Comparative pharmacognostical investigation on four ethanobotanicals traditionally used as Shankhpushpi in 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 alsinoides* (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, tranquilizing, antidepressant, antistress, neurodegenerative, antiemetic, 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...
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. 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 Bhopal 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, Gujrat, 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 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. 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.

**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.

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.

**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.

**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.

**HPTLC studies**

HPTLC equipment 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. 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**

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. 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.

| Table 1: Comparative microscopical character of various ethanobotanicals claims of Shankhpushpi |
| --- |
| **Diagnostic features** |
| **TS of Stem** |
| Outline | Wings absent | Wings absent | Four wings | Wings absent |
| Cuticle | Rridged | Striated | Rridged | Rridged |
| 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 | Coarsely pitted | 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 | Absent | Present | Present |
| Pith | Hollow | Coarsely pitted | Pitted | Pitted |

EA - *Evolvulus alsinoides*; CP - *Convolvulus pluricaulis*; CD - *Canscora decussata*; CT - *Clitoria 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          | EA            | CP            | CD            | CT            |
|-------------------------|---------------|---------------|---------------|---------------|
| Moisture content        | 5.23±0.039    | 7.38±0.034    | 6.34±0.077    | 3.40±0.089    |
| Total ash               | 10.21±0.19    | 18.77±0.26    | 12.44±0.23    | 8.73±0.058    |
| Acid insoluble ash      | 2.49±0.09     | 4.28±0.089    | 5.31±0.056    | 3.84±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    | 3.49±0.177    |
| Water insoluble ash     | 6.49±0.12     | 11.40±0.64    | 11.14±0.084   | 4.86±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 anisaldehyde–sulfuric acid reagent. The results of HPTLC were shown in Figure 3.

**HPLC fingerprinting**
Various HPLC fingerprints of all available Shankhpushpi

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**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 |

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

**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 | Solid      | 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 | Solid    | 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                 |

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