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Research Article

# ESTABLISHMENT OF QUALITY PARAMETERS TO DETECT SUBSTITUTION OF SHATAVARI BY SAFED MUSALI

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**ABSTRACT**

Objectives: Shatavari (tuberous roots of *Asparagus racemosus*, family Liliaceae) is traditionally used as diuretic, tonic, antidiabetic, in gout, female genitourinary tract disorders, as styptic, anti-ulcer, intestinal disinfectant and astringent in diarrhea, nervine tonic, in sexual debility for spermatogenesis, lactic disorders, haematuria, bleeding disorders and hyperacidity. Safed Musali (tuberous roots of *Chlorophytum arundinaceum*, family Liliaceae) is also used traditionally, though only as tonic and aphrodisiac, thereby making it a drug of limited use compared to Shatavari. However, the market samples of Shatavari are often substituted by Safed Musali, though only Shatavari is official in Ayurvedic Pharmacopoeia of India. Also, there is a risk of hormonal imbalance in geriatric and female patients consuming Shatavari if is substituted by Safed Musali. This makes a comparative study of the roots of both these species necessary.

Methods: A detailed study of Shatavari and Safed Musali roots has been performed here, including morphological study, qualitative and quantitative microscopic evaluation, phytochemical screening and Thin-layer Chromatography (TLC) studies.

Results: Morphologically, the two roots show significant differences. Shatavari roots are cylindrical, thicker, almost straight, creamish-white to pale brown in color whereas Safed Musali roots are curved and tapering at both ends, thinner, creamish-white to yellowish-white in color. Microscopically, absence of collenchymatous cortex and absence of lignified endodermis distinguishes Safed Musali from Shatavari. Powder of both Shatavari and Safed Musali show the presence of almost similar characters. However, quantitative microscopy can be useful in distinguishing the powder of the two species. Phytochemical screening showed the presence of saponin glycosides, carbohydrates, mucilage and steroids and triterpenoids in both species. The TLC of both roots is most crucial as the alcoholic extract of Safed Musali reveals the presence of a yellowish spot at $R_f$ 0.35 upon plate development using the mobile phase n-Butanol : Acetic acid : Water (4:1:5) and spraying with 5% Methanol-Sulfuric acid reagent. Such a spot is absent in the TLC of alcoholic extract of Shatavari roots developed using the same system.

Conclusion: The present work can serve as a very useful phytopharmacognostical tool in the standardization of the raw material and prepared formulations of Shatavari and detection of its substitution by Safed Musali. This study can prove to be very useful for herbal industries, traditional healers and for the general public who consume Shatavari.

**Keywords:** *Asparagus racemosus, Chlorophytum arundinaceum,* Liliaceae**,** Safed Musali, Shatavari.

## INTRODUCTION

Shatavari (tuberous roots of *Asparagus racemosus*, family Liliaceae) is also known as Shatmuli or Shatpadi and is found distributed throughout tropical Asia, Africa and Australia. In India, it is found in Himalayas upto an altitude of 1300 to 1400 m and all tropical parts of India. The roots are traditionally used as diuretic, tonic, antidiabetic, in gout, female genitourinary tract disorders, as styptic, anti-ulcer, intestinal disinfectant and astringent in diarrhea, nervine tonic, in sexual debility for spermatogenesis, lactic disorders, haematuria, bleeding disorders and hyperacidity. Safed Musali (tuberous roots of *Chlorophytum arundinaceum*, family Liliaceae) is found in African countries and also in India. In India it is collected from wild. They are also used traditionally, though only as tonic and aphrodisiac, thereby making it a drug of limited use compared to Shatavari [1]. However, the market samples of Shatavari are frequently substituted by Safed Musali, though only Shatavari is official in Ayurvedic Pharmacopoeia of India [2]. This makes a comparative study of the roots of both these species necessary. A detailed pharmacognostic and phytochemical comparison of the two can provide valuable indications regarding substitution with each other as well as establish quality parameters for their respective formulations, which will ultimately benefit the traditional medical practitioners as well as the fast-growing herbal industry.

## MATERIALS AND METHODS

Dried roots of Shatavari and Safed Musali were purchased from Yucca (Mumbai) and voucher specimens [RK/COG/2012/SH] and [RK/COG/2012/SM] of both the respective species were deposited in the college laboratory. The roots were compared morphologically and used for transverse section study. The roots were powdered, stored in airtight containers and used for quantitative microscopy and phytochemical studies. For microscopical studies, safranin was used for staining. Photomicrography of the transverse sections and the powdered drug was performed using camera. Quantitative microscopic study was performed using camera lucida and stage micrometer scale (Table 1)[3]. Phytochemical screening of both the species was performed using the appropriate extract (aqueous and alcoholic) and a battery of chemical tests (Table 2)[4-8]. TLC of alcoholic extracts of both roots was developed using the mobile phase n-Butanol : Acetic acid : Water (4:1:5 v/v) and spraying the developed plates with 5% Methanol-Sulfuric acid reagent, followed by heating of the plates at 110°C in hot-air oven for 10min.

**Fig. 1: It shows Morphology of Shatavari (Left) and Safed Musali (Right)**

**RESULTS**

**Macroscopy**

Shatavari roots are peeled, cylindrical, 4-6cm X 0.5-1cm, almost straight, creamish-white to pale brown in color having rough surface with longitudinal ridges. Safed Musali roots are even more peeled, curved and tapering at both ends, 2-4cm X 0.5cm, creamish-white to yellowish-white in color having rough surface with longitudinal ridges (Fig.1).

**Microscopy: Transverse section**

The market samples of Shatavari roots, being peeled, show no presence of epiblema in the transverse section. The outermost layer is the 6-8 layered cortex having outer cells collenchymatous and inner cells parenchymatous, some of which may be pitted, wavy-walled, overlapping and may have mucilage content. Endodermis is 1-2 layered, lignified, pitted and continuous. Pericycle is 1-2 layered and consists of thin walled parenchymatous cells. Vascular bundles are radial, a characteristic of monocot root. Pith consists of pitted, lignified or non-lignified cells having intercellular spaces (Fig. 2a, 2b).

The market samples of Safed Musali roots, being highly peeled, show no presence of epiblema and collenchymatous cortex. Inner cortical parenchyma may be pitted. Endodermis is 1-2 layered, non-lignified, continuous and parenchymatous whereas pericycle is 1-2 layered and consists of thin walled cells. Vascular bundles are radial having exarch xylem and pith contains non- lignified cells having intercellular space (Fig. 3a, 3b).

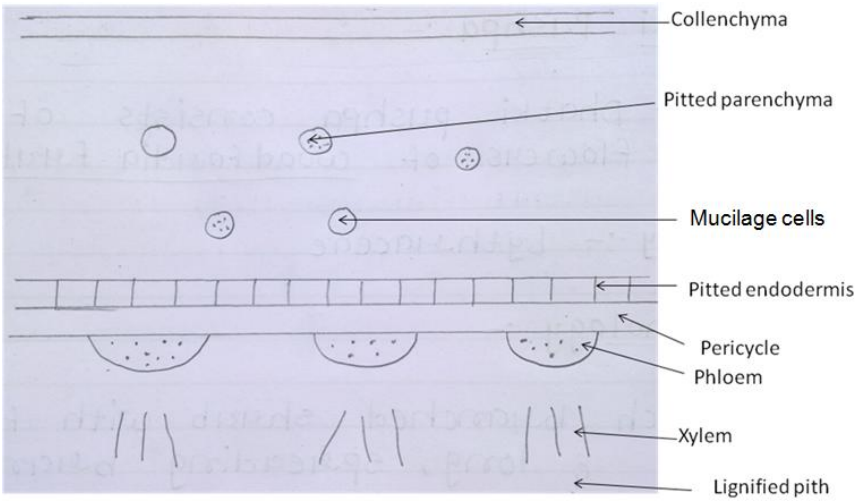



**Fig. 2a: It shows Schematic diagram of Shatavari root**

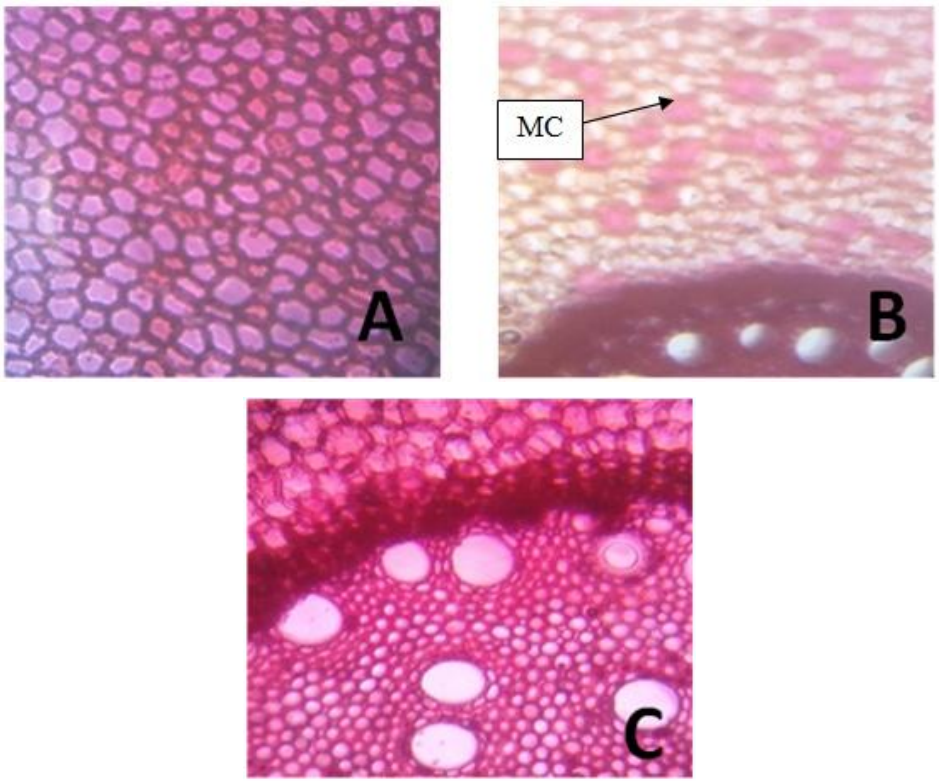



**Fig. 2b: It shows Detailed TS of Shatavari root (100x)**

**A: Cortex; B: Cortex, Endodermis, Pericycle; C: Stele (MC: Mucilage cell)**

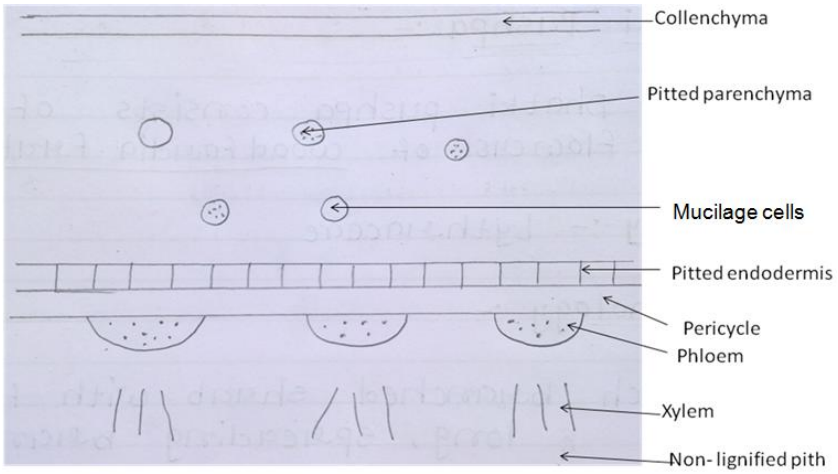



**Fig. 3a: It shows Schematic diagram of Safed Musali root**

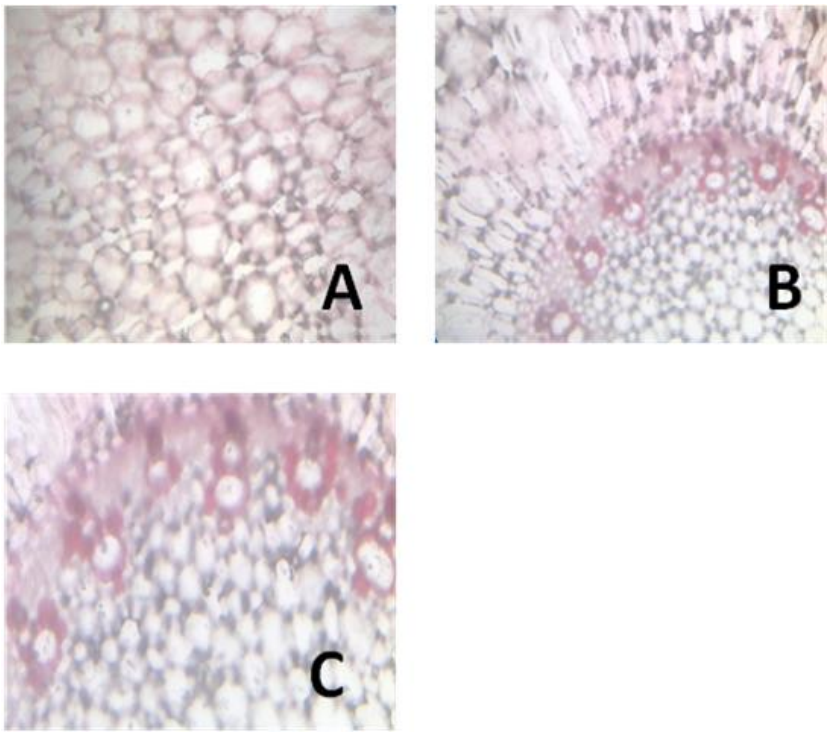
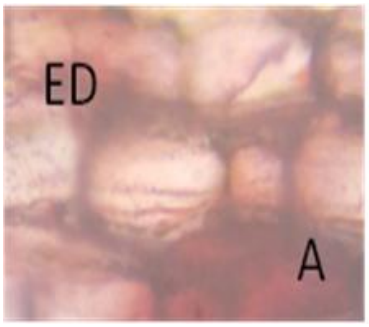


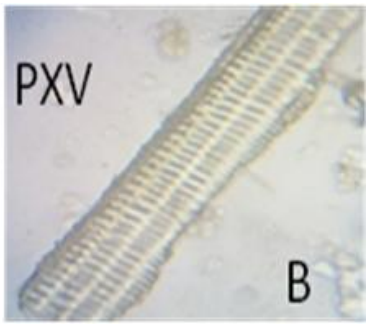

**Fig. 3b: It shows Detailed TS of Safed Musali root (100x)**

**A: Cortex; B: Cortex, Endodermis, Pericycle, Stele; C: Stele & Pith**

**Microscopy: Powder characteristics**

Shatavari powder is creamish-white whereas Safed Musali powder is slightly yellowish-white. Both of them have no distinct odor and mucilaginous taste. The microscopic features of both the powders are very similar and show the presence of pericyclic fibres, prismatic and acicular calcium oxalate crystals (isolated or in bundles); pitted, annular and reticulate-thickened xylem vessels (Fig. 4).



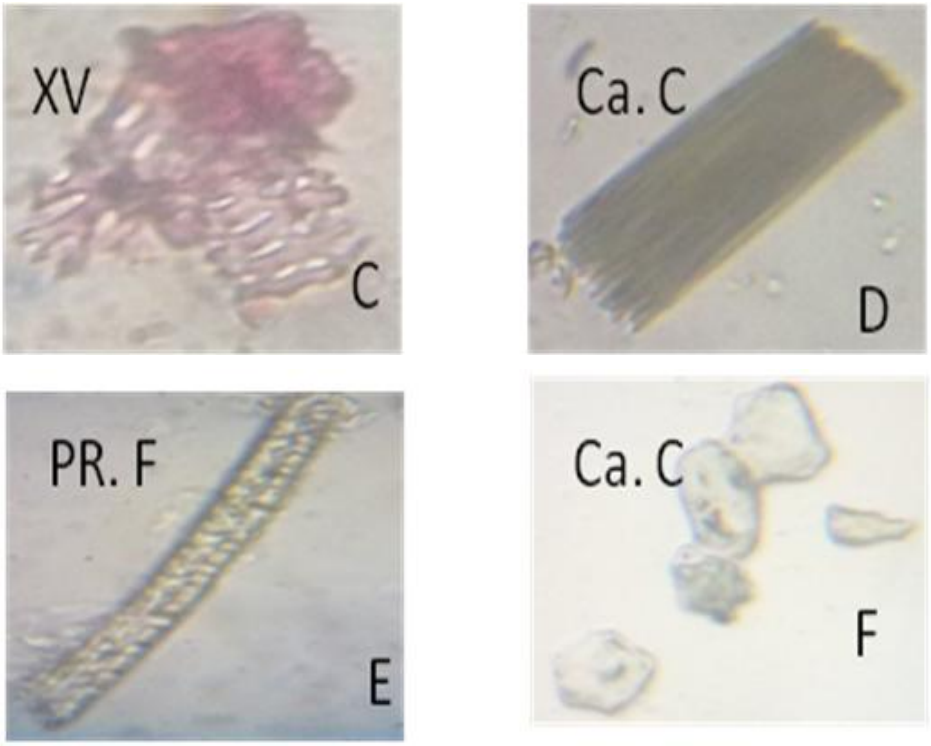



**Fig. 4: It shows Powder characteristics of Shatavari & Safed Musali**

A: Portion of endodermis having mucilage cell (ED); B: Xylem vessel having annular thickenings (PXV); C: Xylem vessel having reticulate thickenings (XV); D: Acicular raphides of calcium oxalate (Ca. C); E: Pericyclic fibre (PR. F); F: Calcium oxalate prisms (Ca. C).

**Thin-Layer Chromatography**

TLC of Shatavari revealed the presence of a conspicuous dark violet spot at $R_f$ 0.30, a conspicuous dark purple spot at $R_f$ 0.38 and an inconspicuous dark brown spot at $R_f$ 0.53. Whereas, TLC of Safed Musali revealed the presence of a single small yellowish spot at $R_f$ 0.35 (Fig. 5).

**Fig. 5: It shows TLC of Shatavari (Left) & Safed Musali (Right)**

**Table 1: It shows Quantitative microscopy**

| Parameters | Measured value(µm) | |
|---|---|---|
| | Shatavari | Safed Musali |
| Length of pericyclic fibres | 175.2 | 159.2 |
| Length of acicular crystals | 108.5 | 96.4 |
| Length of xylem vessels | 122.4 | 105.25 |

Number of observations = 100

**Table 2: It shows Phytochemical screening**

| Phytoconstituents | Tests | Result | |
|---|---|---|---|
| | | Shatavari | Safed Musali |
| Alkaloids | Dragendorff's test | -ve | -ve |
| | Wagner's test | -ve | -ve |
| | Mayer's test | -ve | -ve |
| | Hager's test | -ve | -ve |
| Flavonoids | Shinoda test | -ve | -ve |
| | Lead acetate test | -ve | -ve |
| Steroids & Terpenoids | Salkowski test | +ve | +ve |
| | Libermann Buchard test | +ve | +ve |
| Cardiac glycosides | Legal's test | -ve | -ve |
| | Baljet test | -ve | -ve |
| | Keller Killiani test | -ve | -ve |
| | Kedde's test | -ve | -ve |
| Saponin glycosides | Foam test | +ve | +ve |
| | Lead acetate test | +ve | +ve |
| Carbohydrates | Molisch test | +ve | +ve |
| Mucilage | Ruthenium Red test | +ve | +ve |
| Tannins | Ferric chloride test | -ve | -ve |
| | Catechin test | -ve | -ve |

## DISCUSSION

A detailed comparative phytopharmacognostic study of Shatavari and Safed Musali roots has been performed. Morphologically, the two roots show significant differences. Shatavari roots are cylindrical, thicker, almost straight, creamish-white to pale brown in color whereas Safed Musali roots are curved and tapering at both ends, thinner, creamish-white to yellowish-white in color. Microscopically, absence of collenchymatous cortex and absence of lignified endodermis distinguishes Safed Musali from Shatavari. Powder of both Shatavari and Safed Musali show the presence of pericyclic fibres, prismatic and acicular calcium oxalate crystals (isolated or in bundles), pitted and reticulate-thickened xylem vessels. However, quantitative microscopy can be useful in distinguishing the powder of the two species on the basis of the lengths of pericyclic fibres, xylem vessels and acicular crystals. Phytochemical screening revealed a similar phytochemical profile, showing the presence of saponin glycosides, carbohydrates, mucilage and steroids and triterpenoids in both Shatavari and Safed Musali. The comparative TLC of both roots revealed noteworthy results. The alcoholic extract of Safed Musali reveals the presence of a yellowish spot at $R_f$ 0.35 using the mobile phase n-Butanol : Acetic acid : Water (4:1:5) after spraying with 5% Methanol-Sulfuric acid reagent and heating the plate. Such a spot is absent in the TLC of alcoholic extract of genuine Shatavari roots developed using the same system. Thus, presence of such a spot in the TLC of any powder sample or formulation of Shatavari indicates substitution by Safed Musali. Also, it is advisable to purchase Shatavari in the form of entire crude drug from the market due to striking differences in morphology and transverse section of the roots of both species. Safed Musali is normally used by young or middle-aged men as a tonic or aphrodisiac, whereas Shatavari, apart from the aforementioned uses, is also used by geriatric patients in hyperacidity and by females in genitourinary & lactic disorders. Thus, substitution of Shatavari by Safed Musali can cause severe hormonal imbalance in such patients not intending to use it as an aphrodisiac or tonic. Also, only Shatavari is official in Ayurvedic Pharmacopoeia of India.

## CONCLUSION

Thus,the present work can serve as a very useful tool in the identification, authentication and standardization of the raw material and prepared formulations of Shatavari and distinguishing it from Safed Musali. This study can prove to be crucial for herbal industries, traditional medicinal practitioners and for people who buy and consume Shatavari or its products directly from the market.

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