Shankhpushpi has been reputed as '*Medhya Rasayana*' in ayurveda- meaning a drug that potentiates intellect, learning and memory. The aim of present study is to differentiate various species (botanicals) of *Shankhpushpi* used in traditional medicine on the basis of scientifically evidenced parameters.

Four different herbs in India are known by the name of *Shankhpushpi* viz., *Evolvulus alsinoids* (Convulvulaceae), *Convolvulus pluricaulis (Convululvulaceae), Clitoria ternatea* (Leguminoseae) and *Canscora decussata* (Gentianaceae). Phytochemical investigations were performed on the various extracts of four herbs and the systemic procedure for the same is as follows:

2.1 PLANT MATERIALS

CD was collected from the waterfall of Ninai ghat, Didiya Pada district (Gujarat, India) and identified by Dr. S.C. Agrawal (Department of Botany, CDRI, Lucknow). While CP, EA and CT were collected 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 Lab, Department of Pharmacy, The M. S. University of Baroda, India.

2.2 REAGENTS AND CHEMICALS

DPPH, curcumin, β-carotene, gallic acid and rutin were obtained from Himedia Laboratories Pvt. Ltd., Mumbai. Ascorbic acid, mangiferin, chlorogenic acid, β-sitosterol, querecetin, stigmasterol, urosolic acid, Lupeol, betullinic acid, betaine and scopoletin were procured from Loba Chemie (Mumbai), Sigma Aldrich (USA), Sisco Research Laboratories (Mumbai) and Lailla Imprex Laboratory (Vijayawada) respectively. Other solvents and chemicals were of analytical grade and procured from Sd. Fine (Mumbai). Brain Tab and *Shankhpushpi* syrup was purchased from Baidhyanath Pharmaceuticals. Pre-coated silica gel $60F_{254}$ TLC plates were purchased from Merck (Darmstadt, Germany).

2.3 PHARMACOGNOSTICAL SCREENING

The macroscopical characters, microscopy and physico-chemical parameters such as extractive values, ash values, loss on drying were performed as per the procedure given in WHO guidelines and Indian herbal Pharmacopoeia (Padashetty & Mishra, 2008).

Embedding

These materials were transferred in a glass vials. Then paraffin wax was gradually added till it has been completely filled. Then these vials were placed in an oven to get molten mass of paraffin. During this, paraffin diffuses gradually to all tissues and remaining of TBA was evaporated. Then paraffin mass of these vials were poured in petri dish and cooled. Then, these were attached to wooden blocks and stored separately.

Microtome

Transverse sections were cut with a rotary microtome. Blocks were adjusted in such a position that faces of blocks were parallel with the blade; the carriage was moved forward until the blade rarely touches the paraffin at its lower edge. Then continuously the block was brought towards the knife by turning the wheel. The micron scale was set at 12–15 μm thickness for sectioning.

Mounting

These sections were placed on various slides containing Haupt's adhesive. Xylene was used to remove paraffin before staining. The series of dilutions used for removal of paraffin's are:

1.100 % xylene

2.Equal parts of xylene and absolute ethyl alcohol (1:1).

3.Equal parts of absolute alcohol and ether (1:1).

4.95 % ethanol.

Staining

All staining reagents were prepared in 70 % absolute alcohol. Sections were first stained with concentrated safranin solution and allowed to stand for 5 min. The excessive staining was removed by passing these sections through a series of ethanol solution (35%, 50%, 75%, and 95%). The stained sections were photographed with Mips Olympus camera.

2.3.3.2 Ash values

Total ash

About 4.0 gm of the air-dried powdered materials of various *Shankhpushpi* botanicals were accurately and separately weighed, in a tarred silica crucible. These materials were spreaded in an even layer and ignited by gradually increasing the heat to 500-600°C until it became white, which indicate the absence of carbon. These were then allowed to cool in desiccator and weighed. Content of total ash were calculated in mg/gm with reference to air-dried materials.

Acid insoluble ash

25 ml of HCL (2 N) were separately poured in various crucible containing total ash of various Shankhpushpi botanicals and covered with separate watch glasses. These were gently boiled for 5 minutes. Each watch-glass was rinsed with 5 ml of hot distilled water and these liquids were added to their respective crucible. The insoluble matters on an ash less filter-papers were washed with hot water until the filtrates was neutral. Each filter-paper containing the insoluble matters were transferred to their respective crucible. Each was dried in hotplate, till it ignites to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weigh. The content of acid-insoluble ash in mg/gm were calculated with reference to air-dried material.

Water soluble ash

In crucible containing the total ashes of various *Shankhpushpi* botanicals, 25 ml of water was added and boiled gently for 5 minutes. Insoluble matter was collected on ash less filter paper and washed with hot water until the filtrate became neutral. Transferred the filter-paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite in a crucible for 15 min. at a temperature not exceeding 450°C to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weigh. Weight of the residue was subtracted from weight of total ash. The content of acid-insoluble ash in mg/g were calculated with reference to air-dried material.

2.3.3.3 Extractive values

Alcohol soluble extractive value

About 4 gm of coarsely powdered air-dried materials of *Shankhpushpi* botanicals were weighed, in glass-stopper conical flasks. Macerated these with 100 ml of the methanol for 6 hours, with frequent shaking. These were allowed to stand for 18 hours and rapidly filtered with care of any solvent losses. These filtrates were transferred to 25 ml of tarred flat-bottomed dishes and evaporated to dryness on a water-bath (105°C for 6 hours). The solidified masses obtained by cooling 30 minutes in a desiccator were weighed and percentage yield were calculated in terms of content of extractable matter in alcohol in mg/gm with reference to air-dried materials (Sethiya et al., 2010c).

Water soluble extractive value

About 4 gm of the coarsely powdered air-dried materials of *Shankhpushpi* botanicals were macerated with 100 ml of chloroform: water (1: 99) in a closed flask for 24 hours with frequent shaking initials for 6 hours and allowed to stand for 18 hours. These solutions were filtered rapidly; 25 ml of the filtrate of these were evaporated to dryness in tarred flat bottomed dishes, and dried at 105 ^{o}C. The solidified masses were weighed and percentage yield were calculated in terms of content of water-soluble extractive with reference to the air-dried drug (Sethiya et al., 2010c).

2.3.3.4 Estimation of inorganic elements including heavy metals.

Elemental content of the powdered materials of various *Shankhpushpi* botanicals were estimated by atomic absorption spectrophotometer (Sethiya et al., 2010c).

Heavy Metal Analysis

About 5 gm of all the four powdered drug material were ignited in muffle furnace to obtain total ash. 100 mg of ashes were then dissolved in 10 ml of 1 N HCl and then these solutions were filtered and diluted to 50 ml with distilled water. These solutions were further used for quantitative determinations of heavy metals by absorption spectroscopy. (WHO, 1998)

Extraction with Water

Lastly, the marc was subjected to hot water maceration. The maceration was continued for a period of 24 hrs. The aqueous extract was filtered and concentrated. Various scheme of making different extracts were summarized in figure 2.1.

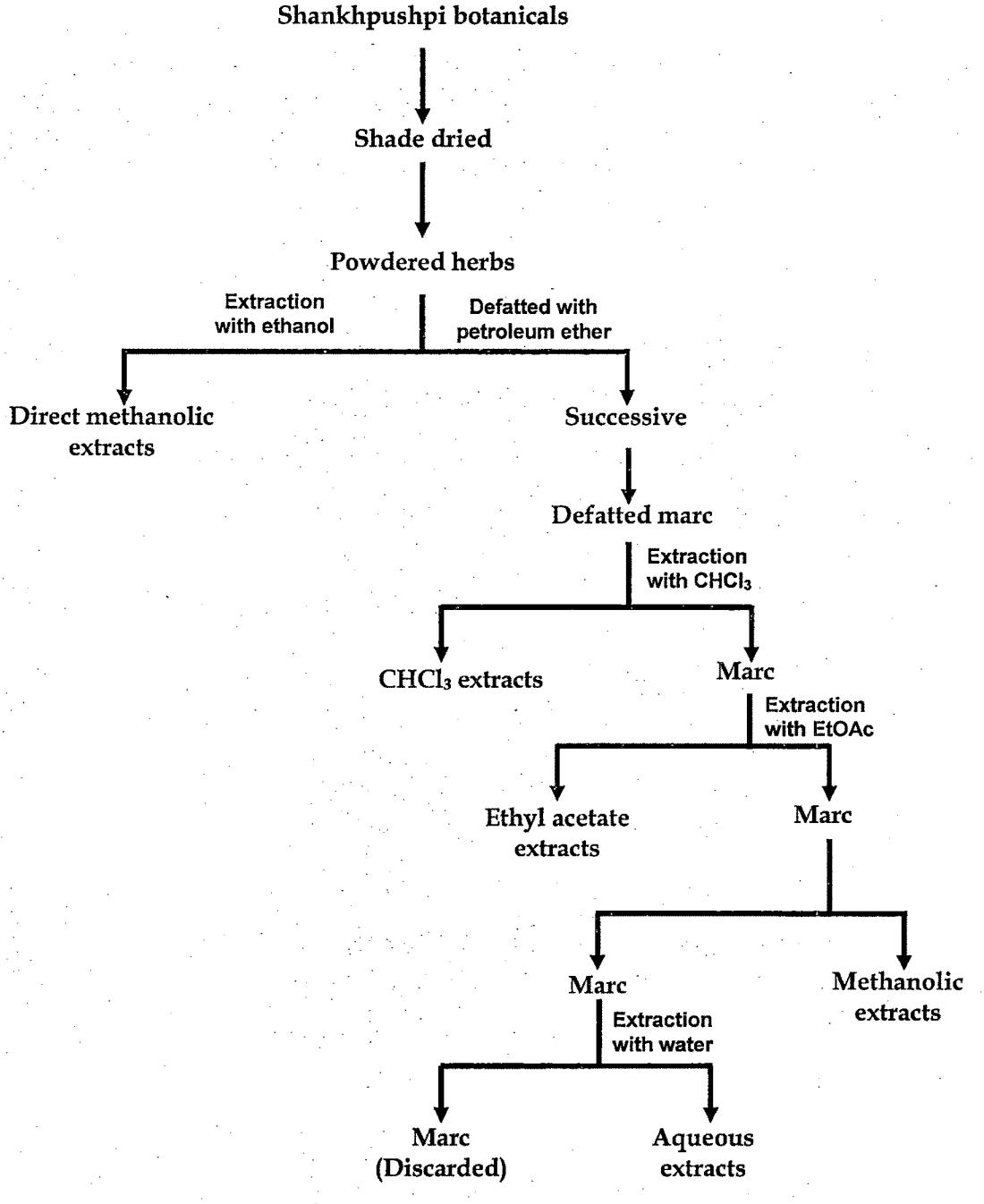


Figure 2.1 Schematic representation of extractions procedure for shankhpushpi botanicals

2.4.1.2 Direct Methanolic Extraction

Defatting of Plant Material

Shade dried plant materials of various *Shankhpushpi* botanicals were coarsely powdered and subjected to extraction with petroleum ether in soxhlet apparatus. The extractions were continued till the defatting of the materials had taken place.

Extraction with Methanol

Defatted marcs of these drugs were subjected to methanol extraction in soxhlet apparatus. The extraction was continued for a period of 6-7 days to achieve complete extraction. These extracts were concentrated and dried to a constant weight.

2.5 PHYSICAL EXAMINATION OF THE EXTRACTS

Dried extracts of the various *Shankhpushpi* botanicals were evaluated for physical parameters such as consistency, color, odor and taste.

2.6 CHARACTERIZATION OF EXTRACTS BY QUALITATIVE CHEMICAL TESTS

Methanolic extracts of various *Shankhpushpi* botanicals were subjected to following chemical tests:

2.6.1 Test for Carbohydrates

Test solutions were prepared by dissolving test extracts in water, hydrolysed with 2N HCl and subjected to following tests:

- **Fehling's Test (for reducing sugars)**

Each test solutions were treated with few drops of Fehling's reagent [34.66 gm of Copper sulphate dissolved in distilled water and was made upto 500 ml (solution A); 173 gm of potassium sodium tartarate, 50 gm of sodium hydroxide dissolved in distilled water and was made upto 500 ml (solution B)]. This gives brick red color on warming shows presence of reducing sugars (Wagner & Baldt, 1996).

- **Molisch's Test**

Each test solutions were treated with few drops of Molisch's reagent (10 g α-naphthol in 100 ml of 95% ethanol). Then 2 ml of conc. sulphuric acid was added slowly from sides of the each test tube. Positive tests show purple ring at the junction of two layers (Wagner & Baldt, 1996).

➢ **Wagner's Test**

Each test solutions were treated with Wagner's reagent (iodine-potassium iodide solution). Positive test gives reddish brown precipitate (Wagner & Baldt, 1996).

2.7.4 Test for Steroids

Methanolic extracts of various *Shankhpushpi* botanicals were dissolved in chloroform and subjected to following tests:-

➢ **Libermann Burchard Test**

To each test solutions few drops of acetic anhydride were added and conc. sulphuric acid added from sides of test tube, shaken and allowed to stand. Lower layer turns bluish green indicating the presence of sterols.

➢ **Salkowski Test**

To each test solutions few drops of conc. sulphuric acid were added, shaken and allowed to stand. Lower layer turns red indicating the presence of sterols.

2.7.5 Test for Glycosides

Test solutions were prepared by dissolving methanolic extracts of various *Shankhpushpi* botanicals in 95% ethanol or aqueous alcoholic solution.

➢ **Keller Killiani Test (for digitoxose)**

Each test solutions were treated with few drops of Ferric chloride solution and mixed, and then sulphuric acid containing crystals of ferric chloride were added, it forms two layers. Lower layer shows reddish brown color while upper layer turns bluish green indicates the presence of glycosides.

➢ **Baljet Test**

Each test solutions were treated with sodium picrate. Formation of yellow to orange colour indicates presence of glycosides.

➢ **Legal Test**

Each test solutions were treated with a drop of 2% sodium nitroprusside and a drop of sodium hydroxide cause production of a deep red color.

2.6.6 Test for Saponins

Test solutions were prepared by dissolving methanolic extracts of various *Shankhpushpi* botanicals in water.

➢ **Foam Test**

Positive test indicates by formation of foam on shaking, which was stable for at least 15-20 minutes.

➢ **Hemolysis Test**

1ml of blood was diluted with 10 ml of sodium citrate (36.5g/l) and separately 10 mg of methanolic extracts of various *Shankhpushpi* botanicals were dissolved in phosphate buffer pH 7.4 and made up to 100 ml. Drop of blood was taken in a slide and observed under microscope (40 x) for the presence of intact RBC, then 1-2 drops of test solutions was put on the blood drop and observed under microscope for hemolysis (Mukherjee, 2002).

2.6.7 Test for Tannins

Methanolic extracts of various *Shankhpushpi* botanicals were dissolved in 95% ethanol.

➢ **Gelatin- Lead Acetate Test**

Each test solutions treated with lead acetate solution. Formation of white precipitate, on addition of 1 % solution of gelatin containing 10% sodium chloride shows presence of tannins.

2.6.8 Test for Phenolics

Methanolic extracts of various *Shankhpushpi* botanicals were dissolved in 95% ethanol.

➢ **Ferric chloride Test**

These test solutions treated with few drops of ferric chloride solution gives dark color.

2.6.9 Test for Pentose Sugar

Test solutions in a test tube with an equal volume of hydrochloric acid containing a little phloroglucinol were heated. Formation of a red color indicates pentoses.

2.6.10 Test for Flavonoids

Methanolic extracts of various *Shankhpushpi* botanicals were dissolved in 95% ethanol.

➢ **Shinoda Test**

Test solutions taken in test tubes. Few magnesium ribbons are dipped and conc. HCl is added over them. Magenta color develops indicating presence of flavonoids.

2.6.11 Test for Triterpenoids

Test solutions were prepared by dissolving methanolic extracts of various *Shankhpushpi* botanicals in chloroform.

➢ **Libermann Burchard Test**

To these test solutions few drops of acetic anhydride were added and mixed. Then few drops of conc. sulphuric acid were added from sides of test tube. Red color is produced in the lower layer indicates the presence of triterpenes.

➢ **Salkowski Test**

Test solutions were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Lower layer turns golden yellow indicating the presence of triterpenoids.

2.7 ESTIMATION OF SECONDARY METABOLITES

2.7.1 Determination of Total Phenolic content

Total Phenolic content was determined as per the reported method described by Singleton and Rossi (1965). This method was performed for total methanolic extracts, ethanolic extracts and water extracts of various Shankhpushpi botanicals.

Preparation of reagents

✓ Folin ciocaltu reagent

1: 2 dilution of the readymade reagent with distilled water was prepared.

✓ 20 % sodium carbonate solution

20 gm of anhydrous sodium carbonate was dissolved in 100 ml of distilled water.

2.7.2.1 Aluminum chloride colorimetric method:

The principle of aluminium chloride colorimetric method is that aluminium chloride forms stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonol. In addition, aluminium chloride forms acid labile complexes with ortho-dihydroxyl groups in the A or B - ring of flavanoids. Though the apigenin, kaempferol and quercetin can be used for the calibration curve, quercetin was used for this purpose in the experiment because of its maximum absorbance after reaction with aluminium chloride in the selected concentration range.

Preparation of reagents

✓ Preparation of standard solution

A stock solution, 1 mg/1 ml of quercetin was prepared in methanol.

✓ 10 % aluminium chloride

10 gm of aluminium chloride was dissolved in 100 ml of distilled water.

✓ 1 M Potassium acetate

9.814 gm of potassium acetate were dissolved in 100 ml distilled water.

✓ Preparation of standard solution

Quercetin was used to make the stock solution (100 µg/mL) for the calibration curve.

✓ Preparation of test samples

Stock solutions of samples were prepared by dissolving 10 mg of extracts of various Shankhpushpi botanicals in 10 ml of methanol.

Preparation of calibration curve

- For the stock solution of standard 0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8 and 1 ml were taken in 25 ml of volumetric flasks which gave 10, 20, 30, 40, 60, 70, 80 and 100 µg/mL concentrations respectively.
- The standard solution were separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate and 208 ml of distilled water.

- Benzene: Acetone (36:13)
- Ethyl acetate: Toluene: Glacial acetic acid (5:3:1)
- Hexane: Ethyl acetate (4:1)
- Ethyl acetate: Acetic acid: Formic acid: Water (100: 11: 11: 26)
- Pet Ether: Ethyl acetate: Toluene (7:2:1)
- Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)

The number of spots and resolution pattern of methanolic extracts of various Shankhpushpi botanicals against these solvent in TLC plates were depicted in table 2.2.

Table 2.2 TLC Profile of methanolic extracts of various *Shankhpushpi* botanicals

Solvent System	Number of Spots	Resolution
EA		
Chloroform: Methanol: Toluene (7:2:1)	14	Very Good
Pet Ether: Ethyl acetate: Toluene (7:2:1)	12	Very Good
Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)	9	Good
Chloroform: Methanol (9:1)	8	Good
Butanol: Glacial acetic acid: Water (4:1:2)	8	Good
Ethyl acetate: Toluene: Glacial acetic acid (5:3:1)	7	Poor
CP		
Chloroform: Methanol: Toluene (7:2:1)	12	Very Good
Pet Ether: Ethyl acetate: Toluene (7:2:1)	10	Very Good
Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)	7	Good
Chloroform: Methanol (9:1)	6	Poor
Butanol: Glacial acetic acid: Water (4:1:2)	5	Poor
Ethyl acetate: Toluene: Glacial acetic acid (5:3:1)	6	Poor

CT		
Chloroform: Methanol: Toluene (7:2:1)	8	Very Good
Pet Ether: Ethyl acetate: Toluene (7:2:1)	9	Very Good
Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)	7	Good
Chloroform: Methanol (9:1)	6	Poor
Butanol: Glacial acetic acid: Water (4:1:2)	5	Poor
Ethyl acetate: Toluene: Glacial acetic acid (5:3:1)	6	Poor
CD		
Chloroform: Methanol: Toluene (7:2:1)	10	Very Good
Pet Ether: Ethyl acetate: Toluene (7:2:1)	14	Very Good
Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)	7	Good
Chloroform: Methanol (9:1)	6	Poor
Butanol: Glacial acetic acid: Water (4:1:2)	6	Poor
Ethyl acetate: Toluene: Glacial acetic acid (5:3:1)	8	Good

2.8.2 Comparative Co-TLC Studies of Different Plants Known by the Name *Shankhpushpi* with Known Markers.

Following solvent systems were found for the best separation of petroleum ether and methanolic extracts of various *Shankhpushpi* botanicals for comparison with known identified chemical markers:

- Hexane: Ethyl acetate (4:1)
- MeOH: Acetone: HCl (90:10:4 v/v/v)
- Pet Ether: Ethyl acetate: Toluene (7:2:1)
- Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5)

2.8.3 HPTLC Fingerprinting with Marker

2.8.3.1 HPTLC equipment

A Camag TLC system equipped with Camag Linomat V an automatic TLC sample applicator, Camag glass twin trough chamber (20X10 cm), Camag scanner 3 and integrated winCATS 4 Software were used for the analysis. TLC was performed on 20X10 cm pre-coated 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 (20X10 cm) which was pre-saturated 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 TLC plate dryer.

2.8.3.2 Preparation of Sample

All herbs of *Shankhpushpi* botanicals were shade dried at room temperature and coarsely powdered. Accurately weighed 5 g of dried coarse powder of CP, EA, CT and CD (whole herb) were extracted separately with methanol (3X50 mL) under reflux (30 min each time) on a water bath. The combined extracts were filtered, concentrated on a rotary evaporator and transferred to a 50 mL volumetric flask and the volume was made up 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. All chemical standard markers, extracts and marketed formulation were prepared in the form of stock solution (10mg/10 ml).

2.8.3.3 Chromatographic Studies

Identified markers and methanolic extracts of shankhpushpi botanicals were separated by using solvent system given in Table 2.3. The various fingerprints with various samples of shankhpushpi and its marketed formulation were given in Figure 2.4.

2.8.4 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). The mobile phase optimized for the fingerprinting of methanolic extracts of Shankhpushpi botanicals were Methanol: water: Acetonitrile (40: 45: 15) with a flow rate: 1 mL/min (Sethiya et al., 2010c).

Adsorption of extract

These extracts were mixed with the adsorbent i.e. silica gel and left for half an hour so that the adsorption of extracts on silica gel takes place completely.

Packing of column

Slurry packing technique was used. Silica gel was suspended in chloroform and added to the column in portions. The stopcock was left open to allow the solvent to flow through the solid material and solvent was added to prevent the column from going dry. When the bed reached the desired height, the stopcock was closed and a layer of mobile phase left at the top of the bed (Gennaro, 2000).

Application of Sample

The prepared sample was then added slowly through a funnel onto the bed of the adsorbent without disturbing the packing of the column.

Then the optimized mobile phase/ gradient basis were added slowly from the walls of the column till it reached a height of 75 cm.

The outlet of the column was then adjusted such that the eluent was continuously released as well as sufficient solvent head was maintained on top of the column.

Collection of eluates

The effluent from the column was collected in fractions as a function of volume i.e. 25 – 100 ml in each fraction taken in volumetric flasks. TLC was performed for each fraction. Fractions showing similar pattern were mixed.

2.9 ISOLATION OF COMPOUNDS

2.9.1 Isolation and characterization of phytochemical marker from EA

2.9.1.1 EA-1

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-9-14, shows a prominent single band of steroids with anisaldehyde Sulfuric acid. These were further eluted with same solvents in column using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml each. Elute no-6-12 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.2.2 Isolation of Scopoletin from CP

Identification of scopoletin in CP was done by Sethiya et al. 2009 on TLC. This leads to perform isolation by the previous reported method as in EA. The methanol extract of CP was subjected to column chromatography using silica gel (80-120 mesh) as adsorbent and chloroform: methanol: toluene (8: 1: 1) as eluent. The fractions (50 ml each) number 12-20 give prominent single spot. This was further purified by preparative chromatography and recrystalized with acetone. A crystalline material was isolated. It gave blue fluorescence in UV and was characterized by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis.

2.9.3 Isolation and characterization of phytochemical marker from CT

2.9.3.1 CT-1

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-9-14, shows a prominent band of steroids with Anisaldehyde Sulfuric acid. These were further eluted with same solvents in column using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml each. Elute no-6-12 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.3.2 CT-2

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-16-20, shows a prominent band of steroids with Anisaldehyde Sulfuric acid. These were further eluted with same solvents in column using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml each. Elute no-9-12 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.3.3 CT-3

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-20-27, shows a prominent band of steroids with Anisaldehyde Sulfuric acid. These were further eluted with same solvents in column

using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml each. Elute no-9-18 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.3.4 Isolation of Rutin from C. ternatea

Plant material was shade dried and ground into a coarse powder. About 1 kg of powdered drug was extracted three times with methanol (5 L, each 48 h) at room temperature. Solvent was removed under reduced pressure at 40 °C, the methanol extract (79.31 gm) was suspended in 1 L of water and partitioned with 1 L of petroleum ether, 1 L of ethyl acetate (EtOAc), 1 L of n-butanol (BuOH) to yield the petroleum ether (12.56 g), EtOAc (10.14 g), BuOH (11.67 g) and water (18.32 g) fractions, respectively. The EtOAc fraction was subjected to preparative thin layer chromatography using n-butanol: acetic acid: water (4:1:2) as the solvent system. A crystalline material melting at 185-190°C was isolated. It gave yellow color with NP/PEG reagent and a light yellow fluorescence in UV at 366 nm. It was characterized as further by mixed melting point (Superfit Melting Point Apparatus), UV absorption maxima and superimposable Fourier Transform Infra Red (FTIR) spectral analysis.

2.9.4 Isolation and characterization of phytochemical marker from CD

2.9.4.1 CD-1

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-9-14, shows a prominent band of steroids with Anisaldehyde Sulfuric acid. These were further eluted with same solvents in column using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml each. Elute no-6-12 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.4.2 CD-2

Unsaponified fraction of Pet ether extract was eluted with hexane: ethyl acetate (4:1), with column using silica 60-120 as a adsorbent. About 30 elute were collected of 25 ml each. Elute no-18-27, shows a prominent band of steroids with Anisaldehyde Sulfuric acid. These were further eluted with same solvents in column using silica 100-200 mesh as adsorbent. Again about 15 elute were collected of 25 ml

each. Elute no-9-18 crystallized. These were mixed and again recrystalized to get needle shaped crystal. This compound was further investigated with HPTLC and IR.

2.9.4.3 Isolation of Mangiferin from *C. decussata*

Extraction and isolation was done according to the method followed in our previous studies. Plant material was shade dried and ground into a coarse powder. 120 gm powdered material was first defatted with petroleum ether (500 ml). The marc was extracted with methanol to obtain the methanolic extract (yield 4.47% w/w). For isolation of this compound, preparative thin layer chromatography was performed using n-butanol: acetic acid: water (4:1:2) as the solvent system. A crystalline material was isolated. It gave apricot green yellow color with 1% ferric chloride reagent and a light yellow fluorescence in UV at 366 nm. It was characterized by mixed melting point (Superfit Melting Point Apparatus), UV absorption maxima and superimposable Fourier Transform Infra Red (FTIR) spectral analysis.

2.9.12 Melting Point Analysis

The melting point analysis of isolated compounds lead us to know the fingerprint match of exact melting point of known standard. This can be performed in melting point apparatus (Superfit Melting Point Apparatus) with known standard that matches in TLC.

2.9.13 Fourier Transform-Infra Red (Ft-Ir) Spectroscopy of the Isolated Compounds

The FT-IR analyses of the isolated compound were crossed checked with FTIR spectrum of predicted compounds standard. These spectrum if found to be matching with predicted spectrum compound then this was superimposed to the sample spectra to get overlay spectrum for exact fingerprinting.

2.10 RESULTS AND DISCUSSION

2.10.1 PHARMACOGNOSTICAL SCREENING

2.10.1.1 Morphological and Microscopical investigation

The major morphological identification parameters observed among plants were similar as reported earlier by Sethiya et al., 2009. The morphological difference among Shankhpushpi botanicals were shown in Figure 2.2. The various microscopical differentiation features were summarized in Table 2.4 and Figure 2.3. 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. They are small herbs with several branches bearing sessile and shortly petioled leaves. Although there are certain salient diagnostic characters (marked by underline) by which these plants can be differentiated from one another (Table 2.4).

Table 2.4 Comparative microscopical character of various ethanobotanicals claims of Shankhpushpi.

Diagnostic Features	EA	CP	CD	CT
		T.S. 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 fibres	Present	Present	Absent	Present
Phloem	Present	Present	Absent	Present
Pith	Hollow	Hollow	Coarsely pitted	Pitted
		T. S. of Root		
Calcium oxalate	Present	Present	Absent	Present
Trichome	Present	Present	Absent	Present
		T.S. 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

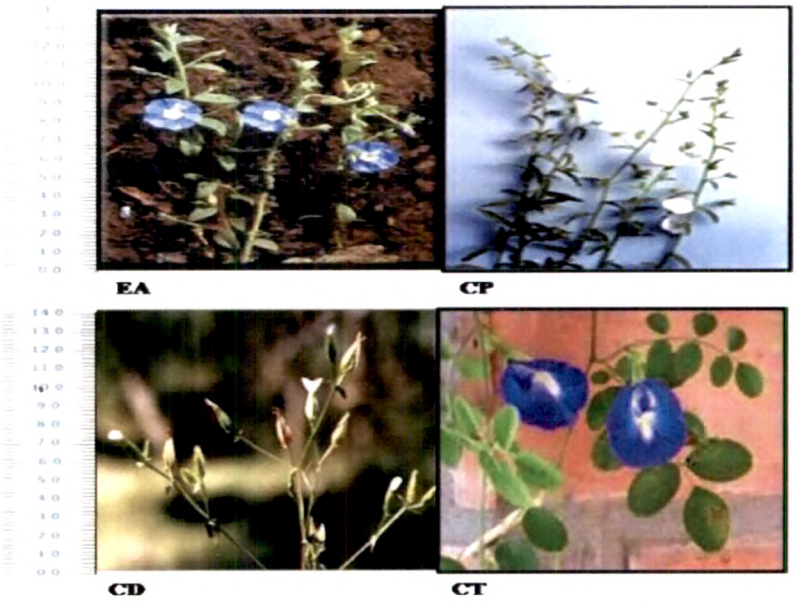


Figure 2.2 Comparative morphological character of various ethanobotanicals claims of Shankhpushpi.

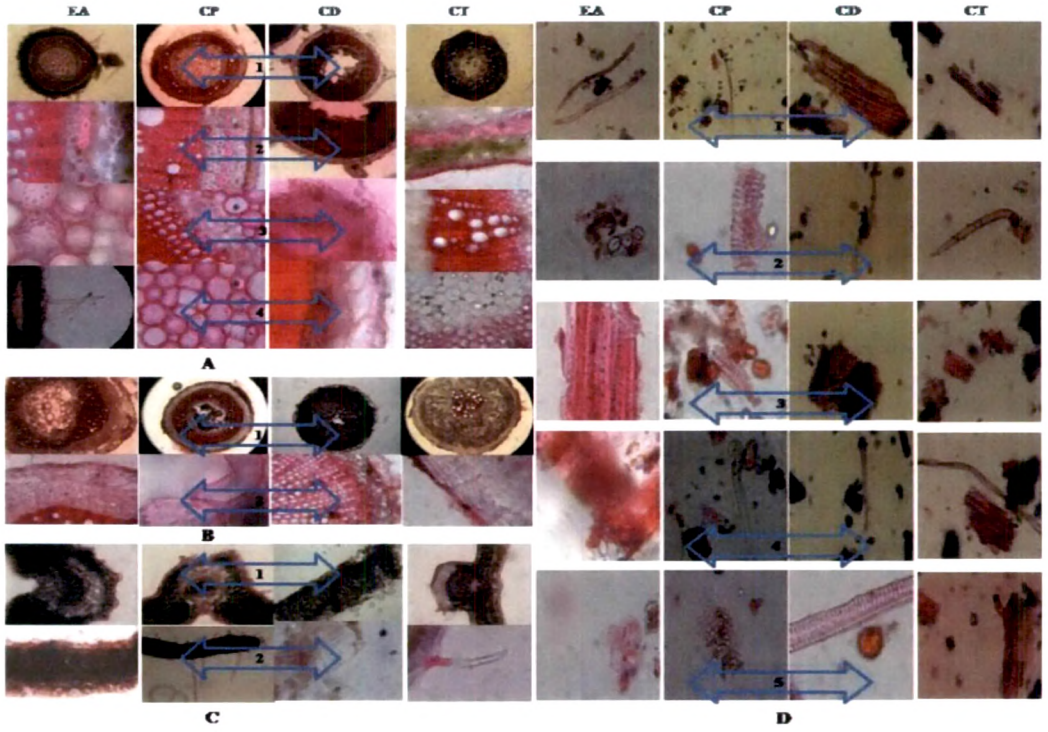


Figure 2.3

(A) Transverse Section (T.S.) of stems [1-Whole section; 2-Chlorenchyma, endodermis, cuticle; 3-Phloem, pith; 4- Trichome]

(B) T.S. of roots [1-Whole section; 2- Calcium oxalate, trichome]

(C) T.S. 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].

Although EA and CP is much similar in characteristic, as they belongs to same family. The marked difference between EA and CP was absence of phloem fiber in former. There is well defined diagnostic profile of CD among all. The CT resemblance in some characters to EA and CP, but differ in lamina and stomata type. Morphologically all plants have distinct pattern of identification characters.

2.10.1.2 Proximate analysis, Elemental analysis and Quantitative microscopy

The various differentiation parameters for analysis of moisture content, ash values, and extractives values were shown in Table 2.5. Quantitative analysis of various elements present in samples of shankhpushpi was shown in Table 2.6. Results of the quantitative microscopy viz, palisade ratio, stomatal index, vein islet and vein termination number were shown in Table 2.7.

Table 2.5 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	18.77±0.26	10.212±0.19	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
Sulphated ash	4.32±0.05	6.24±0.071	3.18±0.033	4.83±0.050
Water soluble ash	8.52 ± 0.05	4.02±0.048	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

*All values are Mean ± SEM (n=3)

Table 2.6: Comparative quantitative elemental analysis

Elements	EA	CP	CD	CT
Sodium (g/kg)	0.50±0.006	0.60±0.006	0.79±0.002	-
Potassium (g/kg)	16.64±0.08	9.99±0.11	15.35±0.008	14.92±0.26
Zinc (ppm)	89.84±0.19	64.35±0.58	78.24±0.30	117.69±2.02
Cupper (ppm)	35.45±0.60	18.59±0.33	34.52±0.67	9.02±0.11
Manganese (ppm)	114.00±0.28	77.59±0.49	122.96±0.93	41.52±0.44
Iron (ppm)	6523.07±96.71	2640.32±24.46	5404.07±17.96	1006.74±7.14
Magnesium (ppm)	526.31±0.61	531.57±0.99	524.96±3.80	512.28±3.51

*All values are Mean ± SEM (n=3)

Table 2.7: 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

2.10.1.3 Physiochemical investigations

All the extracts of Shankhpushpi botanicals were evaluated for physical parameters such as consistency, color, odor and taste. The results of percentage yield, color, odor and consistency of various extract obtained by successive solvent extraction was shown in Table 2.8. Qualitative chemical tests were carried out for the methanolic extracts of various Shankhpushpi botanicals. The results of the tests showed the presence of reducing sugars, proteins, alkaloids, steroids, saponins, glycosides, phenolics, tannins, flavonoids and pentoses in the ethanolic extract of the drugs. Triterpenoids was found to be absent in the drug.

2.10.1.4 Estimation of secondary metabolites

The result of preliminary photochemical analysis shows presence of tannins phenolics, flavanoid, flavonols and sugars and steroids in different botanicals of shankhpushpi extracts and their fractions. The presence of various tannins, flavanoids, flavonols and sugars leads us to evaluate their content. As these components are important for their antioxidant action and an antioxidants impart direct effects on signaling to enhance neuronal communication, can buffer against excess calcium, enhances neuroprotective adaptations, reduces stress signals, regulates extracellular signal for kinase activation, increases insulin-like growth factor I, regulates mitogen-activated protein (MAP) kinase and other signaling pathways at the level of transcription (Lau et al., 2005; Frigo et al., 2002; Mazzucchelli et al., 2000). Shankhpushpi botanicals are also employed for their action on brain and its related disorders. From table 2.9, it was cleared that there is highest content of phenolics, flavanoids and flavonols in EA among other botanicals of shankhpushpi. Whereas the sugar content is higher in CP and CD.

Table 2.9: Total Content of various metabolites by Successive extract

S. No.	Treatment	Total phenolics[a]	Total flavanoids[b] ($AlCl_3$ Method)	Total Flavanols[b]	Total sugar[c]
1.	DME-EA	1.18± 0.35	9.31±0.06	9.35±0.21	0.23±0.09
2.	DME-CP	0.29±0.11	5.01±0.09	7.21±0.23	0.91±0.09
3.	DME-CT	0.51±0.12	4.10±0.03	8.42±0.05	0.46±0.15
4.	DME-CD	0.71±0.15	8.16±0.01	10.11±0.22	0.99±0.06
5.	AE-EA	0.85±0.03	11.04±0.02	11.24±0.16	1.86±0.02
6.	AE-CP	0.31±0.06	9.87±0.08	8.47±0.12	0.52±0.12
7.	AE-CT	0.67±0.03	8.76±0.08	9.14±0.05	0.82±0.02
8.	AE-CD	0.76±0.09	10.54±0.23	14.02±0.11	2.04±0.14
9.	TEE-EA	1.02±0.01	15.07±0.001	12.03±0.08	0.41±0.37
10.	TEE-CP	0.39±0.01	13.02±0.13	13.02±0.02	0.96±0.08
11.	TEE-CT	0.62±0.08	10.41±0.27	13.55±0.03	0.95±0.04
12.	TEE-CD	0.82±0.001	9.94±0.18	14.23±0.01	1.02±0.21
13.	SS	1.11±0.21	10.24±0.11	12.14±0.03	1.14±0.01
14.	BT	1.05±0.08	9.87±0.25	7.95±0.08	0.98±0.03

Where a= mg gallic acid/g; b= mg quercetin/g; c= mg sucrose/g

Abbreviation: DME: Direct Methanolic Extract; AE: Aqueous Extract; TEE: Total Ethanolic Extract.

2.10.2 CHROMATOGRAPHIC STUDIES OF EXTRACTS

The chromatographic studies on TLC plates coated with silica gel G were performed. Initially various solvent systems were tried. Mixtures of two or three solvents produced a satisfactory separation. The criterion for good separation was resolution of distinct and discrete spots.

The methanolic extract of the Shankhpushpi botanicals were chromatographed on TLC plates and best resolution was obtained in the mobile phase, chloroform: methanol: toluene (7:2:1). The TLC plate examined under UV light (both 254 nm and 366 nm) showed the presence of various spots. The same plates were derivatized with anisaldehyde-sulphuric acid reagent and results were compared with UV visualization. (Table 2.10). There is highest numbers of bands found in EA, which shows its higher chemical content nature among others.

Table 2.10: TLC Profile of methanolic extract of *Shankhpushpi* botanicals in Chloroform: Methanol: Toluene (7:2:1)

Spot No.	Rf Value	Color in UV (256nm)[a]	Color in UV (366nm)[a]	Color in Sunlight[b]
EA				
1.	0.19	Yellow	Yellow	Dull Violet
2.	0.21	Yellowish green	Yellowish green	Dull Green
3.	0.27	Yellow	Yellow	Dull Green
4.	0.31	Blue	Blue	Yellowish green
5.	0.35	Yellow	Yellow	Brownish Black
6.	0.41	Blue-Violet	Blue-Violet	Dark Violet
7.	0.49	Orange	Orange	Bluish Green
8.	0.58	Blue	Blue	Dark Purple
9.	0.63	Blue	Blue	Violet
10.	0.69	Brick red	Brick red	Brownish Black
11	0.73	Light blue	Blue Fluorescence	No color
12	0.80	Pink	No color	Green
13	0.86	Reddish brown	Quenching	Red
14	0.91	Orange	Light pink	Green
CP				
1	0.18	Blue	Blue	Dull brown
2	0.21	Yellowish blue	Dull blue	No color
3	0.27	Yellow	Yellow	Dull Green
4	0.31	Blue	Blue	Yellowish green
5	0.35	Yellow	Yellow	Brownish Black
6	0.41	Blue-Violet	Blue-Violet	Dark Violet
7	0.49	Orange	Orange	Bluish Green
8	0.58	Blue	Blue	Dark Purple
9	0.63	Blue	Blue	Violet
10	0.68	Brick red	Brick red	Brownish Black
11	0.73	Light blue	Blue Fluorescence	No color
12	0.88	Blue violet	Blue Violet	Violet

2.10.4 HPLC fingerprinting

Various HPLC fingerprint of all available shankhpushpi samples along with two marketed formulation were shown in Figure 2.5. The fingerprint of EA shown similar profile with Shankhpushpi syrup and the fingerprint of CD also matches in major peaks with Brain tab.

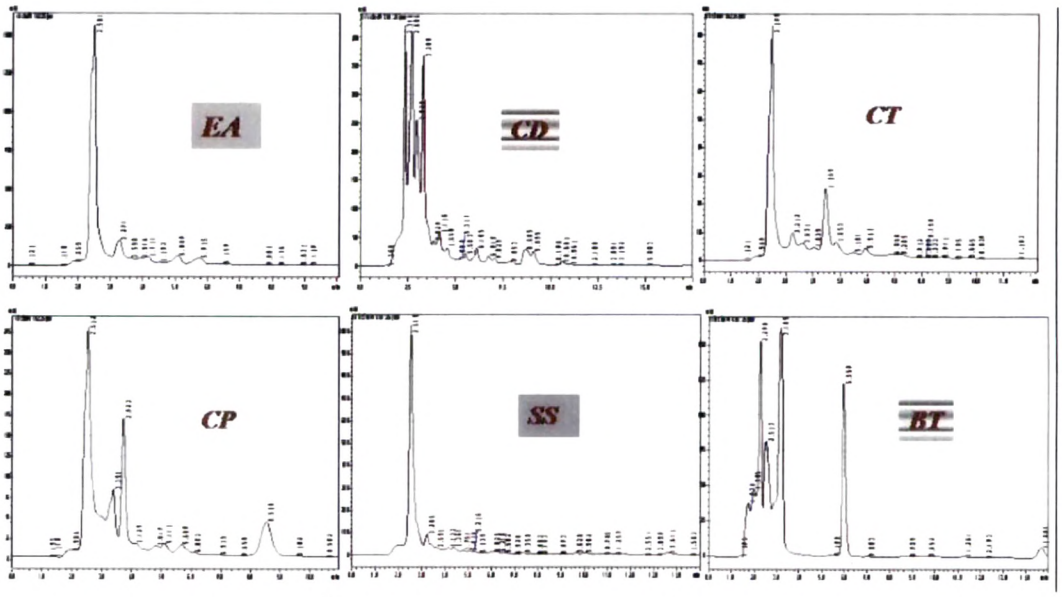


Figure 2.5 HPLC chromatogram of Shankhpushpi sample and marketed formulation

2.10.5 ISOLATION OF COMPOUND

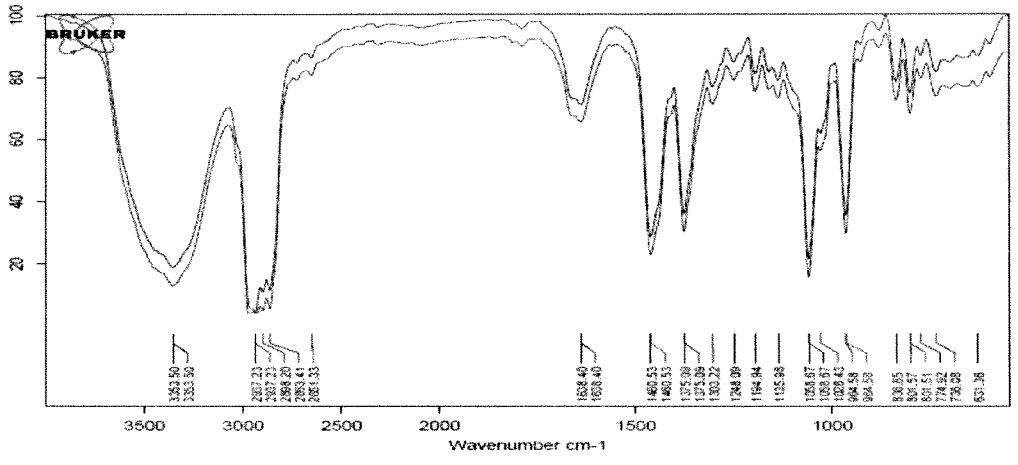
The column chromatography analysis for petroleum ether and methanolic extracts of various *Shankhpushpi* botanicals leads to various fractions, some of which were on further screening shows the presence of a single spot. These fractions were mixed and crystallization was done with methanol. These were further purified with the aid of preparative chromatography or washing in fresh column and ultracentrifugation to get pure compound. Crystals obtained were recrystalized with acetone and collected.

These crystals showed an R_f value similar to standard chemical marker. Further the FT-IR analysis of the isolated compound confirmed its identity with the standard compound. The FTIR analysis of a mixture of pure compound and isolated compound also showed similar peaks. This indicated that the isolated compound is same to that of standard. Furthermore the melting points of the standard and the isolate were found to be in the same range, indicating their similar chemical identity.

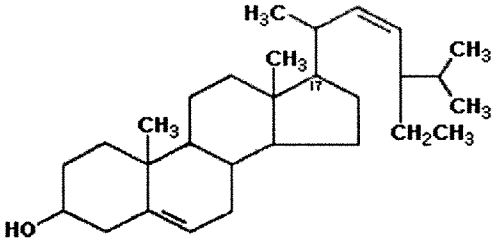
2.10.5.1 Isolation and characterization of phytochemical marker from EA

EA-1

The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.38. A crystalline material melting at 162-163 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as stigmasterol by mixed melting point, HPTLC, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of stigmasterol. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.6 a; b).



(A)

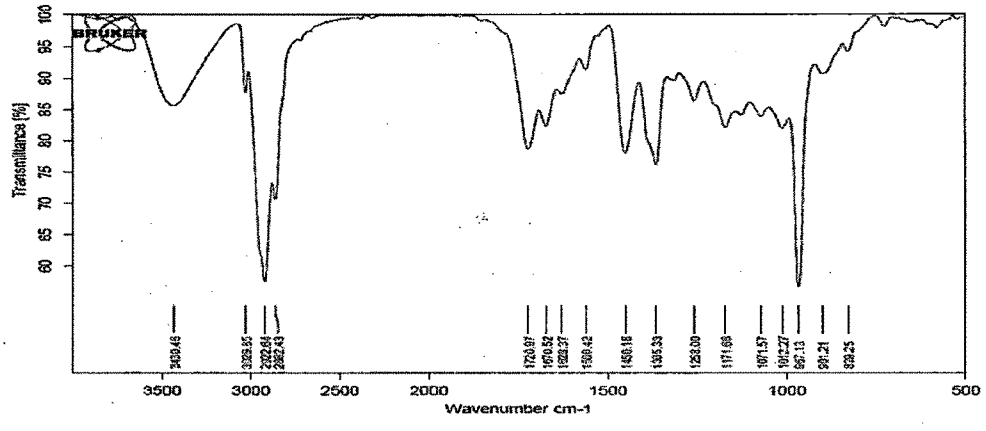


(B)

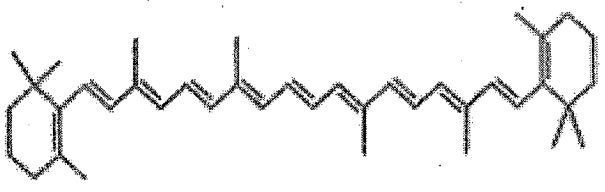
Figure 2.6 (A). Overlay FTIR spectrum of stigmasterol and isolate; (B). Chemical structure of stigmasterol

EA-3

The isolated compound showed a single spot in TLC with Hexane: Ethyl acetate (4:1, v/v), R_f 0.83. A crystalline material melting at 182-182 °C was isolated. It was characterized as β-Carotene by mixed melting point, UV absorption maxima and FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of β-Carotene. (Fig. 2.8 a; b).



(A)

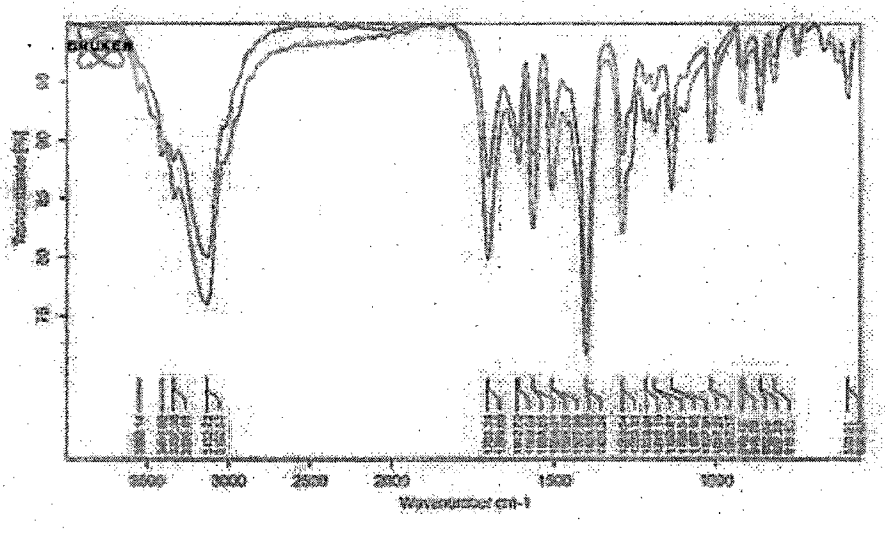


(B)

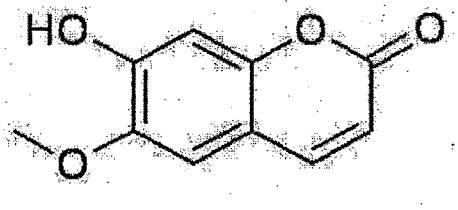
Figure 2.8 (A). FTIR spectrum of β-Carotene; (B). Chemical structure of β-Carotene

Isolation of Scopoletin from *E. alsinoids*

The isolated compound showed a single spot in TLC with Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5, v/v), R_f 0.78. A crystalline material melting at 204 °C was isolated. It gave blue fluorescence in UV and was characterized as scopoletin by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of 6-methoxy, 7-hydroxy coumarin, i.e. scopoletin. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.9 a; b).



(A)



(B)

Figure 2.9 (A). Overlay FTIR spectrum of scopoletin and isolate; (B). Chemical structure of scopoletin

2.10.5.2 Isolation and characterization of phytochemical marker from CP

CP-1

The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.38. A crystalline material melting at 162-163 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as stigmasterol by mixed melting point, HPTLC, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of stigmasterol. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.6a; b).

Isolation of Scopoletin from CP

The isolated compound showed a single spot in TLC with Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5, v/v), R_f 0.78. A crystalline material melting at 204 °C was isolated. It gave blue fluorescence in UV and was characterized as scopoletin by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of 6-methoxy, 7-hydroxy coumarin, i.e. scopoletin. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.9a; b).

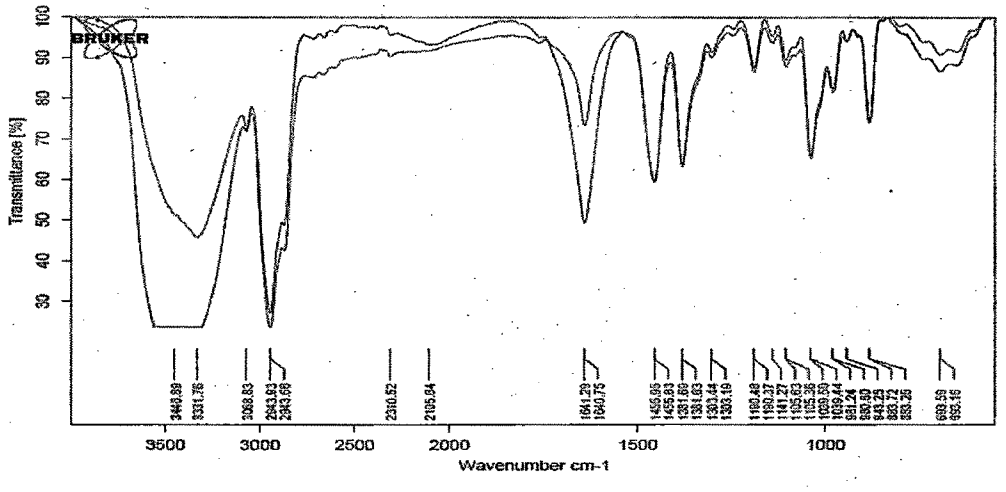
2.10.5.3 Isolation and characterization of phytochemical marker from CT

CT-1

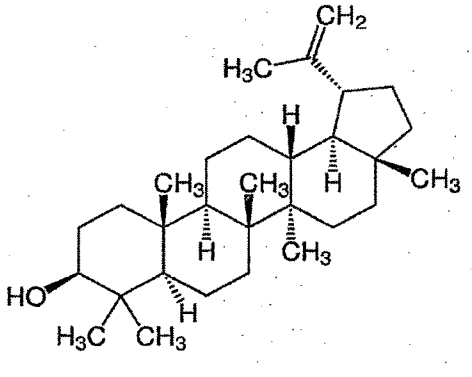
The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.38. A crystalline material melting at 162-163 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as stigmasterol by mixed melting point, HPTLC, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of stigmasterol. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.6a; b).

CT-2

The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.54. A crystalline material melting at 214-215 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as lupeol by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of lupeol. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.10a; b).



(A)

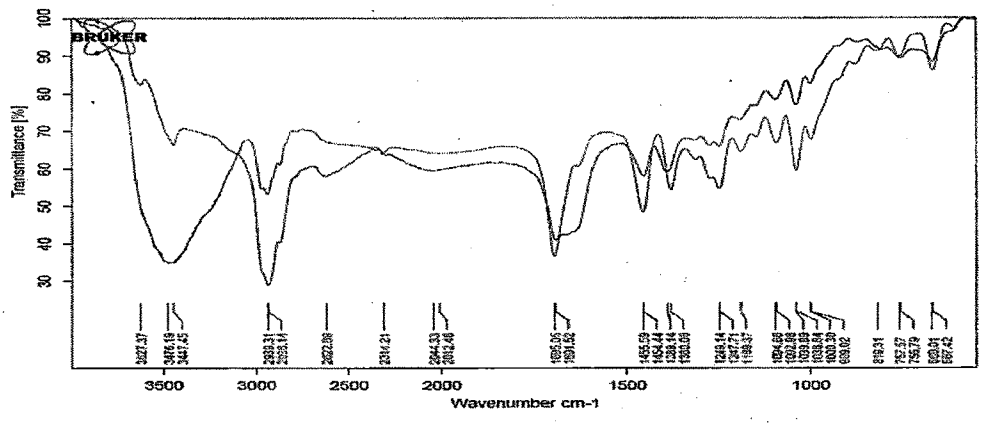


(B)

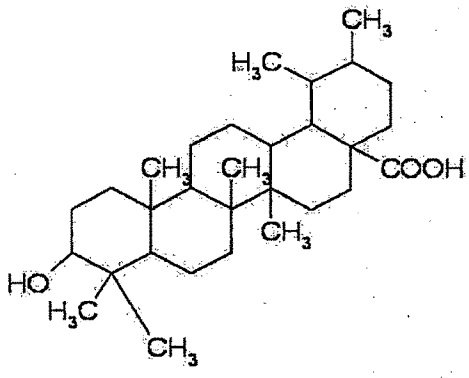
Figure 2.10 (A). Overlay FTIR spectrum of lupeol and isolate; (B). Chemical structure of lupeol

2.10.5.3.3 CT-3

The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.24. A crystalline material melting at 283-284 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as ursolic acid by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of ursolic acid. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.11a; b).



(A)

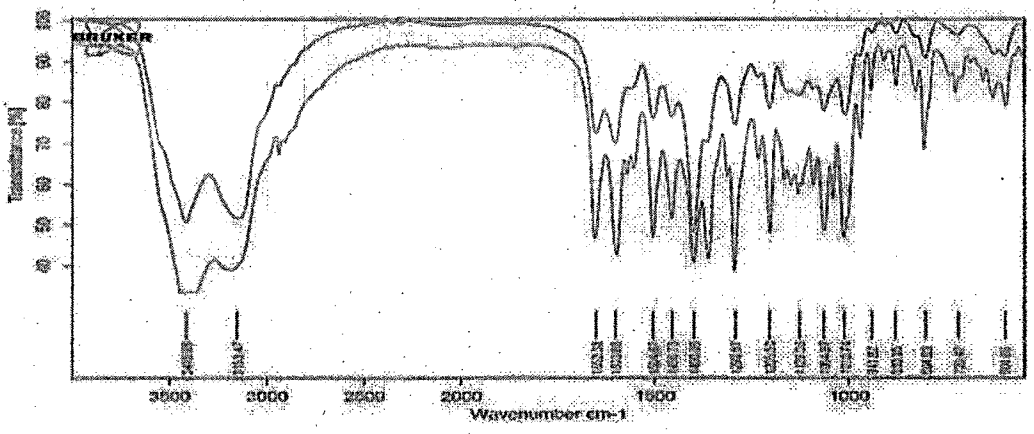


(B)

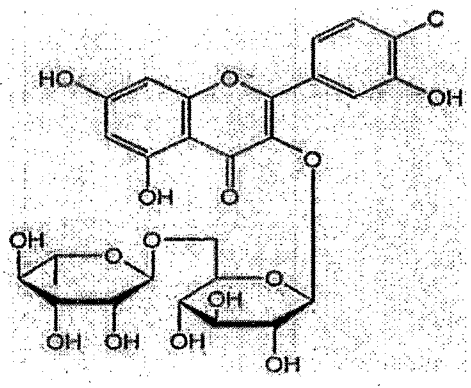
Figure 2.11 Overlay FTIR spectrum of ursolic acid and isolate; (B). Chemical structure of ursolic acid.

2.10.5.3.4 Isolation of Rutin from *C. ternatea*

The isolated compound showed a single spot in TLC with Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5, v/v), R_f 0.09. We have confirmed our isolate as rutin with reference to previous findings, which included melting point determination (range 206-210°C), UV absorption maxima (λ_{max} at 359 nm) and superimposable FTIR analysis with standard rutin (Fig. 2.12a; b).



(A)



(B)

Figure 2.12 Overlay FTIR spectrum of rutin and isolate; (B). Chemical structure of rutin.

2.10.5.4 Isolation and characterization of phytochemical marker from CD

CD-1

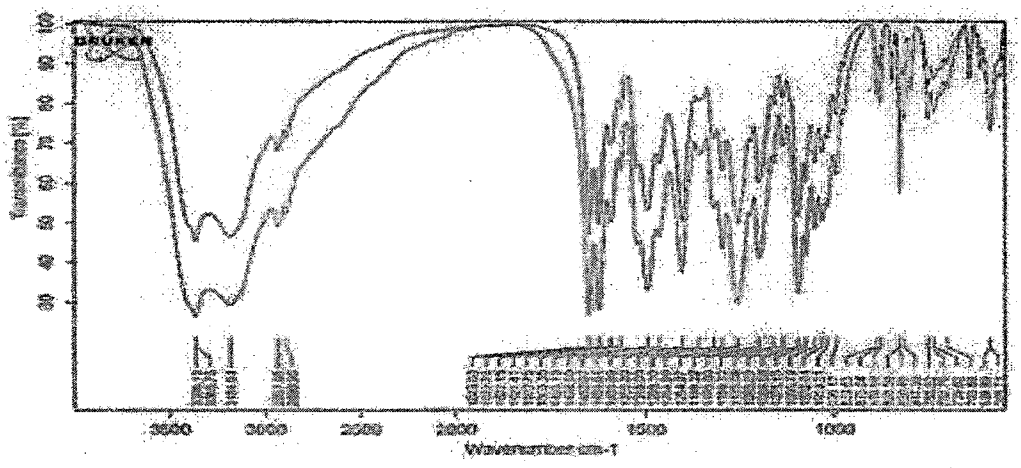
The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.38. A crystalline material melting at 162-163 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as stigmasterol by mixed melting point, HPTLC, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of stigmasterol. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.6a; b).

CD-2

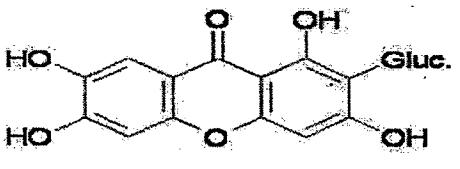
The isolated compound showed a single spot in TLC with Petroleum Ether: Ethyl acetate: Toluene (7:2:1, v/v), R_f 0.24. A crystalline material melting at 284--285 °C was isolated. It gave blue colour after derivatisation with anisaldehyde sulfuric acid and was characterized as ursolic acid by mixed melting point, UV absorption maxima and superimposable FTIR spectral analysis. The FTIR spectrum of the compound is characteristic of ursolic acid. A superimposable FTIR spectrum with the reference standard proved this fact (Fig. 2.11a; b).

2.10.5.4.3 Isolation of Mangiferin from C. decussata

The isolated compound showed a single spot in TLC with Ethyl acetate: Acetic acid: Formic acid: Water (10:0.5:0.5: 1.5, v/v), R_f 0.22. We have confirmed our isolate as mangiferin with reference to previous findings, which included melting point determination (range 273-276°C), UV absorption maxima (315 nm) and superimposable FTIR analysis with standard mangiferin (Fig. 2.13 a; b).



(A)



(B)

Figure 2.13 Overlay FTIR spectrum of mangiferin and isolate; (B). Chemical structure of mangiferin.

In present studies various prescribed protocol of evaluation by WHO has been tried on various collected sample of shankhpushpi in the form of crude, processed and finished herbs from various infield and outfield resources. Although there is great diversity in the Pharmacognosy of all but still due to confusion of name these drugs in the form of powder and processed extract always creates the problems for identification.

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 our work we explored the parameter of differentiation such as pharmacognostical and phytochemical for ayurvedic medicine shankhpushpi, reputed as brain tonic for its proper authentication.

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 then other varieties. There is still very little characters explored for EA, CP and CT. One can also not able to evaluate the parameters, if drug is supplied in the form of extract. The maximum content of iron in EA and CD makes there use as drug of choice for iron deficient diseases. Formulation containing CD, although proves its potential in problems related with post menopausal disorders (Devi and Swarup, 2000). High content of phenolics, flavonols and flavanoids of EA also make its differentiation with other. High content of carbohydrate is also a diagnostic feature for the CD. TLC fingerprinting pattern of all are distinct and may also serve to solve the purpose of identity with reference to standard chromatogram. Chemical markers based identification serves a better tool for the purpose. These distinct identified markers may use as rapid, easy and economic identification of botanical sources.

These studies, thus, proved to be successful for the differentiation of Shankhpushpi botanicals on the basis of their morphological, microscopical and phytochemical basis. These studies paved the way for the development of standardization parameters for the identifications of various Shankhpushpi botanicals viz., EA, CP, CT and CD.