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RESEARCH ARTICLE

Isolation and Characterization of marker compound for detecting Adulteration of *Chlorophytum* Species in *Asparagus racemosus*

Bhindora Jay[1], Bhoot Sagar[1], Nariya Pankaj[2], Vaibhavi Savalia[1]*, Pandya Devang[1]
[1]School of Pharmacy, RK University, Kasturbadham, Rajkot, Gujarat- 360020 India.
[2]N. M. Virani Science College, Kalavad Road, Rajkot, Gujarat- 360005 India.
*Corresponding Author E-mail: v**aibhavi.savalia@rku.ac.in**

ABSTRACT:
Shatavari (*Asparagus racemosus*) is used by children for increasing strength, in young and middle-aged men as an aphrodisiac, in mothers as a galactogogue and in old patients as an anti-ulcer. Whereas, Safed Musali (*Chlorophytum* species) is primarily used only as an aphrodisiac due to its high steroidal content, which may cause severe side-effects when consumed by children, women and geriatric patients. However, market formulations of *A. racemosus* are often mixed with *Chlorophytum* species, and vice-versa. The present work aims at chromatographic detection and isolation of a marker compound from *Chlorophytum* species, so that its adulteration in *A. racemosus* can be detected. Petroleum ether extracts of market samples of *A. racemosus* and *Chlorophytum* species were subjected to TLC using the mobile phase *n*-hexane: diethyl ether: glacial acetic acid (7:3:0.1). This was followed by preparative TLC of *Chlorophytum* species extract, isolation of the spotted marker, its H^1 NMR & GC-MS study, and finally its structure elucidation. A spot was observed in TLC of *Chlorophytum* species extract but not in *A. racemosus* extract, indicating it to be the marker which distinguishes the two species. Spectral analysis revealed the isolated marker to be 2, 4, 6, 10, 18, 22- tetracosahexaene. This work will be very useful to herbal industries and testing laboratories in detection of adulteration of *A. racemosus* formulations by *Chlorophytum* species, which will also benefit the patients. Such measures of standardization and quality control are also necessary to justify the authenticity of the Indian traditional system of medicine.

KEYWORDS: 2, 4, 6, 10, 18, 22 – Tetracosahexaene, *Asparagus racemosus*, *Chlorophytum*, Safed Musali, Shatavari, Standardization, Quality control.

INTRODUCTION:
Shatavari (*A. racemosus* Willd.*;* Family: Liliaceae/ Asparagaceae), being a 'Rasayana' drug, has several uses. In children it is used for improving their physical strength and body structure, in young and middle-aged men as an aphrodisiac, in mothers as a galactogogue and in old patients as an anti-ulcer. It is mentioned in Charak Samhita as well as Sushrut Samhita, also official in Indian and British Pharmacopoeias[1,2,3,4].

Whereas, Safed Musali (commercially available as a mix of *Chlorophytum* species *mainly C. arundinaceum* Baker, *C. tuberosum* Baker, *C. borivilianum* Sant and Fern and *C. indicum* Willd. syn. *C. attenuatum* Baker; Family: Liliaceae) is mainly used in sexual debilities like physical weakness, impotency, as an aphrodisiac, revitalizer, general sex tonic and spermatogenic. This is mainly due to its high steroidal content comprised of triterpenoid saponins[5,6,7,8].

This indicates that *Chlorophytum* species may cause severe side-effects when consumed by children, women and geriatric patients. But nowadays, crude drugs and market formulations of *A. racemosus* are often mixed with *Chlorophytum* species, and vice-versa, thereby making it necessary to detect and prevent this practice which may harm the health of a large section of society.

It is difficult to differentiate powders or extracts of the two species on the basis of microscopy or phytochemical screening[9]. Thus, the present work aims at TLC detection, isolation, spectroscopic characterization and structure elucidation of a marker from *Chlorophytum*

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species, so that its adulteration in *A. racemosus* can be ascertained.

Figure 1: Marketed samples of Shatavari and Safed Musali
a. *Asparagus racemosus* -Shatavari root, b. *Chlorophytum* species - Safed Musali root market samples

MATERIAL AND METHODS:

Chemicals and reagents:

All chemicals petroleum ether, n-hexane, diethyl ether, and, glacial acetic acid used were of analytical grade (Molychem chemicals, Mumbai, India). All other chemicals and reagents used were analytical grade unless otherwise indicated.

Collection and authentication of samples:

Dried roots of *A. racemosus* sand *Chlorophytum* species were collected from local market (Kadar Vora Gandhi) of Rajkot, Gujarat, India. (Fig. 1) The samples were authenticated by Dr. Kunjal Soni, Botanist, School of Science, RK University, Rajkot.

Preparation of extract:

10g roots of each species were powdered and macerated separately with 50ml of petroleum ether for 24h. This process was performed repetitively until the sufficient amount of pure extract was obtained. The collected samples were stored in air tight container and used for further study.

Thin Layer Chromatography:

After performing several pilot TLC, solvent system n-hexane: diethyl ether: glacial acetic acid (7:3:0.1) gave a clear spot in petroleum ether extract of root of *Chlorophytum* species extract but not in petroleum ether extract of root of *A. racemosus* at UV 254nm, indicating it to be the marker which distinguishes the two species.

Isolation of Spot by Preparative TLC:

Preparative TLC was performed with Silica gel G 60 F254 pre-coated plates (Merck®) using the above designed solvent system n-hexane: diethyl ether: glacial acetic acid (7:3:0.1). The band observed in petroleum ether extract of *Chlorophytum* species at UV 254nm was scrapped using scalpel and stored in air tight container.

Spectral Analysis:

The scrapped silica gel from preparative TLC was extracted with toluene and filtered. The filtrate was evaporated, and the residue was sent to National Facility for Drug Discovery, Department of Chemistry, Saurashtra University, Rajkot, to perform H^1 NMR & GC-MS analysis. Identification of compound on basis of GC-MS was done using the GC-MS database of National Institute Standard and Technology (NIST) library. This was followed by structure elucidation on the basis of spectral data obtained.

RESULTS AND DISCUSSION:

Thin Layer Chromatography:

A spot was observed in TLC of *Chlorophytum* species petroleum ether root extract at 0.82 Rf value but not in *A. racemosus* petroleum ether root extract, indicating it to be the marker which differentiates one species from the other (Fig. 2).

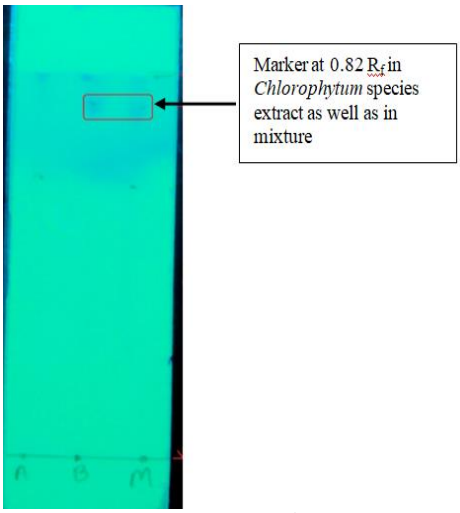

Figure 2: Thin layer chromatography of petroleum ether extract of root of Shatavari and Safed Musali at UV254nm
Where, sample A. Petroleum ether extract of root of *Asparagus racemosus*, B. Petroleum ether extract of root of *Chlorophytum* species, M. Mixture of both extracts

Spectral analysis:

Data from the H^1 NMR (Fig. 3) suggests that the isolated fraction from a marker compound is having frame work of aromatic (singlet peaks 1.2328, 1.2811) and 1' alkyl (singlet peak 3.3531) compound. Analysis of GC-MS (Fig. 4) indicates base peak of a compound having molecular weight 410, retention time 17.620 and area% 20.85. Identification of compound on basis of GC-MS was done using database of National Institute Standard and Technology (NIST) library. These data suggest that the isolated marker from petroleum ether extract of root of *Chlorophytum* species is 2, 6, 10, 14, 18, 22-tetracosahexaene ($C_{30}H_{50}$, *trans* form of Squalene, Fig. 5), which was not detected in petroleum ether extract root of *A. racemosus* using the same mobile phase *n*-hexane: diethyl ether: glacial acetic acid (7:3:0.1).

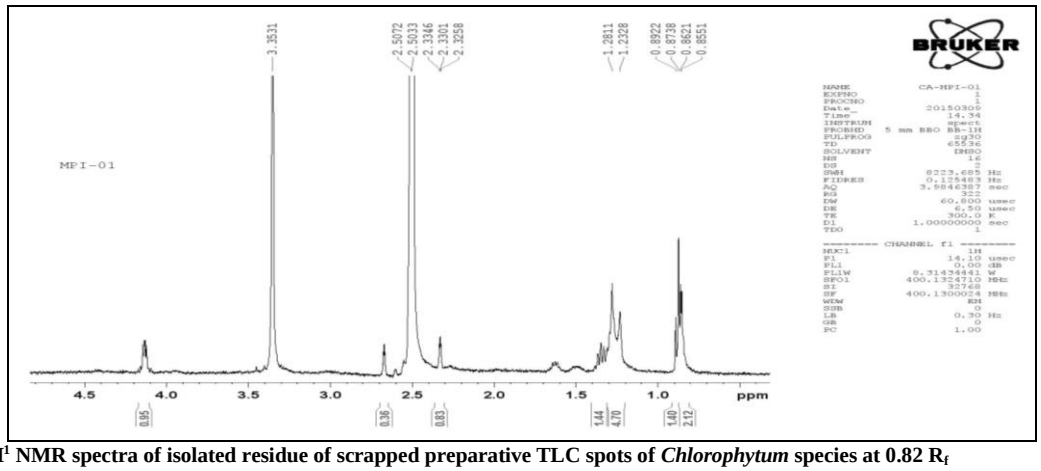

Figure 3: H^1 NMR spectra of isolated residue of scrapped preparative TLC spots of *Chlorophytum* species at 0.82 R_f

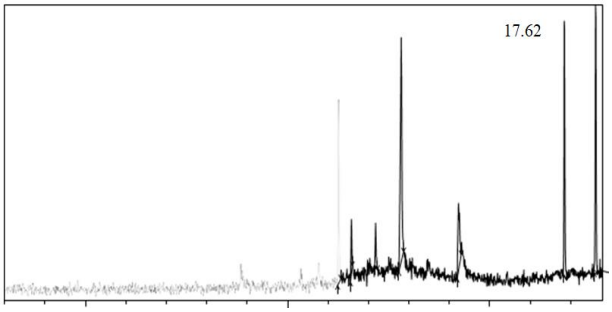

Figure 4: GC-MS of spectra of isolated residue of scrapped preparative TLC spots of *Chlorophytum* species at 0.82 R_f

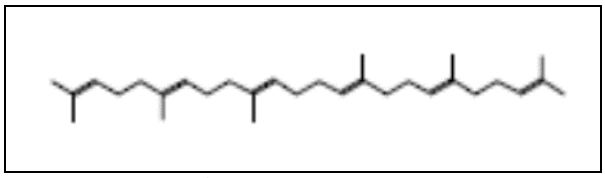

Figure 5: Structure of isolated compound- 2, 6, 10, 14, 18, 22 – Tetracosahexaene

A. racemosus root powder is often adulterated with the powder of root of *Chlorophytum* species. The reason for it might be related to potentiating the effect of *A. racemosus*, without keeping in mind the adverse effects it may have on various other age groups.

CONCLUSION:

The present work will be very useful to industries in detection of adulteration of *A. racemosus* formulations by *Chlorophytum* species, thereby helping in its prevention. The work will be helpful to ensure the safety of children, women and the elderly consuming *A. racemosus* products. The simple methods of marker isolation and its TLC method development can also provide crucial help in standardization of *A. racemosus* as well as *Chlorophytum* species formulations easily and cheaply, without any expensive techniques. It leaves scope for further phytochemical research on both species.

Such quality control is necessary to uplift the legacy of the Indian traditional system of medicine which is otherwise being questioned in the modern scientific world, mostly due to unstandardized, spurious, adulterated herbal products available in the market in the name of Ayurveda.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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