ORIGINAL ARTICLE

Comparative pharmacognostical investigation on four ethanobotanicals traditionally used as Shankhpushpi in India

Neeraj K. Sethiya, Ashish Trivedi, Mayur B. Patel, S. H. Mishra

Department of Pharmacy, Herbal Drug Technology Laboratory, The Maharaja Sayajirao University of Baroda, GH Patel Pharmacy Building, Donor's Plaza, Fatehgunj, Vadodara, Gujarat, India

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ABSTRACT

People in Indian region often apply Shankhpushpi and other Sanskrit-based common name to *Evolvulus alsinoides*, *Convolvulus pluricaulis*, *Canscora decussata*, and *Clitorea ternatea*. These are pre-European names that are applied to a medicinal plant. Before the establishment of British rule, like the other books, ayurvedic treatises were also hand written. This might be one of the reasons due to which ayurveda could not stand parallel to the western medicine and an ambiguity is reflected in the interpretation of names and description of drugs found in the books like *Charaka Samhita* and *Sushruta Samhita*. The most widespread application of Shankhpushpi is for mental problems, but they have been considered for an array of other human maladies. The present investigation deals with the comparative pharmacognostical evaluation of four ethanobotanicals of Shankhpushpi. A comparative morphoanatomy of the root, stem, and leaves has been studied with the aim to aid pharmacognostic and taxonomic species identification. Various physicochemical, morphological, histological parameters, comparative high-performance thin-layer chromatography (HPTLC), and comparative high-performance liquid chromatography (HPLC), chromatogram of methanolic extract presented in this communication may serve the purpose of standard parameters to establish the authenticity of commercialized varieties and can possibly help to differentiate the drug from the other species. All the parameters were studied according to the WHO and pharmacopoeial guidelines.

Key words: Ethanobotanicals, HPLC, HPTLC, physiochemical, Shankhpushpi

INTRODUCTION

Shankhpushpi is considered as "medhya rasayana" in ayurvedic texts. Shankhpushpi is a word of Sanskrit which means "the plant with flowers shaped like a conch." The conch or *Shankha* is one of Lord Shiva's sacred instruments often used in ritual worship. Shankhpushpi of Ayurvedic Pharmacopoeia of India consists of the whole plant of *Convolvulus pluricaulis* (CP) Choisy (Convulvulaceae) syn; *Convolvulus microphyllus* Sieb. ex Spreng. Plants other than C. pluricaulis like *Evolvulus alsinoids* (EA) Linn. (Convulvulaceae), *Clitorea ternatea* (CT) Linn. (Papilionaceae) and *Canscora decussata* (CD) Schult. (Gentianaceae) were also used as Shankhpushpi by some practitioners.[1-5] Indian Council of Medical Research has given quality standards for *C. pluricaulis* drug in its publication. Although these plants proved their scientific potential in CNS depression, anxiolytic, tranquillizing, antidepressant, antistress, neurodegenerative, antiamensic, antioxidant, hypolipidemic, immunomodulatory, analgesic, antifungal, antibacterial, antidiabetic, antiulcer, anticatatonic, and cardiovascular activity. These are reported to contain several types of alkaloids, flavanoids, and coumarins as active chemicals that bring about its biological effects.[6-11] Botanical identification data and phytochemical characterization of a medicinal plant provides authentic

Address for correspondence

Neeraj K. Sethiya, Department of Pharmacy, Herbal Drug Technology Laboratory, The Maharaja Sayajirao University of Baroda, GH Patel Pharmacy Building, Donor's Plaza, Fatehgunj, Vadodara - 390 002, Gujarat, India. E-mail: nscognosy2006@gmail.com

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means to use these as drug or raw material for medicinally important formulation. Sethiya *et al*. compiled the various pharmacognostical characters from various database in a review, although the previous reported work lacks modern methods of characterization and there is no lead for strict comparison between botanical of Shankhpushpi.[7] The present study is based on preliminary pharmacognostic, microscopical, and phytochemical investigation with reference to high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) on Shankhpushpi.

MATERIALS AND METHODS

Plant Material

CD was collected from the outskirts of Raipur from December to February, 2009 (Chattisgarh, India) and identified by Dr. S.C. Agrawal (Department of Botany, CDRI, Lucknow, India). While CP, EA, and CT were collected in the month of January to March, 2009, from Bhapel village near Sagar, India, and identified in the Department of Botany, Dr. Hari Singh, Gour Vishwavidyalaya, Sagar. Voucher specimens of all four plants (No. Pharmacy/EA/09-10/10/NS, Pharmacy/CP/09-10/11/NS, Pharmacy/CT/09-10/12/NS, and Pharmacy/CD/09-10/13/NS) have been deposited in Herbal Drug Technology Department, The M. S. University of Baroda, Gujarat, India.

Reagents and Chemicals

All solvents and chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany). Two polyherbal marketed formulation containing Shankhpushpi as ingredient viz., Brain tab and Shankhpushpi syrup was purchased from Baidhyanath Pharmaceuticals. Precoated silica gel $60F_{254}$ TLC plates were purchased from Merck (Darmstadt, Germany).

Morphological and Microscopical Investigation

The macroscopic features of the fresh plant of EA, CP, CD, and CT were determined using the methods of Evans.[12] Anatomical sections, surface preparations of the fresh leaves, stems, roots, and powdered samples for the microscopy were carried out according to the methods reported earlier.[13-16]

Proximate analysis, elemental analysis, and quantitative microscopy

Whole plants of all four above mentioned plants were dried in shade and powdered plant materials were used for analysis of moisture contents, ash values, and extractives values.

For elemental analysis, 5 g of all the four powdered drug material were ignited in muffle furnace to obtain total ash; 100 mg of ash then dissolved in 10 ml of 1 N HCl, solutions were filtered and diluted to 50 ml with distilled water. These solutions were further used for the determination of sodium, potassium, zinc, copper, manganese, iron and magnesium by absorption spectroscopy.[14]

The quantitative microscopy on the anatomical section and the epidermal layers of the fresh leaf of the plant to determine the palisade ratio, stomatal index, vein islet, and vein termination number were carried out as described in WHO guideline for medicinal plant material.[15-17]

Preparation of extracts and phytochemical investigations

For phytochemical screening, the powdered drug of all mentioned plants were subjected to successive solvent extraction, with petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and water. After complete extraction, all the extracts were evaporated under reduced pressure, and the percentage yield, color, and consistency were determined.[18-22]

Thin layer chromatographic studies of extracts

Thin layer chromatographic (TLC) studies were performed using various solvent systems, and finally chloroform: methanol: toluene (7:2:1) was found to be suitable mobile phase for the proper separation of phytoconstituents. Anisaldehyde–sulfuric acid was used as the spraying agent.[23-24]

HPTLC studies

HPTLC equipment [24-26]

A CAMAG TLC system equipped with CAMAG Linomat V, an automatic TLC sample spotter, CAMAG glass twin trough chamber (20×10 cm), CAMAG scanner 3, and integrated winCATS 4 Software were used for the analysis. TLC was performed on 20×10 cm precoated plate. Samples and standards were applied on the plate as 8 mm wide bands with an automatic TLC sampler (Linomat V) under a flow of nitrogen gas, 10 mm from the bottom and 10 mm from the side, and the space between two spots were 15 mm of the plate. The linear ascending development was carried out in a CAMAG twin trough chamber (20×10 cm) which was presaturated with 20 mL mobile phase for 20 min at room temperature (25±2°C and 40% relative humidity). The length of the chromatogram run was 8 cm. Subsequent to chromatographic development, TLC plates were dried in current air with the help of a dryer.

Sample preparation

Accurately weighed 2 g of methanolic extract of EA, CP, CD, and CT were dissolved in 20 ml of methanol and refluxing for 30 min on water bath at 60–70°C. The extract was cooled, filtered, and finally the volume was made up to 20 mL with methanol.[24] For polyherbal marketed formulation, 5 g of Brain tab was extracted with methanol and 5 g of dried concentrated syrup was taken in 50 ml of methanol for extraction.

Sample application

The sample was applied on TLC plate in the form of band using an automatic sample application device (Linomat V, CAMAG) with band width of 9 mm. The quantity of sample applied was 10 µL.

Development

The plate was developed by placing in presaturated chamber up to the height of 8 cm. It was developed in the optimized mobile phase, chloroform: methanol: toluene (7:2:1). The plate was dried using air dryer. Then it was derivatized with anisaldehyde in sulfuric acid, followed by heating at 110°C for 5 min and scanned at 580 nm.

HPLC fingerprinting[27]

HPLC was done using Shimadzu Prominence UFLC (Pump: LC-20 AD; Detector: SPD-20 AV; Column: Phenomenex 5 µ, C-18, 4.6 X 250 mm) and mobile phase optimized was methanol:water: acetonitrile (40:45:15) with a flow rate 1 mL/min (detection; λ_{max} – 254 nm).

RESULTS

Morphological and Microscopical Investigation

The detailed systematic pharmacognostical and phytochemical evaluation of plant and plant material provides means of standardization of a herb that can be used as drug or as raw material.[28-29] The major morphological identification parameters observed among plants were similar as reported earlier by Sethiya *et al*.[7] The morphological difference among Shankhpushpi claimants is shown in Figure 1. Various microscopical differentiation features are summarized in Table 1. On comparison with the observations made on EA, CP, CD, and CT usually available in commerce as Shankhpushpi, it becomes evident that there is a great similarity in habit, habitat, and in the macro and microscopical features of their stem, leaves, and root [Figure 2]. They are small herbs

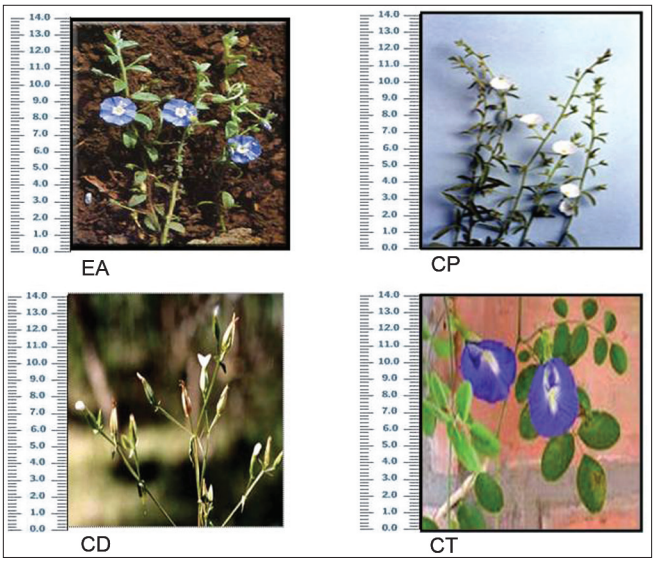

Figure 1: Comparative morphological character of various ethanobotanical claims of Shankhpushpi

Table 1: Comparative microscopical character of various ethanobotanicals claims of Shankhpushpi

Diagnostic features	EA	CP	CD	CT
TS of Stem				
Outline	Wings absent	Wings absent	Four wings	Wings absent
Cuticle	Ridged	Striated	Ridged	Ridged
Trichome	Present	Present	Absent	Present
Chlorenchyma	Present	Present	Absent	Present
Endodermis	Indistinct	Indistinct	Distinct	Indistinct
Pericyclic fibers	Present	Present	Absent	Present
Phloem	Present	Present	Absent	Present
Pith	Hollow	Hollow	Coarsely pitted	Pitted
TS of root				
Calcium oxalate	Present	Present	Absent	Present
Trichome	Present	Present	Absent	Present
TS of leaf				
Calcium oxalate	Present	Present	Absent	Present
Lamina	Isobilateral	Isobilateral	Dorsiventral	Dorsiventral
Trichome	Present	Present	Absent	Present
Stomata	Anisocytic and paracytic type	Anisocytic and paracytic type	Anisocytic	Subcoriaceous
Powder microscopy				
Xylem fiber	Present	Present	Absent	Present
Phloem fiber	Absent	Present	Absent	Present
Pith	Hollow	Hollow	Coarsely pitted	Pitted

EA - *Evolvulus alsinoides*; CP - *Convolvulus pluricaulis*; CD - *Canscora decussata*; CT - *Clitorea ternatea*

with several branches bearing sessile and shortly petioled leaves. Although there are certain salient diagnostic characters by which these plants can be differentiated from one another.

Proximate analysis, elemental analysis, and quantitative microscopy

Various differentiation parameters for the analysis of moisture content, ash values, and extractives values are shown in Table 2. Quantitative analysis of various elements present in samples of Shankhpushpi is shown in Table 3. Results of the quantitative microscopy viz., palisade ratio, stomatal index, vein islet, and vein termination number are shown in Table 4.

Phytochemical investigations (physiochemical values)

The results of percentage yield, color, odor, and consistency of various extract obtained by successive solvent extraction is shown in Table 5.

Table 2: Comparative proximate analytical parameters

Determinations	Average value (% w/w)			
	EA	CP	CD	CT
Moisture content	5.234±0.039	7.38±0.034	6.34±0.077	3.404±0.089
Total ash	10.212±0.19	18.77±0.26	12.44±0.23	8.732±0.058
Acid insoluble ash	2.49±0.09	4.28±0.089	5.31±0.056	3.842±0.065
Sulfated ash	4.32±0.05	6.24±0.071	3.18±0.033	4.83±0.050
Water soluble ash	4.02±0.048	8.52±0.05	7.88±0.033	3.493±0.177
Water insoluble ash	6.49±0.12	11.40±0.64	11.14±0.084	4.866±0.039

EA - *Evolvulus alsinoides*; CP - *Convolvulus pluricaulis*; CD - *Canscora decussata*; CT - *Clitorea ternatea*; *All values are mean±SEM (n=3)

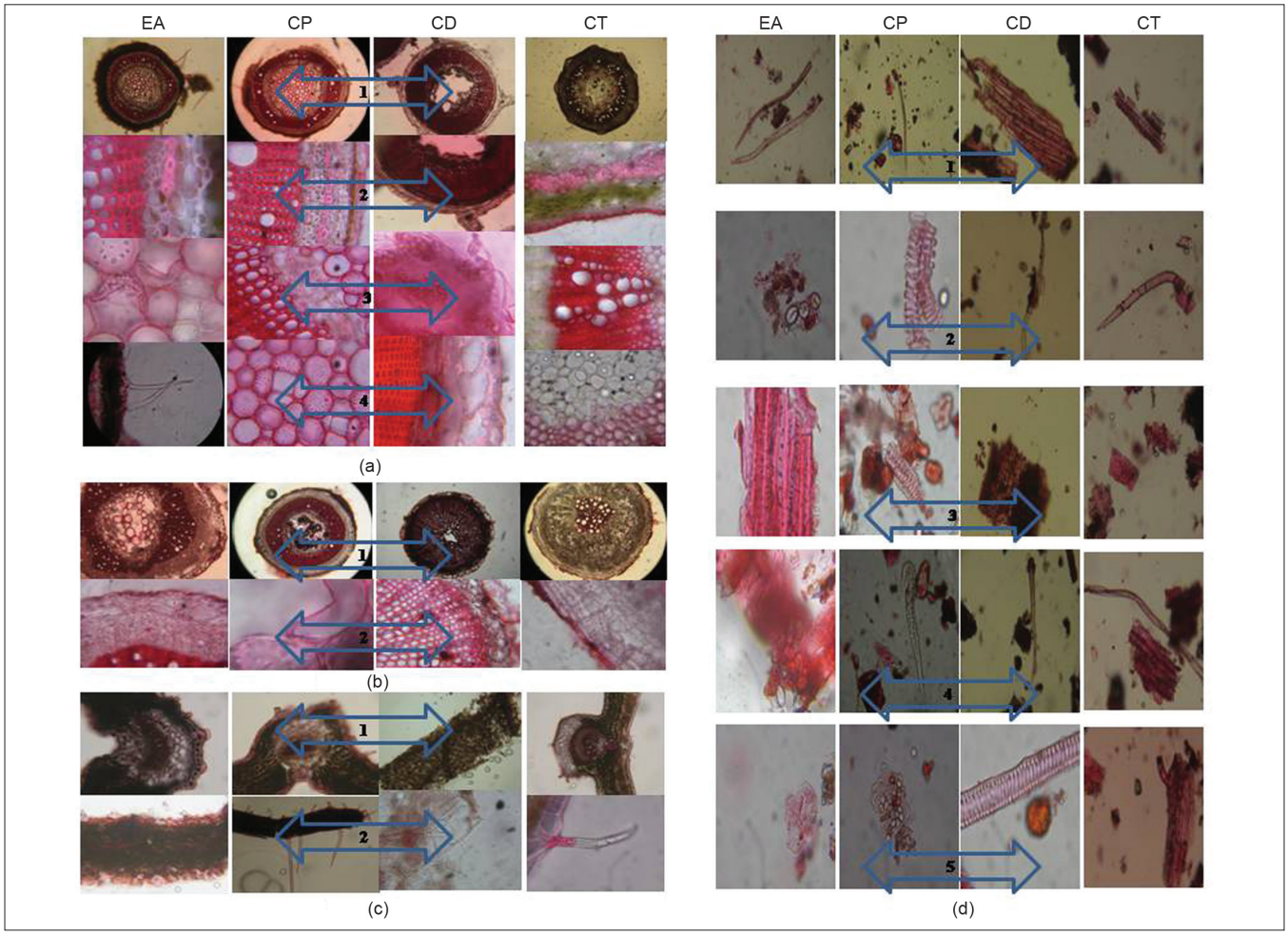

Figure 2: (a) Transverse section (TS) of stems [1, whole section; 2, chlorenchyma, endodermis, cuticle; 3, phloem, pith; 4, trichome], (b) TS of roots [1, whole section; 2, calcium oxalate, trichome], (c) TS of leaves [1, whole section; 2, lamina, trichome, stomata], (d) Powder microscopy of whole plants [1, xylem fiber; 2, phloem fiber; trichome; 3, stomata, pith; 4, starch grains, trichome; 5, pericyclic fiber]

HPTLC studies

Different proportions of hexane, toluene, chloroform, ethyl acetate, methanol, and water were tried; among these chloroform:methanol:toluene (7:2:1) was found to be most suitable solvent combination for separation and differentiation of various constituents among above mentioned claims of whole plant powder methanolic extracts. Detection was carried out by scanning plates at 254 and 366 nm and then at 580 nm post derivatized with anisaldehayde–sulfuric acid reagent. The results of HPTLC were shown in Figure 3.

HPLC fingerprinting

Various HPLC fingerprints of all available Shankhpushpi

Table 3: Comparative quantitative elemental analysis

Elements	EA	CP	CD	CT
Sodium	0.50±0.006 g/kg	0.60±0.006 g/kg	0.79±0.002 g/kg	–
Potassium	16.64±0.08 g/kg	9.99±0.11 g/kg	15.35±0.008 g/kg	14.92±0.26 g/kg
Zinc	89.84±0.19 ppm	64.35±0.58 ppm	78.24±0.30 ppm	117.69±2.029
Copper	35.45±0.60 ppm	18.59±0.33 ppm	34.52±0.67 ppm	9.02±0.11
Manganese	114.00±0.28 ppm	77.59±0.49 ppm	122.96±0.93 ppm	41.52±0.44
Iron	6523.07±96.71 ppm	2640.32±24.46 ppm	5404.07±17.96 ppm	1006.74±7.14 ppm
Magnesium	526.31±0.61 ppm	531.57±0.99 ppm	524.96±3.80 ppm	512.28±3.51 ppm

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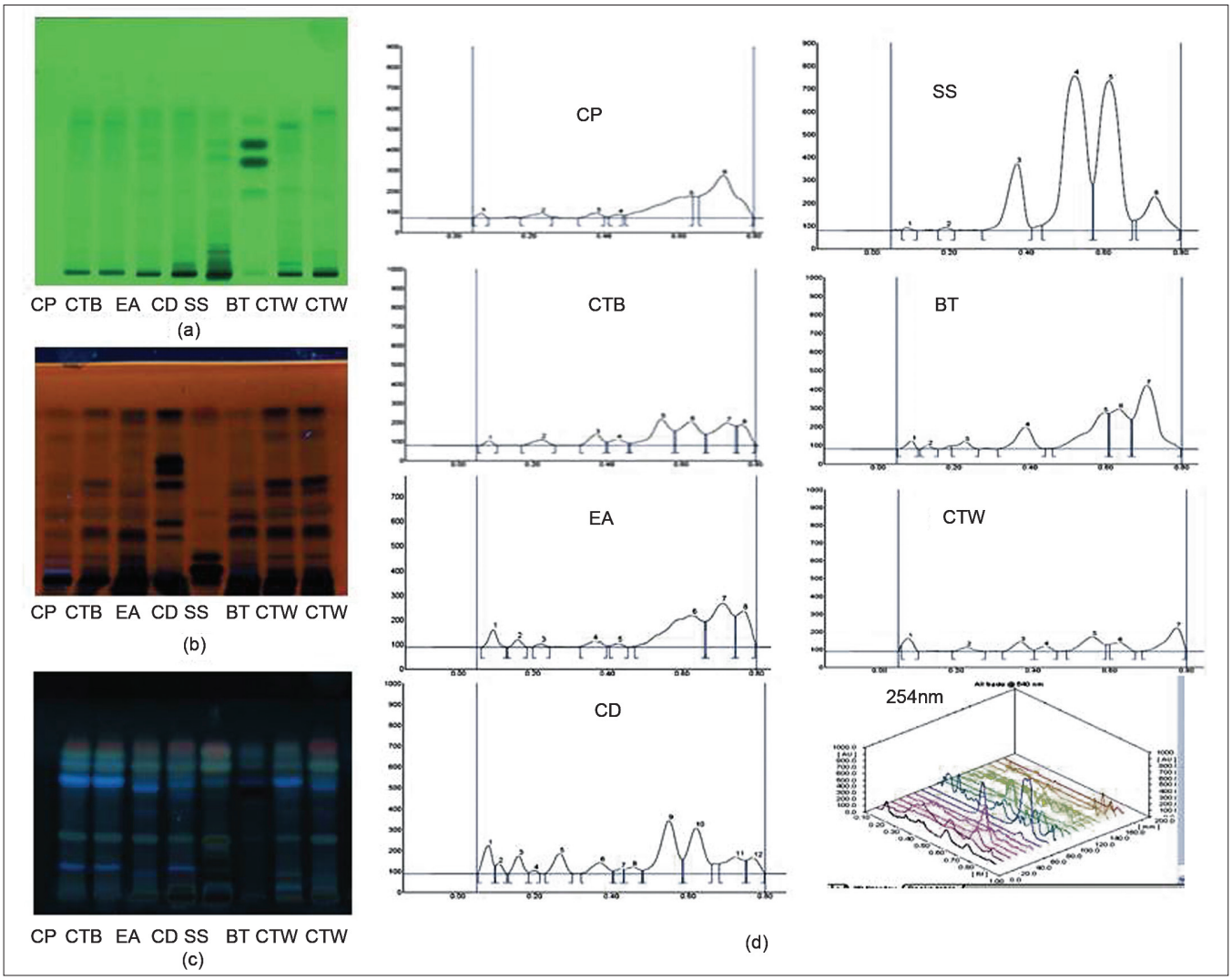

Figure 3: (a) HPTLC photographs at 254 nm, (b) HPTLC photographs after derivatization with AS (580 nm), (c) HPTLC photographs after derivatization with AS (366 nm), (d) HPTLC densitogram at 580 nm (for individual sample) and 254 nm (3-dimension view); (CTW - Clitorea ternatea white flower, CTB - Clitorea ternatea blue flower)

samples along with two marketed formulation shown in Figure 4. The fingerprint of EA showed similar profile with Shankhpushpi syrup, and the fingerprint of CD also matches in major peaks with Brain tab.

DISCUSSION

In order to assure the efficacy of Ayurveda, a critical study is essential for exploring its full strength.[7] Evaluation of plant materials and their derived products has always been an important part of the professional expertise of workers in the field of discovery of phytopharmaceuticals. A big quantum of research work in the area of authentication of correct plant source has been undertaken to provide means of differentiation among many controversial available plants sources.[28-29] In the work, we explored the parameter of differentiation such as pharmacognostical and phytochemical for ayurvedic medicine Shankhpushpi (a brain tonic). Morphologically all the four plants are distinct in their appearance and can be easily identified. But raw material is sold either by common name or in the form of powder or extract, which further necessitates the identity problem. Based on microscopical characters, one can identify CD from other varieties.[10] There are still very little characters explored for EA, CP, and CT. Present work solves the vicinity of standardization, even if the drug is supplied in the form of extract. The maximum content of iron in EA and CD makes their use as the drug of choice for iron-deficient diseases. Formulation containing CD although proves its potential in problems related with

Table 4: Comparative quantitative microscopical parameters

Parameters	EA	CP	CD	CT
Stomatal number				
Upper	280-328-405	202-216-238	291-342-411	Very few
Lower	270-336-424	184-212-248	188-223-251	52-72-108
Stomatal index				
Upper	14.5-15.5-16.5	17.0-18.0-19.9	16.9-18.0-19.1	Very few
Lower	15.7-17.0-18.7	13.8-15.8-16.9	14.8-16.3-17.2	16.9-21.0-24.6
Vein-islets number	18.0-19.0-20.0	21.0-23.0-25.0	7.5-8.0-9.0	1-2,5-3,25

EA - *Evolvulus alsinoides*; CP - *Convolvulus pluricaulis*; CD - *Canscora decussata*; CT - *Clitorea ternatea*; *All values are mean±SEM (n=3)

Table 5: Comparative physiochemical parameters

Botanicals	Consistency	Color	Odor	Taste	Extractive values (% w/w)
EA					
	Petroleum ether extract Semisolid	Dark-green	Characteristic	Bitter	1.78±0.001
	Chloroform extract Solid	Dark-green	Characteristic	Bitter	1.72±0.0006
	Ethyl acetate extract Solid	Brownish	Characteristic	Bitter	1.98±0.003
	Methanolic extract Semisolid	Greenish black	Characteristic	Bitter	4.88±0.03
	Aqueous extract Semisolid	Dark-brown	Characteristic	Sweet	9.33±0.07
CP					
	Petroleum ether extract Semisolid	Brown-green	Characteristic	Bitter	1.79±0.002
	Chloroform extract Semisolid	Dark-green	Characteristic	Bitter	0.72±0.004
	Ethyl acetate extract Semisolid	Dark-green	Characteristic	Bitter	1.98±0.02
	Methanolic extract Semisolid	Greenish dark	Characteristic	Bitter	5.07±0.026
	Aqueous extract Solid	Dark-brown	Characteristic	Pungent	4.25±0.067
CD					
	Petroleum ether extract Solid	Dark-brown	Characteristic	Bitter	2.25±0.092
	Chloroform extract Solid	Greenish	Characteristic	Bitter	4.24±0.058
	Ethyl acetate extract Semisolid	Brownish	Characteristic	Bitter	1.68±0.042
	Methanolic extract Semisolid	Greenish black	Characteristic	Bitter	7.61±0.0061
	Aqueous Extract Solid	Brownish red	Characteristic	Sweet	10.83±0.33
CT					
	Petroleum ether extract Semisolid	Dark-green	Characteristic	Bitter	1.23±0.057
	Chloroform extract Semisolid	Dark brown	Characteristic	Bitter	0.68±0.015
	Ethyl acetate extract Semisolid	Dark brown	Characteristic	Bitter	1.93±0.04
	Methanolic extract Semisolid	Brownish dark	Characteristic	Bitter	3.42±0.06
	Aqueous extract Solid	Dark brown	Characteristic	Sweet	5.28±0.02

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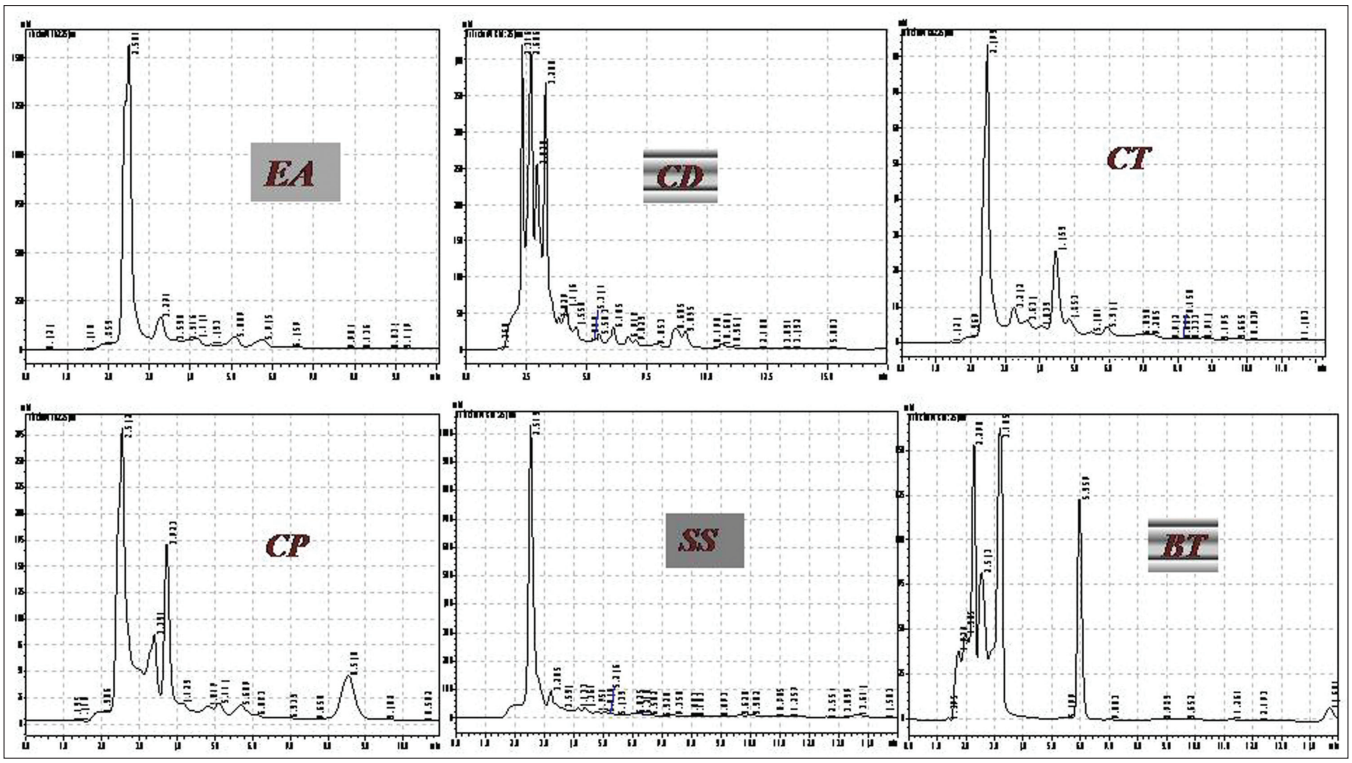

Figure 4: HPLC chromatogram of Shankhpushpi sample and marketed formulation

postmenopausal disorders.[30] TLC fingerprinting pattern of all is distinct and may also serve to solve the purpose of identity with reference to standard chromatogram. HPLC chromatogram also serves the purpose of differentiation among all.

CONCLUSION

Shankhpushpi is a well-known and extensively used plant in Ayurveda with therapeutic potential as memory enhancer. Ample use of same synonym for various botanicals raises the controversy regarding its identification. The present work provides a means of differentiation, as well as evaluation of herbal preparation consisting any of these plants. This work ultimately enriches the knowledge and may also contribute in near future to fix the limits for identification of Shankhpushpi in official compendium. There is still need to evaluate each plants for their comparative chemical markers based identification and their comparative biological potency.

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