Pharmacognostic study of *Chlorophytum tuberosum* Baker

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**ABSTRACT**

*Chlorophytum tuberosum* Baker belongs to family Liliaceae and is being used in the indigenous systems of medicine as a galactogogue and aphrodisiac. It is being sold in the market under the common name *safed musali*. The white tuberous roots of this plant are the medicinally useful parts. The tuberous roots of other species of *Chlorophytum*, *Asparagus*, *Bombax* and Orchids are also sometimes called *safed musali* leading to confusion. In order to ensure correct botanical standardization to remove the controversy, a detailed pharmacognostic study on tuberous roots of *Chlorophytum* has been carried out in this study.

**Key words:** *Chlorophytum tuberosum*, High Performance thin layer chromatography, pharmacognostic standardization, phytochemical analysis

**INTRODUCTION**

*Chlorophytum tuberosum* Baker 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 2800 m). This is an erect plant growing up to a height of 1.5–2 ft 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. The tuberous roots are medicinally important and are known commonly as *safed musali*. *Safed musali* is used as an aphrodisiac and galactogogue as well as for its nutritive, health promoting properties and immunoenhancing, hepatoprotective and antioxidants activities. The tubers are also used in fever, leucorrhoea and also as an aphrodisiac.

The species *Asparagus*, *Bombax* and Orchids are also known as *safed musali* in literature. It is therefore important to define specifications that will allow correct identification of the plant that 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.

We choose *Chlorophytum tuberosum* 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.

**MATERIAL 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. 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 specimen number is PAVICH2/2009.

**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. The macroscopic evaluation was studied by the method of Trease...
and Evans\cite{14} and Wallis.\cite{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.\cite{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 taid containers for phytochemical screening. Ash and percentage extractive content was measured by following the standard pharmacopeial techniques.\cite{17} Fluorescence analysis was carried out as per Chase and Pratt.\cite{18} Qualitative phytochemical tests were carried out by standard methods of Harborne\cite{19} and Trease and Evans.\cite{14} Quantitative phytochemical analysis were determined for proteins, carbohydrates and saponins by the methods of Lowry et al.,\cite{20} Nelson\cite{21} and Obadoni and Ochuko.\cite{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.\cite{23,24}

**RESULTS AND DISCUSSIONS**

**Macroscopic evaluation**

The details of the macroscopic examination are mentioned in Table 1 and illustrated in Figures 1 and 2.

**Microscopic characters**

The transverse section of the root 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

| Table 1: Macroscopic examination of *safed musali* |
|-----------------------------------------------|
| **Herb** | 1.5–2 ft. in height. |
| **Roots** | Fleshy, tuberous with ellipsoid tubers hanging from them, 10–12 cm long, 1–1.9 cm diameter. |
| **Leaves** | Green, 6–12 (8–14 also) 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.) |
| **Scape** | Unbranched, naked, 3–12 in. long. |
| **Flower** | White in simple racemose, 3–6 cm long, dense flower. |
| **Bract** | Lanceolate, acuminate, lower 0.8–0.5 cm long. |
| **Pedicels** | Ascending, 0.5–0.7 cm long, jointed below the middle. |
| **Perianth** | Segments, less than 0.7 cm long by 0.5 cm broad. Oblong, lanceolate, sub-acute, 7–9 nerved. |
| **Stamen** | 0.5–1 cm long, anther 0.5–0.8 cm long, linear, shining transversely veined |
| **Style** | 1, stigma minute. |
| **Capsule** | Obovoid – sub-globose, 0.8–1.2, transversely veined, emarginate. |
| **Seeds** | Black, irregularly orbicular, 0.3–0.5 cm diameter |

| Table 2: Histochemical study of *C. tuberosum* Baker |
|-----------------------------------------------|
| **Test** | **Reagents** | **Color** | **Tissue** |
|-------|-----------------|--------|---------|
| **Starch** | I, KI | Blue | Endo., Peri., Phlo. |
| **Protein** | Potassium ferrocyanide + water + acetic acid + 60% alcohol + FeCl₃ | Blue | Epi., Cort., Peri., Phlo., xy. |
| **Tannin** | Acidic FeCl₃ | Light brown | Cort. Phlo., xy., peri. |
| **Saponin** | Conc. H₂SO₄ | Yellow | Endo., xy. |
| **Fat** | Sudan III | Pink | Epi., endo., phlo., xy. |
| **Sugar** | 20% aq. NaOH | Yellow | Epi. xy. |
| **Glycosides** | Guignard’s test | Brown | Epi., xy., Phlo. |
| **Alkaloids** | Mayer’s reagent | Colorless | Hair, Epi., Cort xy. Phlo. |
| | Wagner’s reagent | Orange | Epi., Endo., peri., phlo., xy. |
| | Dragendorff’s reagent | Orange to dark brown | Epi., Endo., Peri., Phlo., xy. |
| | Tannic acid | Buff color | Peri., phlo. |
| | Hager’s reagent | Yellow | Cort. |

I: KI: Potassium iodide, FeCl₃: Ferric chloride, Conc. H₂SO₄: 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 FeCl₃.
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**Table 3: Ash and acid insoluble ash of *C. tuberosum* Baker**

| Parameter              | Results               |
|------------------------|-----------------------|
| Total Ash              | 13.2% dry wt.         |
| Acid Insoluble Ash     | 4.8% total ash        |

**Table 4: Percentage extractives of *C. tuberosum* Baker**

| Solvent used       | Extract (%) |
|--------------------|-------------|
| Distilled water    | 2.395%      |
| Absolute alcohol   | 0.275%      |
| Petroleum ether    | 0.260%      |
| Benzene            | 0.210%      |
| Chloroform         | 9.960%      |
| Diethyl ether      | 0.430%      |
| Acetone            | 0.470%      |

**Table 5: Fluorescence analysis of *C. tuberosum* Baker at 230 nm**

| Treatments                                      | Color emits       |
|------------------------------------------------|-------------------|
| Powder as such                                  | Yellowish brown   |
| Powder as such in UV-light                      | Pale yellow       |
| Powder + Nitrocellulose                         | Grayish white     |
| Powder + 1 N NaOH in methanol dry for 30 min. + | Grayish green     |
| Nitrocellulose                                   |                   |

**Figure 1:** Habit of *Chlorophytum tuberosum*

**Figure 2:** Tuberous roots of *Chlorophytum tuberosum*

**Figure 3:** Transverse section of root of *Chlorophytum tuberosum* (10x x 3.3x)

**Figure 4:** Detection of saponins by high performance thin layer chromatography techniques

**Figure 5:** Detection of stegmasteroids by HPTLC techniques
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 3].

Histochemical screening

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

Phytochemical studies

The tuber had a total ash content of 12.6%, the acid insoluble ash being 5.6% [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 as yellowish brown in color. The powder was treated with nitrocellulose, I N

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
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.

Qualitative analysis of the root indicated the presence of proteins, reducing and non-reducing sugars, saponins, fats, tannin, glycoside and alkaloids [Table 6]. The quantity of proteins is higher than saponins and carbohydrates [Table 7]. 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.\textsuperscript{[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 stegmastroids. 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.\textsuperscript{[23,24]}

**Analytical studies (Saponins)**

The HPTLC analysis showed that the saponins from the *C. tuberosum* 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 366 nm after derivatization. The table also indicates the starting Rf values and end Rf values [Figure 4; Graph 1–3; Table 8–10].

**Analytical studies (Stegmastroids)**

In HPTLC analysis, stegmastroids 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 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; Tables 11 and 13].

**Conclusions**

The plant *C. tuberosum* 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 plant. As in case of saponins and stegmastroids, 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 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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