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Pharmacognostical study of medicinal plants of Western Ghats: *Chlorophytum kolhapurens* and *Chlorophytum bharuchae*

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Abstract
The tuberous roots of *Chlorophytum borivilianum* is widely used by the folk people in the treatment of variety of diseases and as a galactogogue and aphrodisiac. Commonly known as 'Safed musli'. The tuberous roots of other species such as *Aspargus* and *Orchids* are sometimes also called as safed musli, leading to confusion. In order to ensure correct botanical standardization, the detailed pharmacognostic study was carried out in present study on the other species of *Chlorophytum* viz. *Chlorophytum kolhapurens* and *Chlorophytum bharuchae*. Macroscopy and microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardization were performed. Physicochemical tests like moisture content, ash value, extractive values and phytochemical screening showed presence of alkaloids, carbohydrates, proteins and saponins gives supplement information concerning its identification. Chromatographic analysis (TLC) confirms the presence of saponins which is a remarkable identification. The microscopic constants, and other physicochemical examinations of *Chlorophytum* species will be useful in standardization, hence would be of immense value in authentication of plant.

Keywords: *Chlorophytum*, Pharmacognostic standardization, Phytochemistry, TLC

Introduction
Plants as natural medicines to benefit humans have a long history and ancient civilization, particularly in some nations such as India, China, Egypt, and Greece. The majority of world populations still rely on plant based medicine because of several reasons such as long history of safe use, affordability and easy availability. The growing interest in drugs of plant origin may also be due to several other reasons such as a large section of world's population does not have access to conventional pharmacological treatment and side effects and other problems of synthetic drugs due to incorrect use [1]. Natural product remains a prolific source of discovery of new drugs from the ancient *Vedic* period. India has long history of management of human health through *Ayurveda* which came in to existence more than 6000 yrs ago. *Charak* and *Sushurt* had contributed a lot in the development of plant based medicine and surgery [2]. In recent years, there has been growing interest in complementary medicine, functional and therapeutic uses of natural products, especially those derived from plants. Thus, natural products including terrestrial and marine plant extracts have become a source of optimism for drug discovery. The rich biodiversity of India has attracted the attention of researcher, which remained untouched as far as the new drug discovery is concerned [3]. Bioactive natural products are generally originates from microbes and plants. As chemicals, natural products include belong to diverse classes viz. terpenoids, steroids, saponins, phenols, flavonoids, alkaloids, amino acids, proteins, carbohydrates, lipids, nucleic acid bases, etc. [4] and some 75% of these were discovered by examining the use of these plants in traditional medicine.
Species of *Chlorophytum* are sold in the market as 'Safed musli' due to its white tuberous roots widely used in herbal drug industries. *Chlorophytum* species plants are found in India, Nepal and Myanmar [5]. In India they are found wild in natural forest and Hilly areas Rajasthan, Gujarat, Madhya Pradesh, Bihar Orissa and West Bengal [6]. They belong to family Asparagaceae are small perennial herbs and are considered to be valuable nervine and general tonic for strength and vigor. Leaves species are edible and use as a vegetable [7]. The aim of present study was to evaluate the various Pharmacognostical characteristics and phytochemicals from *Chlorophytum* species viz. *Chlorophytum kolhapurens* and *Chlorophytum bharuchae* which will help in their identification and authentication.

Materials and methods

Plant material

Chlorophytum kolhapurens and *Chlorophytum bharuchae* were collected from natural population from Western Ghats of Maharashtra during rainy season. Collected species were identified and confirmed with the herbarium species at Botanical Survey of India (BSI), Pune, Maharashtra.

Microscopic and Macroscopic evaluation

These fresh leaves were used for morphological characters by microscopic and macroscopic methods as studied in Trease and Evans [8]. Thin hand transverse section of fresh leaves of plants were cut and stained with different staining regents and observed under microscope at 45 X objective.

Physicochemical parameter

The various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content, specific gravity, extractive value (water and alcohol) have been studied [9].

Preliminary phytochemical analysis

The tubers (roots) of the plants were washed thoroughly with running water to remove dirt. The tubers were than shade dried, powdered using blender and stored in air tight containers. Dried powder (5g) was extracted in Soxhlet reflux extractor with petroleum ether to remove lipids and fatty acids, ethyl acetate and chloroform to remove proteins and hexane to remove fats. The defatted powder was then extracted with methanol for 48 hours [10]. The extracts were concentrated under vacuum rotary evaporator, dried, weight and stored for further investigation. Methanol extracts were chosen for phytochemical screening. Preliminary phytochemical screening was performed to identify phytochemicals present in plant extract using standard procedures [11].

Thin Layer Chromatography (TLC)

The methanolic fractions were further separated by column chromatography on silica gel. The fractions obtained were analyzed by TLC. Analytical TLC plates were prepared by pouring silica gel G and GF slurry on the glass plates. The plates were allowed to dry in air for 30 minutes and then kept in oven at 110^0C for 30 minutes. The plates were placed in the developing jar with different solvent system. The resultant chromatograms were observed in UV/VIS. The spots were identified and Rf values were calculated [12].

Spot visualization

Concentrated H2SO4 and Ehrlich reagent were used as spraying reagent. TLC plates were heated at 100^0C after spraying the reagent. Pinkish-violet spots of saponin were observed under UV/VIS [13].

Screening for Saponin

Foam test: For the test of saponins, extracts were diluted to 5 mL with distilled water and was shaked vigorously. Appearance of stable foam was regarded as the presence of saponins.

Froth test: The extracts were diluted with 20 ml distilled water in a test tube and were shaked vigorously for 30 seconds. The tube was allowed to stand in vertical position for 30 minutes. If froth was observed above the surface of liquid after 30 min the sample confirms the presence of Saponin.

Heamolytic test: For haemolytic test, the extracts were added to one drop of blood placed on glass side, heamolytic zones appeared was confirmation of heamolysis activity.

Results and Discussions

Chlorophytum bharuchae was distributed in Kolhapur and Aurangabad district in Maharashtra and Karnataka. It was reported as rare species in Maharashtra. *Chlorophytum kolhapurens* was found to be reported from Kolhapur, Maharashtra. Figure 1 and 2 represents the inflorescence of *C.bharuchae* and *C.kolhapurens*.

Macroscopic and Microscopic evaluation

Leaves of Chlorophytum species showed similar morphological and microscopic characters with some differences in leaf length, leaf margin, perianth and number of vascular bundle, number and arrangement of xylem strand. The details of macroscopic evaluation are mentioned in the Table no 1. T.S. of leaf showed its typical dorsoventral nature. Upper and lower epidermis, lamina, mesophyll, and midrib region were observed as important diagnostic characters. Midrib shows central nonlignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma, illustrated in figure 3 and 4.

Fig 1: *C. bharuchae*

Fig 2: *C. kolhapurens*

Table 1: Macroscopic evaluation of *Chlorophytum* species

Characters	*Chlorophytum bharuchae*	*Chlorophytum kolhapurens*
Herb	2 - 2.5 ft. in height	1.5 - 2.5 ft. in height
Roots	Tuberous, cylindrical, 15 - 34 cm long, 5 – 15 numbers, 1-1.8 cm diameter.	Non-tuberous, cylindrical, 15 - 47 cm long, 5 – 15 numbers, 0.4-1.0 cm diameter.
Leaves	5 - 8 in number, thick, acute to acuminate, 15 – 20 × 1.5 – 3 cm, margin wavy, hyaline, green at the base.	6 - 13 in number, thick, lanceolate to loratete, 8 – 40 × 1 – 3 cm, margin wavy, hyaline, green at the base.
Scape	Branched, 1 – 2 ft. height	Branched, upto 2 ft. height
Flower	White, yellow brown spot at the tip, racemose,alternate or sub-opposite, cluster of 4-8 flowers	Greenish white, racemose,alternate or sub-opposite, cluster of 2-4 flowers
Bract	Ovate lanceolate	linear-lanceolate
Pedicels	Ascending, geminate, 0.5 – 1 cm long	Ascending, terete, 0.8 – 1 cm long
Perianth	White with green blotch at the apex, 3 nerved, lanceolate.	Dark green with greenish-white at the apex, 3 nerved, lanceolate.
Stamen	6 in number, 0.5 – 0.8 cm long, anther dorsifixed – 0.6 cm long	6 in number, 0.7 – 0.8 cm long, anther dorsifixed – 0.3 cm long
Style	1, 0.5 – 0.8 cm long	0.6 – 0.7 cm long
Capsule	3 lobed, 0.6 – 1 cm long, greenish, obchordate.	3 lobed, 0.6 – 1 cm long, greenish, triquetrous, obchordate.
Seeds	Black, solitary flattened.	Black, discoid.

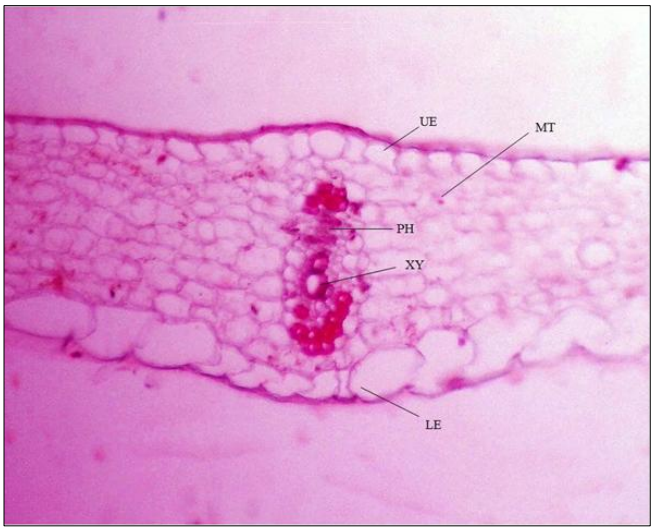

Fig 3: *Chlorophytum bharuchae*

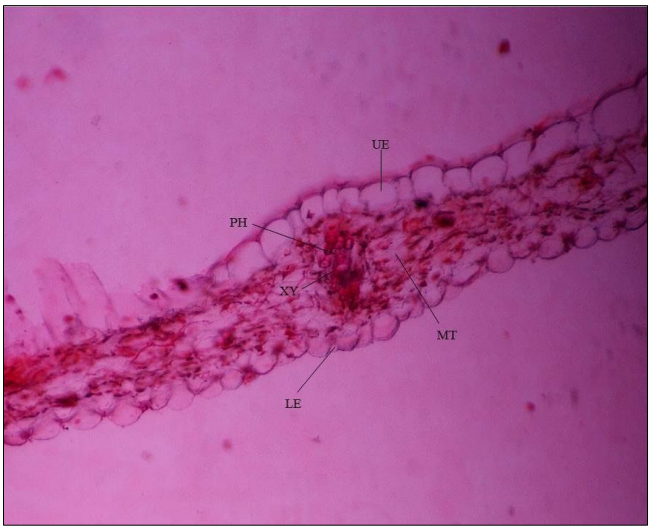

Fig 4: *Chlorophytum kolhapurens*

Whereas, UE= Upper Epidermis, LE = Lower Epidermis, XY = Xylem, PH = Phloem and MT= Mesophyll Tissue.

Physicochemical parameter

Physicochemical study is important, as it helps in characterization of constituent or group constituents that frequently lead to establish the structure-activity relationship and likely mechanism of action of the drug. The results are summarized in table 4; the values are expressed in terms of Mean±SD.

Table 2: Physico-chemical analysis of *Chlorophytum* species

S. No	Physico-chemical Parameter	*C.kolhapurens* Mean+SD	*C.bharuchae* Mean+SD
1	Loss of weight on drying at 105 °C (%)	24.53 ± 0.02	24.98 ± 0.95
2	Specific Gravity	1.320 ± 0.01	1.27 ± 0.05
3	Moisture content (%)	11.53±0.08	14.80 ± 0.02
4	**Ash value in (%)**		
	Total Ash	1.27 ± 0.05	1.12 ± 0.00
	Acid Insoluble Ash	0.23 ± 0.01	0.20±0.04
	Water Soluble Ash	0.49±0.01	0.44 ± 0.00
5	**pH value**		
	pH at 1%	3.86±0.70	3.81 ± 0.00
	pH at 10%	3.72 ± 0.00	3.12±0.01
6	**Solubility (%)**		
	Alcohol Soluble extractive	62.65 ± 0.88	61.59±0.80
	Water Soluble extractive	58.56 ± 01.20	45.88 ± 0.85

Preliminary phytochemical analysis

The qualitative analysis of methanol extract of *Chlorophytum* species showed the presence of medicinally active constituents such as alkaloids, carbohydrates, proteins and saponins. Table 2 shows the various phytochemicals present in *Chlorophytum kolhapurens* and *Chlorophytum bharuchae*.

Table 3: Preliminary Phytochemical screening of *Chlorophytum*

S. No	Phytochemicals	Test/reagent	*Chlorophytum kolhapurens*	*Chlorophytum bharuchae*
1	Alkaloids	Mayer's test	+	+
2	Carbohydrates	Molish test	+	+
3	Reducing sugar	Benedicts test	+	+
4	Starch	Iodine test	+	+
5	Protein	Biuret test	+	+
6	Tannins	Lead acetate test	+	+
7	Saponin	Foam test	+	+
		Froth test	+	+
		Haemolytic test	+	+

Thin Layer Chromatography

Chlorophytum species were subjected to thin layer chromatographic analysis to find the presence of number of chemicals and also to identify the type of saponins. The best separation was observed in solvent system containing $CHCl_3$: MeOH. The respective solvent systems are depicted in Table 3. Saponins were visualized by spraying 10% sulphuric acid in ethanol and Ehrlich reagent. Saponin produced violet and dark violet spots with 10% sulphuric acid in ethanol whereas Ehrlich's reagent showed pink or red colored spots, which confirms the presence of saponins (Figure 5).

Table 4: Solvent system of *Chlorophytum species*

S. No.	Species	Solvent system	Ratio
1	*Chlorophytum kolhapurens*	$CHCL_3$: MeOH	15:1
2	*Chlorophytum bharuchae*	$CHCL_3$: MeOH	11:1

The species showed the correct taxonomy which is useful for the standardization of drug. The morphological characters, ash analysis, phytochemical screening and the saponins identification test with respect to their Rf values (Table 5). All these investigations will be useful for correct botanical identification and authentication. Also if data is comparable with the above mentioned species of safed musli, the *Chlorophytum* species can be used as a substitute for them.

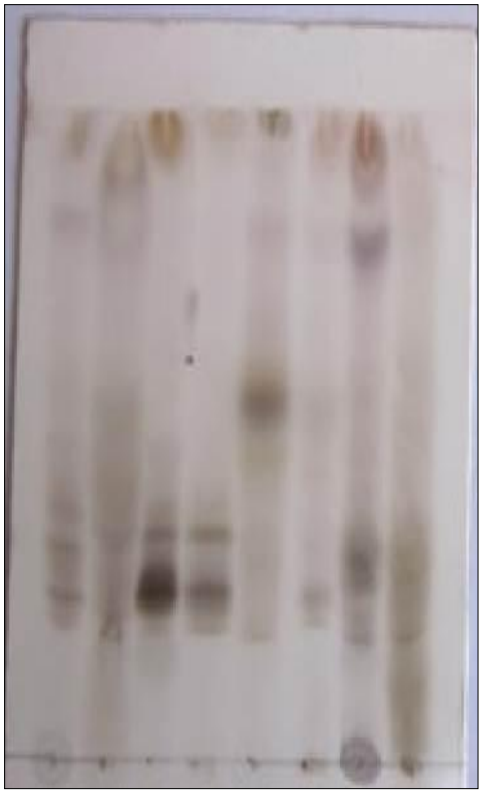

Fig 5: Violet and Pink spots of Saponins

Table 5: Rf value of *Chlorophytum kolhapurens* and *Chlorophytum bharuchae*

S. No.	Name	Rf Value
1	*Chlorophytum borivilianum*	0.9
2	*Chlorophytum kolhapurens*	0.8
3	*Chlorophytum baruchii*	0.7

Conclusions

The detailed study of Pharmacognostic parameters of *C.kolhapurens* and *C.bharuchae* setup the standards which could be beneficial and serves as diagnostic tool for proper authentication of this medicinally important plant.

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