market under the common name safed musali because of its white tuberous roots.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant materials were collected from in and around Pune district of Maharashtra during the rainy season for correct botanical identification. Efforts were made to collect the plants in flowering and fruiting condition for the correct botanical identification. It was identified with the help of Flora of The Presidency of Bombay[2]. Herbarium specimens were prepared and authenticated from Botanical Survey of India, Western Circle, Pune (India). It is housed in Botanical Garden of Botany Department, Pune. The voucher specimens number for *C. tuberosum* Baker and *C. laxum* R. Br. are PAVICH2/2009 and PAVICH5/2009 respectively[12].

Microscopic and macroscopic evaluation

Thin (25µ) hand cut sections were taken from the fresh tuberous roots, permanently double-stained and finally mounted in Canada balsam as per the plant microtechniques method of Johansen[13]. The macroscopic evaluation was studied by the 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 roots were dried under the shade so as to avoid the decomposition of chemical constituents, powdered in a blender and finally stored in dry air tied containers for phytochemical screening. Ash and percentage extractive content was measured by following the standard pharmacopoeial techniques[17]. Fluorescence analysis was carried out as per Chase and Pratt[18]. Qualitative phytochemical tests were carried out by standard methods of Harborne[19] and Trease and Evans[14]. Quantitative phytochemical analysis was 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 done

by the High Performance- Thin Layer Chromatography (HPTLC). HPTLC study was carried out on 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].

RESULTS AND DISCUSSION

Macroscopic evaluation

The details of the macroscopic examination are mentioned in Table 1 and illustrated in Figures 1 (a & b) and 2 (a & b).

Table 1: Macroscopic examination of *Chlorophytum spp*

Characters	*C. tuberosum* Baker	*C. laxum* R. Br.
Herb	1.5–2 ft. in height.	Slender, up to 1 ft. in height.
Roots	Fleshy, tuberous with ellipsoid tubers hanging from them, 10–12 cm long, 1–1.9 cm diameter	Tuberous roots are slender with pendulous ellipsoid tubers (actually fusiform i.e. tapering towards both the end) and are measuring 6-10 cm long, 1-1.5 cm diameter.
Leaves	Green, 6–12 (8–14 also) in number, sessile shorter than the scape, falcate, recurved, acuminate or acute at the apex, margin undulate (12 – 28 × 1.2 – 1.6 cm long.)	Green grass like, 6-12 in number, linear or linear lanceolate, 22.5 - 45 × 1.2 – 2.5 cm. acute with 15 – 20 distinct immersed veins.
Scape	Unbranched, naked, 3–12 in. long.	Naked, slender, flexuous, 1 – 3 in. long, winged.
Flower	White in simple racemose, 3–6 cm long, dense flower.	Few, racemose, greenish white up to 1 in. apart, branched or unbranched.
Bract	Lanceolate, acuminate, lower 0.8–0.5 cm long.	Ovate lanceolate, 0.8 – 1.2 cm long at the base of the branches when forked, acuminate.
Pedicels	Ascending, 0.5–0.7 cm long, jointed below the middle.	Short, jointed about the middle.
Perianth	Segments, less than 0.7 cm long by 0.5 cm broad. Oblong, lanceolate, sub-acute, 7–9 nerved.	Segments hyaline, 3 nerved, oblong, obtuse.
Stamen	0.5–1 cm long, anther 0.5–0.8 cm long, linear, shining transversely veined	Alternately short and long, longer 0.8 cm and the shorter 0.6 cm long, anther-0.5 cm long green.
Style	1, stigma minute.	1, 0.5 cm long
Capsule	Obovoid – sub-globose, 0.8–1.2, transversely veined, emarginate.	Up to 1 cm longer, broadly obchordate, 3 winged, obtusely trigonous.
Seeds	Black, irregularly orbicular, 0.3–0.5 cm diameter	0.7 – 1cm black, angular

Microscopic characters

In both the species, transverse section of the roots had a circular outline. The outermost layer is the epidermis consisting of uniseriate trichomes followed by a very large zone of the cortex. The outermost layer of the cortex just below the epidermis consists of cells which are mostly rectangular, appearing longer than wide. The rest of the cortex are rounded to polygonal parenchymatous cells and have no intercellular spaces. The innermost layer of the cortex is a single-layered endodermis. The stellar structure shows that the endodermis is followed by the pericycle layer. The xylem is exarch variety and the phloem is in between the xylem along with the parenchyma. The central region is occupied by large pith mostly polygonal in shape (Figure 3a & b respectively).

Figure 1a: Habit of *C. tuberosum*

Figure 1b: Habit of *C. laxum*

Figure 2a: Tuberous roots of *C. tuberosum*

Figure 2b: Tuberous roots of *C. laxum*

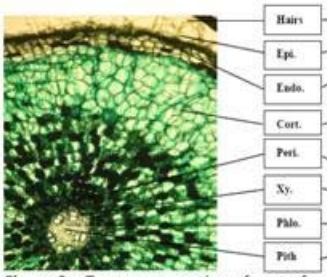


Figure 3a: Transverse section of root of *C. tuberosum* (10× × 3.3×)

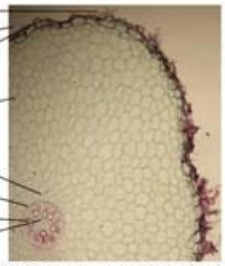


Figure 3b: Transverse section of root *C. laxum* (10× × 3.3×)

Histochemical screening

Histochemical screening showed the presence of starch, protein, fat, saponins, tannin, sugars and alkaloids (Table 2).

Phytochemical studies

The tubers had a total ash acid insoluble ash content is more in *C. tuberosum* as compare to *C. laxum* (Table 3). The values of percentage extractives were higher in chloroform and lower in benzene solvent (Table 4). Fluorescence analysis was carried out to check the purity of the drug. The powder drug was observed in visible light, and then powder was treated with nitrocellulose, 1 N sodium hydroxide, 1 N sodium hydroxide in nitrocellulose and dried for 30 min. After this it was observed under ultraviolet light and it emits the color as shown in Table 5 for both the species. Qualitative analysis of the roots indicated the presence of proteins, reducing and non-reducing sugars, saponins, fats, tannin, glycoside and alkaloids (Table 6).

Table 2: Histochemical study of *Chlorophytum spp*

Test	Reagents	Results	Tissue	
			C. *tuberosum* Baker	C. *laxum* R. Br.
Starch	Iodine K_2KI	+	Endo., Peri., Phlo.	Epi, Peri. Endo.
Protein	Potassium ferrocyanide & $FeCl_3$	+	Epi., Cort., Peri., Phlo., xy.	Epi., Cort., Xy. Pith.
Saponin	$Ba(OH)_2$, $CaCl_2$, & $K_2Cr_2O_7$	+	Endo., xy.	Cort., Xy., Phlo.
Tannin	10% Aq. $FeCl_3$	+	Cort. Phlo., xy., peri.	Epi., Peri., Phlo., xy.
Fat	Sudan III	+	Epi., endo., phlo., xy.	Hair, Epi., Pith.
Sugar	20% aqueous NaOH & Copper tartarate	+	Epi., xy.	Epi., Peri., Xy. Phlo.
Glycoside	1% aq. picric acid & 10% aq. Na_2CO_3	+	Epi., Xy., Phlo.	Epi., Xy., Phlo.
Mayer's Reagent	Mayer's Reagents	+	Hair, Epi., Cort xy. Phlo..	Hair, Epi., Cort xy. Phlo..
Wagner's Reagent	Wagner's Reagents	+	Epi., Endo., peri., phlo., xy.	Endo., Peri., Xy.
Dragendorff's Reagent	Dragendorff's Reagents	+	Epi., Endo., Peri., Phlo., xy.	Hairs, Epi., Endo., Peri., Phlo., xy.
Tannic acid	Tannic acid	+	Peri., phlo.	Hairs, Epi., Cort.
Hager's	Hager's Reagent	+	Cort.	Cort.

Table 3: Ash and acid insoluble ash of *Chlorophytum spp.*

Parameter	Results	
	C. *tuberosum* Baker	C. *laxum* R. Br.
Total Ash	13.2 %	11.5 %
Acid Insoluble Ash	4.8 %	3.7 %

Table 4: Percentage extractives of *Chlorophytum spp.*

Solvent	Extract (%)	
	C. *tuberosum* Baker	C. *laxum* R. Br.
Distilled Water	2.395 %	3.45 %
Absolute Alcohol	0.275 %	0.245 %
Petroleum ether	0.26 %	0.22 %
Benzene	0.21 %	0.175 %
Chloroform	9.96 %	7.49 %
Diethyl ether	0.43 %	0.305 %
Acetone	0.47 %	0.36 %

Table 5: Fluorescence analysis of *Chlorophytum spp.* at 230 nm

Treatments	Color emits	
	C. *tuberosum* Baker	C. *laxum* R. Br.
Powder as such	Yellowish brown	Brownish yellow
Powder as such in UV-light	Pale yellow	Grayish green
Powder + Nitrocellulose	Grayish white	Grayish yellow
Powder + 1 N NaOH in Methanol	Grayish green	Blackish green
Powder + 1 N NaOH in Methanol dry for 30 min. + Nitrocellulose	Grayish black	Blackish gray

The quantity of proteins is higher than saponins and carbohydrates in *C. tuberosum* as compare to *C. laxum* (Table 7). Saponins are the important chemical and justify the use of tubers of these plants and are used as a well-known health tonic, aphrodisiac and galactogogue[3,4,6,25]. In HPTLC study, the methanolic extract is ultrasonic for 15 min and filtered. The filtrate is used as an application for saponins and stegmasteroids. For each application 20 µl, 10 µl and 5 µl 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].

Table 6: Phytochemical study of *Chlorophytum spp.*

Compound	Reagents	Results	
		C. *tuberosum* Baker	C. *laxum* R. Br.
Water Extracts			
Starch	K_2KI	+ ve	+ ve
Protein	Millon's reagent	+ ve	+ ve
Tannins	Acidic FeCl3	+ ve	+ ve
Saponin	Distilled water	+ ve	+ ve
Anthroquinone's	Benzene + 10% Ammonium hydroxide	-ve	-ve
Sugars	Benedict's reagent	+ ve	+ ve
Fats	Sudan III	+ ve	+ ve
Flavonoids		-ve	-ve
Steroids		+ ve	+ ve
Alcoholic extracts			
a	Mayer's Reagent	+ ve	+ ve
b	Wagner's Reagent	+ ve	+ ve
c	Dragendorff's Reagent	+ ve	+ ve
d	Tannic acid	+ ve	+ ve
e	Hager's Reagent	+ ve	+ ve
f	Folin-Phenol Reagent	+ ve	+ ve
Glycosides	Benzene	+ ve	+ ve

Table 7: Quantitative estimation of *Chlorophytum spp.*

Quantitative estimation	(mg/g)	
	C. *tuberosum* Baker	C. *laxum* R. Br.
Protein	3.61	2.82
Reducing Sugar	0.98	0.86
Non - Reducing Sugar	1.98	0.14
Starch	2.82	1.90
Saponins	1.15	1.49

Analytical studies (Saponins)

The HPTLC analysis showed that the saponins are confirmed from *C. tuberosum* and from *C. laxum* root samples. The plates were scanned at 254 and 366 nm. When images were compared with the graph and table

values, it showed maximum area at 366 nm after derivatization. The table also indicates the Rf values (Figure 4; Graph 1; Table 8).

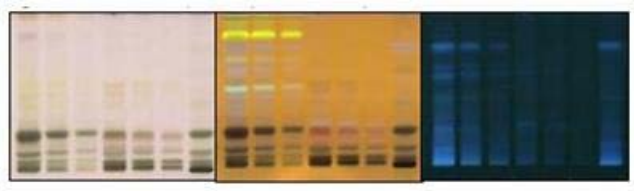
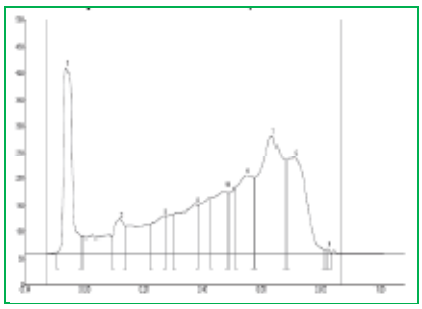


Figure 4: Detection of saponins by HPTLC techniques



Graph 1: Peak for saponins

Table 8: Peak values for saponins

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	1.4	0.08	41.5	9.96	0.10	8.6	535.9	4.27
2	0.10	8.8	0.12	26.5	6.37	0.15	2.4	526.1	4.19
3	0.20	0.8	0.23	11.3	2.71	0.25	6.5	268.8	2.14
4	0.40	8.5	0.45	42.3	10.16	0.49	23.9	1562.1	12.44
5	0.49	24.5	0.53	52.7	12.67	0.54	35.5	1094.5	8.72
6	0.59	44.1	0.61	67.7	16.25	0.64	50.1	2133.2	16.99
7	0.64	50.5	0.68	85.4	20.52	0.72	28.7	3168.3	25.24
8	0.75	22.0	0.80	45.7	10.97	0.83	35.4	1854.7	14.78
9	0.86	37.3	0.86	43.3	10.40	0.92	0.0	1408.8	11.22

Analytical studies (Stegmasteroids)

In HPTLC analysis, revealed the presence of stegmasteroids in both the species. The plates were scanned at 254 and 366 nm. It covered the area indicated in the table 9. The tables also indicate the Rf values for all the peaks scanned by "WINCATS" software (Figure 5; Graph 2; Tables 9).

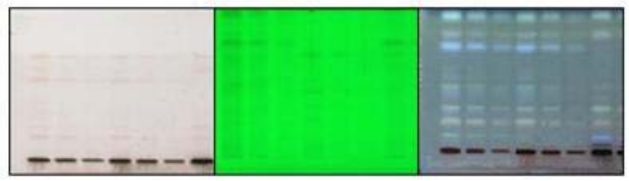
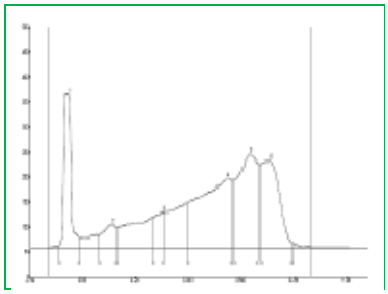


Figure 5: Detection of stegmasteroids by HPTLC techniques



Graph 2: Peak for stegmasteroids

Table 9: Peak values for stegmasteroids

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.6	0.11	17.2	7.49	0.14	4.5	360.5	5.77
2	0.23	5.4	0.25	15.3	6.64	0.27	1.5	198.9	3.18
3	0.29	4.5	0.33	14.1	6.14	0.35	7.3	357.3	5.72
4	0.39	10.5	0.44	38.2	16.64	0.47	18.1	1279.7	20.47
5	0.56	19.9	0.59	40.4	17.59	0.62	21.9	1256.3	20.10
6	0.63	21.6	0.65	32.6	14.16	0.66	26.9	768.4	12.29
7	0.68	22.4	0.68	23.4	10.19	0.72	5.4	505.4	8.09
8	0.74	7.4	0.78	20.7	9.00	0.80	16.5	670.4	10.72
9	0.82	20.5	0.85	28.0	12.16	0.89	2.1	853.8	13.66

CONCLUSION

The plant *C. tuberosum* and *C. laxum* showed the correct taxonomy which is helpful for the standardization of drug. The morphological characters and histochemical study with double staining of the root, percentage extractives, fluorescence and ash analysis and the phytochemical screening of the plants. As in case of saponins and stegmasteroids, the peaks are denoted by the Rf values. These investigations will be useful for the correct botanical identification and authentication of the drug. After getting the overall results of *C. tuberosum* and *C. laxum* and if data is comparable with the above mentioned species of safed musali, it can be used as a substitute for them.

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