AkiNik

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (5): 89-94
Received: 05-12-2013
Accepted: 17-12-2013

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Simple and rapid methods to identify the botanical source of South Indian Shankhapushpi in medhya rasayana tablet - a novel ayurvedic compound nootropic drug

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Abstract

Introduction: Shankhapushpi is one of the highly regarded Medhya Rasayana (nootropic herb) widely used in Ayurveda since antiquity with multi fold benefits, specifically to improve memory and intellect by their Prabhava (specific action) namely Medhya (Nootropic). It is used as both mono therapy or in combination. Controversy prevails owing to different botanical source and geographical variation. Though pharmacognostical standardization of shankhapushpi is available, difference of opinion in its usage is still in existence. There is a need to develop standardization protocols to identify and validate the botanical source of shankhapushpi used in the preparation of poly herbal compound preparations.

Methods: Medhya Rasayana (MR) tablet, a poly herbal preparation containing shankhapushpi was compared to *Clitoria ternatea* and *Evolvulus alsinoides* – two controversial source of shankhapushpi of south India. For this purpose, methods employed are Microscopic characterization, HPTLC fingerprint profile and similarity index.

Results: Comparative microscopic characterisations along with HPTLC finger prints were developed. The finger print of *Evolvulus* species was found more super imposable with that of Medhya Rasayana than *Clitoria ternatea*.

Conclusions: The methods employed may be used as standard to validate shankhapushpi in poly herbal formulations.

Keywords: *Clitoria ternatea*, *Evolvulus alsinoides*, HPTLC, Medhya Rasayana, similarity index, trichomes, validation.

1. Introduction

Ayurveda, the Indian system of medicine is the first recorded medical science widely practiced in India since ancient times. The increasing acceptance of this holistic science owing to its effectiveness and safety in the global scenario has created great need to standardize herbal medicines. There are many herbs labelled as controversial either due to different botanical source or due to use of substitutes and sometimes adulterants too. Thus such herbal drugs need validation to meet up to the standards of the reliable quality control protocols.

Shankhapushpi is one such important nootropic herbs widely used in Ayurveda with multi-fold benefits, specifically to improve Medha (memory, intellect and other higher mental faculties) by its Prabhava (specific action) [1]. This drug finds its application as medicine right from the neonatal period to boost Medha till management of ailments like Unmada (Psychosis), Apasmara (Seizure disorders) and other neuro-psychiatric conditions. The herb is largely used either as a mono-therapy or in poly-herbal and herbo-mineral formulations. Few important formulations that involve Shankhapushpi as an ingredient are Brahmi ghrita [2-3] Astanga ghrita [4] Brahma rasayana, [5] Agastya haritaki rasayana, [6] Naladadi ghrita [7] Manasa mitra vati, [8] Apatantrakari vati, [9] Abhayamalaka rasayana, [10] Ayushman-8, [11] BR-16A (mentat), [12] Thyrocap, [13] MAK-4 and MAK-5 [14] and so on. The controversy prevailing in Shankhapushpi is mainly because of usage of different botanical sources by Ayurvedic physicians in various parts of India [15-16].

The contents of the compound formulation Medhya Rasayana include Mandukaparni (*Centella asiatica* Linn.), Yastimadhu (*Glycyrrhiza glabra* Linn.), Guduchi (*Tinospora cordifolia* (Wild) Miers.) and Shankhapushpi. Plants such as *Convolvulus microphyllus* Sieb. ex Spreng and *Evolvulus alsinoides* L. both from Convolvulaceae, *Clitoria ternatea* L. (*Papilionaceae*) and *Canscora diffusa* L. (Gentianaceae) are in use as Shankhapushpi in different parts of India. Among them, *C. microphyllus* is exclusively used in north India, *E. alsinoides* and *Clitoria ternatea* in south India, while *Canscora diffusa* in eastern India particularly in West Bengal [15, 17]. Few Authors have tried to resolve this controversy by developing pharmacognostical standards especially for *C. microphyllus* and *E. alsinoides* [18]. Comparative phytochemical evaluation of different sources of shankhapushpi is also documented [19]. Few concluded *C. microphyllus* to be the reliable source [20]. But validation of shankhapushpi in a compound formulation is not available as use of *E. alsinoides* and *C. ternatea* is still continued in south India. The present study aims at validation of shankhapushpi used in compound preparation Medhya Rasayana (MR) in tablet form with the help of few simple and rapid tests.

2. Methodology

2.1 Materials

Medhya rasayana (MR) tablet was obtained from SDM Ayurveda Pharmacy, Kuthpady, Udupi. Authentic sample of *E. alsinoides* was collected from M/s Dhanwantarivana, the Garden for Medicinal plants in Bangalore, Government of Karnataka undertaking. An authentic sample of *C. ternatea* was collected from medicinal plant garden at SDM Ayurveda Pharmacy, Kuthpady, Udupi.

2.2 Materials and Methods

Protocols used were based on the reference standards provided by WHO, [21] the Ayurvedic Pharmacopeia of India [22] and Indian Pharmacopeia [23]. TLC characterization of MR tablet was done as per standard procedures [24-25].

To identify and validate the botanical source of Shankhapushpi in MR the air dried samples of *C. ternatea* and *E. alsinoides* were finely powdered. The tablet of MR was washed in water 3 times collecting the debris using a centrifuge. The pinch of powder and the debris were stained using saffranine and slides prepared as per standard protocol [26]. The selected diagnostic characters were photographed under suitable magnification using camera (Zeiss AxioCam ERc 5s) attached to trinocular microscope (Zeiss Axio Lab.A1). For HPTLC fingerprint profile, 3 g each of powdered *C. ternatea*, MR and *E. alsinoides* were extracted with 10 ml ethanol 2 times by cold percolation for 48 hrs. Filtered and made up to 30 ml. 20 µl of the above extracts were applied on a precoated silica gel F_{254} on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in toluene – ethyl acetate (7:2.5) and the developed plates were visualized and scanned under UV 254, 366, 540 nm and after derivatisation in vanillin-sulphuric acid spray reagent at 620 nm. R_f, colour of the spots, densitometric scan and superimposability of densitogram were recorded.

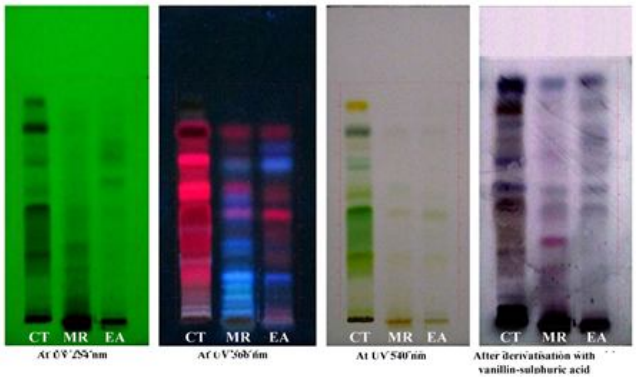
2.3 Similarity index

The R_f values obtained by TLC and HPTLC are considered as a diagnostic parameter to assess the similarities and differences in terms of percentage (%). Only the R_f values in between 0.05 and 0.95 are considered for the statistical analysis because there are chances of merging of different compounds due to polarity factor. In TLC, spots with same R_f as well as same or similar colours are only considered. R_f value different by 0.01 is also considered because the difference in concentration of same compound might alter the R_f value to a small extent. The percentage similarity was calculated by dividing the number of spots / peaks with same R_f (and colour in case of TLC) in each shankhapushpi sample by the total number of spots / peaks in MR.

3. Results

Powder microscopy of the MR in comparison to the powder of *C. ternatea* and *E. alsinoides* did yield a diagnostic information to identify the botanical source of shankhapushpi in MR. The presence of branched, smooth surfaced trichome was detected in *E. alsinoides* and MR inferring the botanical source of shankhapushpi in the MR sample is *E. alsinoides*. In contrast, presence of un-branched, warty trichomes in *C. ternatea* can be a diagnostic identification character to detect the botanical sources rapidly (Figure 1). The TLC photo documentation and comparative R_f values of the spots and their colours are summarized in Figure 2 and Table 1 to 4. Under UV 254 nm there were 5 spots in MR, a single spot was common to both shankhapushpi samples revealing a similarity index (SI) of 20%. Under UV 366 nm there were 14 spots in MR, out of them 5 spots were common with *E. alsinoides* showing a similarity of 35.71% and only 2 spots were common with *C. ternatea* revealing similarity of 14.29% only. Under white light, MR showed 5 spots, 3 and 2 being common with *E. alsionides* and *C. ternatea* respectively. Hence the SI of *E. alsinoides* was higher by 20% with respect to MR. After derivatisation the SI was 44.44 and 22.22 respectively for *E. alsionides* and *C. ternatea*.

Fig 1: Powder microscopy of Shankhapushpi and Medhya Rasayana



Track CT - *C. ternatea* 20ul, Track MR- Medhya Rasayana 20ul, Track EA- *E. alsinoides* 20ul
Solvent system- toluence: ethylacetate (7: 2.5)

By HPTLC densitometric scan, the SI was equal for the botanical sources of shankhapushpi at UV 254 nm. At 366 nm the SI turned to be 58.33 and 41.67% for *E. alsionides* and *C. ternatea* respectively. Densitometric scan at 540 nm pre-derivatisation revealed SI of 66.67 % in both the samples, while densitometric scan after derivatisation the SI was 87.50% for *E. alsinoides* but as low as 50.00% only in case of *C. ternatea*. The results suggest the test of MR is prepared using *E. alsinoides* as shankhapushpi not *C. Ternatea* (Table 1 and 2, Figure 3 and 4).

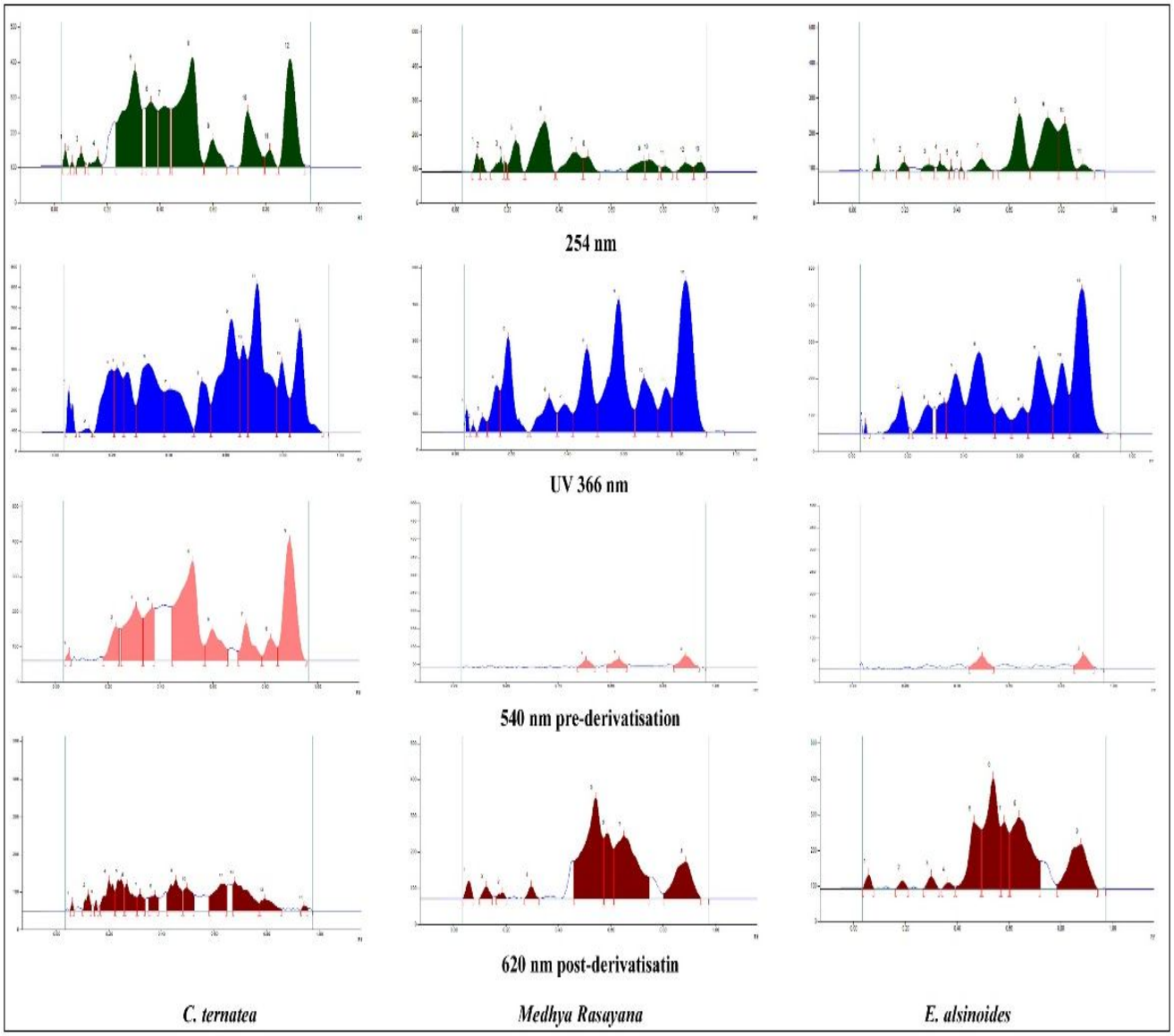


Fig 3: Densitograms of Shankhapushpi and medhya Rasayana

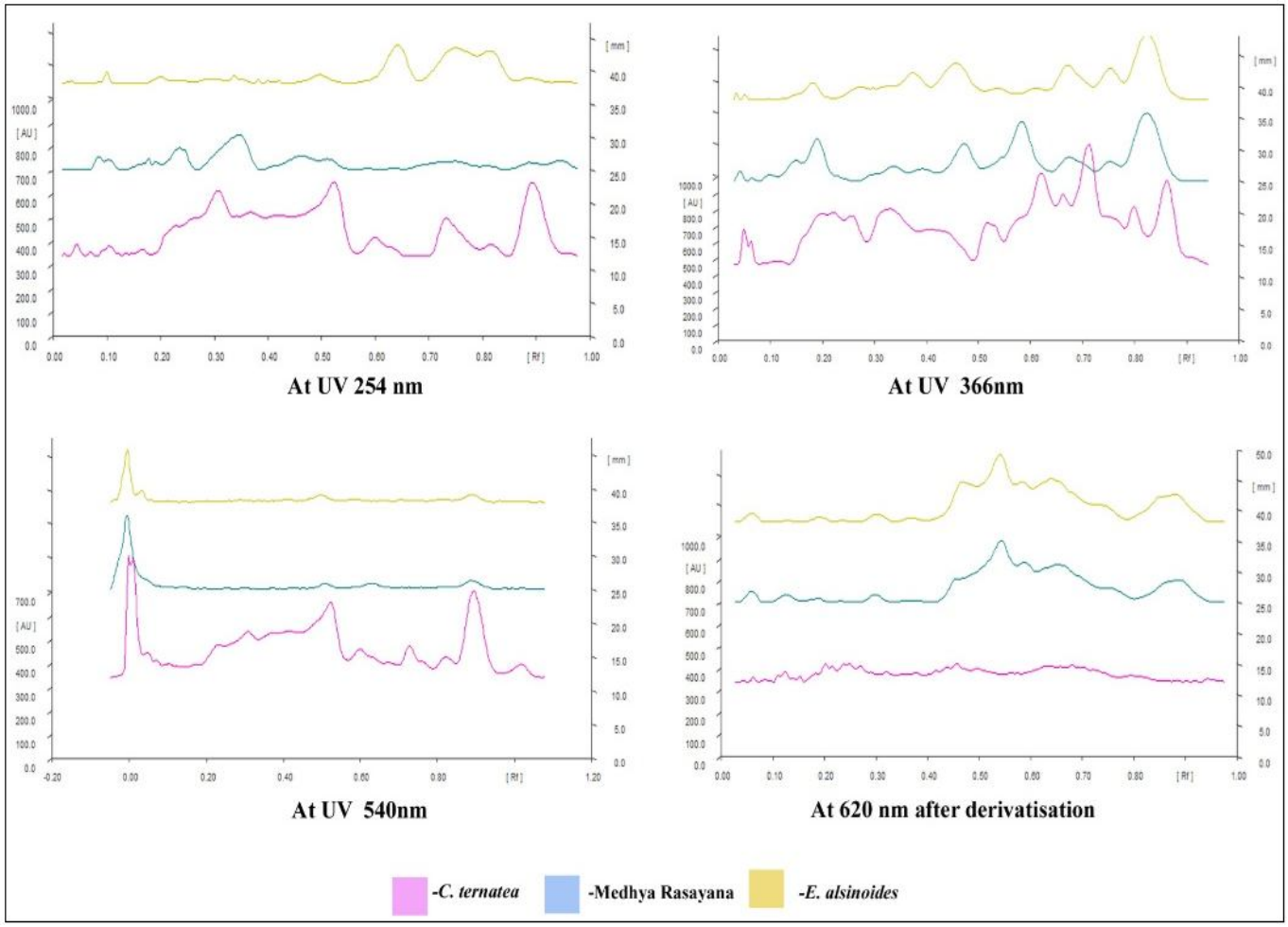


Fig 4: 3D Display of tracks of C. ternatea, Medhya Rasayana and E. alsinoides

Table 1: R_f values of Medhya Rasayana (MR) in comparison with *C. ternatea* and *E. alsinoides* by TLC photo-documentation

At 254 nm			At 366 nm			Under white light			After derivatisation		
C. ternatea	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***
--	--	0.02 Green	0.03 F. Yellow	0.03 F.Blue	0.03 F.Pink	0.03 Light green	0.03 Yellow	0.03Yellow	0.11 Purple	--	--
0.04 Green	--	--	0.05 F.Pink	--	--	0.27 Green	0.27 Yellow	0.27 Yellow	--	0.14 Purple	--
0.10 Green	0.09 Green	--	0.06 F.Yellow	0.06 F.Blue	--	0.45 D.Green	0.45 Green	0.45 Green	--	0.21 Pink	--
--	0.20 Green	--	0.07 F.Pink	--	--	0.52 Green	--	0.51 Green	0.27 Purple	--	--
0.26 Dark green	--	--	--	0.09 F.Blue	--	0.55 Yellow	0.55 Green	--	--	0.32 Pink	--
--	0.28 Green	--	--	0.13 F.Blue	--	0.63 Green	--	--	--	0.41 Light pink	--
--	0.42 Green	0.42 Green	0.19 F.Pink	0.18 F.Blue	0.18 F.Blue	0.66 Green	--	--	0.44 Brown	0.44 Brown	0.44 Brown
0.45 Dark green	--	--	--	--	0.26 F.Pink	0.72 Yellow	--	--	0.51 Violet	0.51 Brown	0.51 Brown
0.52 Green	--	--	--	0.28 F.Green	--	0.78 D.Green	0.78 Green	0.78 Green	--	--	0.58 Brown
--	--	0.55 Green	0.31 F.Red	0.31 F.Blue	--	0.88 Yellow	--	--	0.61 Violet	--	--
0.62 Green	--	--	--	0.37 F.Red	0.37 F.Red	--	--	--	--	0.64 Pink	--
--	--	0.64 Green	0.44 F.Red	0.44 F.Red	0.44 F.Red	--	--	--	0.69 L.Brown	--	0.69 Brown
--	--	0.71	--	0.50	--	--	--	--	0.75	--	--

		Green		F.Violet					Purple		
0.76 Green	0.76 Light green	--	0.53 F.Orange	--	0.53 F.Pink	--	--	--	0.81 Brown	0.81 Brown	0.81 Brown
0.87 Green	--	--	--	0.55 F.Pink	--	--	--	--	0.90 Brown	0.90 Purple	0.90 Purple
--	--	--	--	0.64 F.Blue	0.64 F.Blue	--	--	--	--	--	--
--	--	--	0.67 F.Orange	0.67 F.Pink	--	--	--	--	--	--	--
--	--	--	--	--	0.72 F.Purple	--	--	--	--	--	--
--	--	--	0.78 F.Green	0.78 F.Pink	0.78 F.Pink	--	--	--	--	--	--
--	--	--	0.86 F.Blue	--	--	--	--	--	--	--	--
--	--	--	0.92 F.Blue	--	--	--	--	--	--	--	--
20.00% ←% Similarity→ 20.00%			14.29% ←% Similarity→ 35.71%			40.00% ←% Similarity→ 60.00%			22.22% ←% Similarity→ 44.44%		

Table 2: R_f values of Medhya Rasayana (MR) in comparison with *C. ternatea* and *E. alsinoides* by HPTLC densitometric scan

At 254 nm			At 366 nm			At 540			After derivatisation at 620 nm		
C. ternatea	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***	***C. ternatea***	**MR**	***E. alsinoides***
0.04 (0.72%)	--	--	0.05 (1.76%)	0.04 (0.65%)	0.05 (0.27%)	0.05 (0.44%)	--	--	0.06 (0.68%)	0.06 (2.01%)	0.06 (1.49%)
0.07 (0.16%)	0.08 (3.56%)	--	--	0.07 (0.22%)	--	0.23 (5.05%)	--	--	0.12 (3.20%)	0.13 (1.75%)	--
0.10 (1.01%)	0.10 (2.57%)	0.10 (1.92%)	--	0.10 (1.00%)	--	0.37 (8.49%)	--	--	0.15 (0.88%)	--	--
0.17 (0.86%)	0.18 (4.15%)	--	0.12 (0.24%)	--	--	0.52 (30.57%)	0.51 (23.11%)	0.50 (51.95%)	0.20 (9.96%)	0.19 (0.81%)	0.19 (0.97%)
--	0.19 (1.27%)	0.20 (2.45%)		0.15 (4.15%)		0.60 (7.38%)	--	--	0.25 (8.68%)	--	--
--	0.23 (12.36%)	--	0.20 (6.59%)	0.19 (10.43%)	0.18 (4.77%)	--	0.63 (31.77%)	--	0.27 (8.12%)	--	--
0.31 (22.23%)	--	0.30 (2.51%)	0.22 (4.56%)	--	--	0.73 (6.48%)	--	--	--	0.30 (1.68%)	0.30 (1.73%)
--	0.35 (33.15%)	0.34 (2.38%)	0.26 (4.79%)	--	0.27 (4.13%)	0.82 (3.57%)	--	--	0.32 (3.95%)	--	--
0.37 (9.53%)	--	0.38 (0.41%)	0.33 (12.38%)	0.34 (5.24%)	0.33 (33.80%)	0.90 (23.78%)	0.89 (45.12%)	0.89 (48.05%)	--	--	0.36 (0.90%)
0.41 (8.58%)	--	0.42 (0.37%)	--	--	0.37 (9.89%)	--	--	--	--	0.47 (11.88%)	0.46 (17.50%)
--	0.46 (13.32%)	--	0.40 (7.70%)	0.39 (3.61%)	--	--	--	--	--	--	0.36 (0.09%)
0.52 (25.88%)	0.51 (5.94%)	0.50 (5.16%)	--	0.47 (11.88%)	0.46 (17.50%)	--	--	--	0.38 (5.08%)	--	--
0.60 (3.95%)	--	--	0.52 (4.94%)	--	--	--	--	--	0.46 (13.43%)	--	0.47 (15.00)
--	--	0.64 (25.18%)	--	--	0.54 (4.02%)	--	--	--	0.50 (8.06%)	--	--
0.73 (9.01%)	0.73 (5.52%)	--	--	0.58 (23.40%)	--	--	--	--	--	0.54 (37.37%)	0.54 (26.31%)
--	--	0.75 (35.45%)	0.62 (16.63%)	--	0.61 (3.99%)	--	--	--	0.64 (13.57%)	0.65 (3.36%)	0.64 (27.63%)
0.82 (1.67%)	0.81 (1.89%)	0.82 (21.26)	0.66 (5.19%)	0.67 (9.02%)	0.67 (14.25%)	--	--	--	0.79 (5.83%)	--	--
0.89 (16.39%)	0.89 (4.96%)	0.88 (2.92%)	0.71 (19.93%)	--	--	--	--	--	0.68 (17.49%)	--	--
--	0.95 (5.65%)	--	--	0.75 (4.89%)	0.75 (10.04%)	--	--	--	0.79 (5.83%)	--	--
--	--	--	0.80 (5.42%)	--	--	--	--	--	--	0.89 (14.57%)	0.88 (16.77%)
--	--	--	--	0.82 (22.50%)	0.82 (27.63%)	--	--	--	0.94 (1.08%)	--	--
--	--	--	0.86 (9.96%)	--	--	--	--	--			
50%	←% Similarity→	50.00%	41.67% ←% Similarity→ 58.33%			66.67% ←% Similarity→ 66.67%			50.00% ←% Similarity→ 87.50%		

4. Discussion

Despite the pharmacognostical and phytochemical characterization of Shankhapushpi, the controversy prevails in botanical identification and usage of drug source based on the regional variation. Even with continued boost up on efficacy of this drug, its validation in compound formulations using standard protocols is not available. Considering this fact, validation of Shankhapushpi in MR was proposed. Few diagnostic characters for rapid identification of two species of Shankhapushpi namely *Clitoria ternatea* and *Evolvulus alsinoides* in Medhya Rasayana tablet are validated in the present study. On evaluation, it was found that the finger print of *Evolvulus* species was more superimposable with that of Medhya Rasayana than *Clitorea ternatea*. Thus it can be concluded that the Shankhapushpi used in MR was confirmed as *Evolvulus alsinoides* by the proposed methodology. The microscopic features, R_f values, and the densitogram obtained can be used as standard to identify and validate Shankhapushpi in poly herbal combinations.

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