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Study on Identification and Extraction of Adulterants from Herbal Medicines

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In modern time, usage of herbal medicines has increased all over the world. Herbal medicines are use for treatment of primary health needs and conventional treatment too. Due to increased demand of these medicines, maniac people have started production of adulterants/ inappropriate chemical properties/ low grade drugs in products. In this concern, examination including, chemical tests, analytical tests, phytochemicals screening tests are performed to determine the purity of products, quantitative analysis of heavy metals, presence of drug. This chapter is mainly focused on the implementation of various analytical techniques i.e. HP-TLC, FTIR etc. for identification of derivatization of adulterants from herbal drugs. Extracted adulterants from these herbal medicines include, guanine, flavonoids, alcohol ferric chloride etc. were attained.

Keywords: Adulterants; extraction; recognition; herbal medicine etc.

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1. INTRODUCTION

In recent years, demand of herbal medicines has increased throughout the world. Herbal medicines are active ingredients of plants attained from various parts including roots, leaves, stem flowers, seeds and fruits. Production of synthesized medicines brought a revolution to traditional medicines for medical care. As per WHO, approx. 90% Indians are using herbal medicines for their primary health care needs and 80% of the country's population is leaning towards conventional treatment. Many of these medicines focuses on their organic components i.e., essential oils, glycosides, alkaloids, vitamins, antioxidant chemicals, and their pharmacological efficacy and any potential toxicities. In order to increase efficiency, any product with similar therapeutic/ chemical properties, low grade drugs are added in products [1]. To recognize adulterants, present in medicines various analytical and chemical methods are used. Chemical test is performed in evaluation of resins, acid, value of volatile oil to tests acid value and saponification value. Phytochemical screening is a qualitative method for the identification of chemical constituents present in the drug. While analytical methods such as TLC, HPLC, GC etc. are used to determine purity of products, quantity of heavy metals and presence of contaminants. This chapter is basically a discussion on adulterations in herbal medicines-, types of adulterants and various methods used to quantify the adulteration [2].

1.1 Sarpagandha

Sarpagandha as potential value in ayurveda and widely known remedy to control high blood pressure, to cure fever, wound and worm infection, to detoxify snakes, scorpions and spider poison. It has astringent properties**;** also used as a sedative and tranquillizer agent. It consists ophioxylin, resin, starch and wax and the total alkaloid content in plant is 0.8%.

1.2 Macroscopic Characteristics

It is an evergreen perennial shrub that usually grows up-to to a height of 60cm and found in moist deciduous forest/shady areas. Its root is of pale color and leaves are found bright green and whorls of three leaves. Its flower is of white, while peduncles are long, pedicles are stout with red calyx [3]. The active ingredients present in sarpagandha are alkaline resprine which help to cure insomnia, hypertension etc. It is prepared from the sarpagandha plant which consist various therapeutic properties.

Sarpagandha chemical constitution: Ajmalicidine, Ajmalicine, Rouhimbine, Indobinine, Reserpiline, Reserpine, Sarpagine, Serpentine, Serpentinine, Yohimbine, Ajmalimine, Ajmaline, Rauwolfinine (Perakenine), Sandwicolidine, Serpinine etc.

Various standardization technique is followed to extract these additional elements present in herbal medicines such as HP-TLC, GC-MS, FTIR and HP-LC. This study is an approach for quantitative and qualitative analysis of the chemically active components in herbal medicines.

2. METHODOLOGY

Qualitative analysis was determined by HP-TLC and certain parameters was affixed to assess.

Determination of the total ash; 1 gm. of test medicine is taken in a crucible at 450^0C in a muffle furnace and cooled. This sample was weighed and total percentage of ash was calculated.

Determination of alcohol soluble extracts; Powdered medicine was mixed with 100 ml of alcohol in a closed flask for 24 hours and left for more 18 hours than filtered. 25ml of each filtrate was evaporated and dried in a porcelain dish at 105^0C to constant weight and the alcohol soluble extracts are calculated [4].

Determination of water- soluble extractives: The powder test drugs were macerated with 100 ml of water in a close flask for 1 hour. Then, it was boiled gently for another hour in a water bath, cooled and weighed and weight was re-adjusted. 25 ml of each filtrate was evaporated to dryness in a porcelain dish at 105^0C to constant weight. The percentage of water-soluble extractive was calculated.

Extraction of alkaloids; "1 gm. of each test powdered drug and 0.1g of Sarpagandha root powder were collected. Each material was refluxed with 10 ml methanol containing 0.1M HCl in a water bath for an hour. Then the sample solution was cooled, filtered and liquid-liquid separation was performed using n-hexane. The residual matter after hexane extraction was concentrated in reduced pressure and the

residue was then dissolved in methanol-chloroform (98:2, v/v) in a 10 ml volumetric flask. Each sample solution was filtered through 0.22µm filter force using HTLC analysis" [5].

Loss of drying: 2 gm. of powdered drug was accurately weighed and was taken in porcelain dish, the taken porcelain dish was kept in an open vacuum oven and the sample maintained at a temperature of 100^0C followed by room temperature this procedure is repeated until constant weight is observed.

% Loss on drying = Loss in weight of sample/weight of sample× 100

Phytochemical analysis: The phytochemical screening of prepared samples was carried out to test the presence of tannins, saponins, flavonoids, carbohydrates, phenol, and steroids.

2.1 HP-TLC Analysis

HP-TLC profile is a major quality control aspect to reveal phytochemical components of the formulations and efficacy. In this method, peak profiles and their intensities provide both quantitative and qualitative result in comparison with reference standards while the images will give percentage of purity and minimum content information can also be obtained. HP-TLC analysis of the sarpagandha medicine shown that, sarpagandha churna exhibits 11 peaks while sarpagandha Ghana vati has 17 peaks that indicate fingerprint of test drugs [6]. The individual peaks in chromatograms usually depend on chemically distinct components and concentration of these chemically active components are not only responsible for its therapeutic properties but also different symptomatic and non-symptomatic side effects.

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Table 1. Description of test drug formulations

Test drug/ formulations	Ingredients	Quantity (%)
Sarpagandha Churna	Sarpagandha root powder	100
Sarpagandha Ghana vati	Sarpagandha ghansatwa	50
	Khursaniajawain ghansatwa	10
	Jatamansi ghansatwa	5
	Bijay ghansatwa	5
	Pippalimool churna	25
	Excepients	5
M-Sarpagandha Mishran	Sarpagandha root churna	15.6
	Jatamansi root churna	15.6
	Vacha leaf churna	15.6
	Punarnava whole plant Churna	15.6
	Brahmi whole plant churna	15.6

Table 2. Comparison of chromatographic techniques

Parameters	HPLC	HP-TLC	GC
StationeryPhase	Column	Paper/Glass	Liquid /Solid
Mobile Phase	Solvent Mixture	Solvent Mixture	Pure Inert Gas
Sample	One At One Run	Many at a Single run	One At One Run
Pressure	High	Normal	Closed
Results	System Peaks	System Peaks VisualBy Bands	System Peaks
Resolution	High To Very High	Moderate To High	High To Very High
Time	2-60 Min	1-30min	2-60 Min
Temperature	Constant	Constant	Increasing

2.2 Shankhpushpi Churna

Shankhpushpi (*Convolvulus pluricaulis)* is an herb that has extensively been a topic of investigation for its therapeutic and pharmacological effects. Shankhpushpi is considered as Medhya Rasayana in Ayurvedic texts and plant extraction are used for central nervous system depressants, anxiolytics, sedatives, antidepressants, antistress agents, neurodegenerative agents, antianemics, antioxidants, lipid-lowering agents, immunomodulators, analgesics, it has demonstrated scientific potential in antifungal, antibacterial, antidiabetic, antiulcer, antitonic, and cardiovascular activities. It is extremely beneficial for increasing memory, concentration, and learning capacities, along with treating mental stress, insomnia, anxiety, and depression. Among these phytoconstituents, some are present in higher concentrations (nearly 20% w/w). known as phytoconstituents [7]. This plant contains kaempferol, β-sitosterol, N-hexacosanol, taraxerol, taraxerone, delphinidin and hydroxy-cinnamic acid. In addition, an alkaloid, i.e. Sankhpuspine, has also been isolated from this plant helps as a chemical marker for species. CP also contains other alkaloids (convolamine, convosine, convoline etc.) anthroquinones; carbohydrates (D-glucose, sucrose); coumarin (ayapanin, scopolyne); flavonoids, glycosides (geranilan-3-ol-1-carboxylate-1-O-β-D- xylopyranosyl- (2 ′ → 1 ′) - O-β-D-xylopyranoside; phenols; steroids; and terpenoids. CP is also known for its rich source of vitamins and minerals like phosphorus, manganese, calcium, copper, iron, zinc, sulphur, vitamin C and vitamin E.

2.3 Macroscopic Characteristics

These plants are grown in rocky soil along/found on road side in north India. Its identical characteristics include cylindrical, ribbed and light-yellow color root with a hairy texture [8]. Stem are prostate/ascending slender and the leaves are ex-stipulate, sessile, simple, linear to oblong in shape, alternate phyllotaxy, reticulate venation. In florescence, 1-3 flowers in axillary heads along with ebracteate, monoecious flowers are observed.

2.4 HP-TLC

“During this process, 1gm. of powdered sample was diluted in 10mL of ethanol and was kept for cold percolation for 24 hours before filtration. By using a Lino mat 5 TLC applicator, 4, 8, and 12 µL of the sample were applied to a precoated silica gel F254 on aluminum plates to a bandwidth of 7 mm” [9,10]. “Plate was developed in n-butanol: acetic acid: water (4:1:1) developed plates were scanned under short ultraviolet/ long ultraviolet. It was then derivatized using vanillin sulfuric acid reagent and scanned under 254, 366 nm and white light at 620 nm. The densitometric scan, retention factor (Rf), and color of the spots were noted” [11].

Table 3. Phytochemical screening of alkaloids

Test	Experiment	Observation
Dragendroff’s test	A few mg of the HACP extractdissolved in alcohol + Few drops of acetic acid +Dragendroff’s reagent	Orange red precipitation
Wagner’s test	Few mg of HACP extract dissolved in acetic acid +fewdrops of Wagners’s reagent	Reddish brownprecipitate.
Mayer’s test	Few mg of HACP extractdissolved in acetic acid +Mayer’s reagent	Dull white precipitate
Hager’s test	Few mg of extract dissolved inacetic acid + 3ml of Hager’s reagent	Yellow precipitate

Table 4. Carbohydrates

Test	Experiment	Observation
Molisch’s Test	In a test tube, HACP extract is taken + 1ml α- naphthol solution + conc. Sulfuric acid (along thesides of tube)	Violet colour formed at thejunction of 2 liquids
Fehling’s Test	Few mg of HACP + equalamount of Fehling’s solutions A and B. Warm the mixture in a water bath.	Brick red precipitate.
Benedict’s Test	Few mg of HACP extract +5ml of Benedict's reagent. Boil for 2 mins and cool.	Red precipitate

Table 5. Steroids

Test	Experiment	Observation
Liebermann Burchard test	Few mg of HACP extract dissolved in chloroform + 1ml of acetic acid + 1ml of acetic anhydride. Heat on awater bath and cool. Add a few drops of conc.Sulphuric acid along thesides.	Bluish green color solution
Salkowski's test	Few mg of HACP extractdissolved in chloroform +equal volume of conc. Sulfuric acid	Bluish red to cherry red colour in chloroform layerand green fluorescence in layer

Table 6. Saponins

Experiment	Observation
0.5ml of extract + 5- 10 drops of dilute HCland ZnCl. Boil the solution for a few minutes	Reddish pink or dirty brown color solution

Table 7. Tannins

Experiment	Observation
Extract + drop f dil. ferric chloridesolution	Dark blue color

Table 8. 1f Flavonoids

Test	Experiment	Observation
Shinoda's Test	0.5ml of extract + 5-10 drops of dilute HCl and ZnCl. Boil the mixture for afew minutes	Reddish pink or dirtybrown color

Table 9.1g Phenol

Experiment	Observation
HACP extract in alcohol + 2 drops ofalcoholic ferric chloride	Blue to black color solution

Table 10. Carboxylic acid

Experiment	Observation
HACP extract dissolved in water and treatwith sodium bicarbonate	Brisk effervescence

Table 11. Quinine

Experiment	Observation
Few mg of HACP extract + 0.5% NaOH	Deep coloration like pink/purple/ red.

Table 12. Preliminary phytochemical tests

Test	Shankapushpi
Alkaloid	+
Carbohydrate	+
Tannin	+
Terpenoid	+
Phenol	+

Table 13. Confirmation of additives in extraction

Sl. No.	Test	Colour if positive	Shankapushpi
1	**Alkaloid**		
	Dragendrof's test	Orange precipitate	Orange precipitate
	Wagner's test	Red precipitate	Red precipitate
	Mayer's test	Dull white precipitate	Dull whiteprecipitate
	Hager's test	Yellow precipitate	
2	**Steroids**		
	Liebermann burchardtest	Bluish red	Light red color
	Salkowski's test	Bluish red to cherry red	Cherry red color in chloroform and colorless in the acidform.
3	**Carbohydrate**		
	Molish's test	Violet ring	Violet ring
	Fehling's test	brick red precipitate	ink blue solution
	Benedict's test	red precipitate	bluish green solution
4	**Tannin**		
	With $FECL_3$	dark blue or green or brown	Brown
5	**Flavonoids**		
	Shinoda's test	red to pink	colourless solution
6	**Saponins**		
	With $nahco_3$	stable forth	no forth
7	**Triterpenoids**		
	Tin and thionyl chloride test	red	light pink
8	**Coumarins**		
	With 2n naoh	yellow	light brown
9	**Phenols**		
	With	blue to black, brown	Brown
10	**Carboxylic acid**		
	With water and $nahco_3$	Brisk effervescence	No effervescence
11	**Resin**		
	With aqueous acetone	Turbidity	No turbidity
12	**Quinone**		
	5% Naoh	Pink/ purple/ red	Light brown
13	**Amino acids**		
	Ninhydrin reagent	Purple color	Colorless

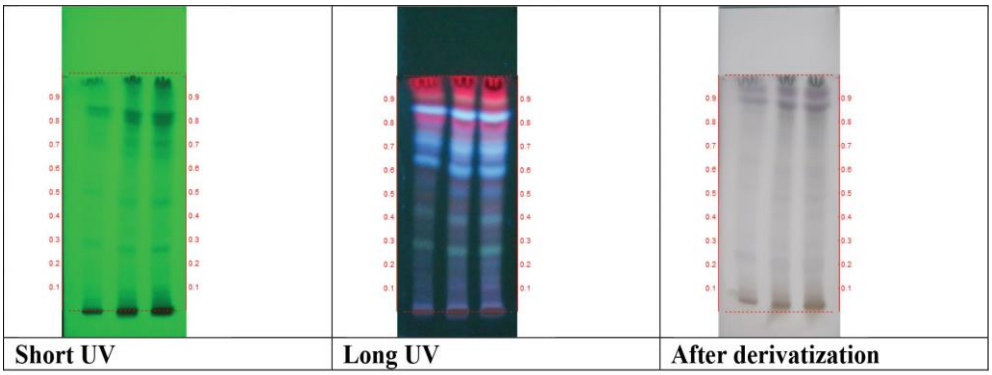
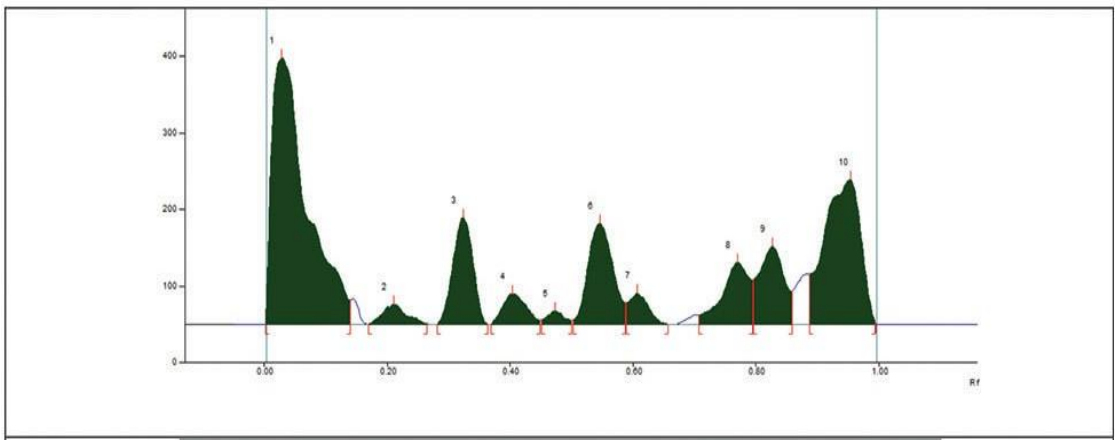


Fig. 1. PTLC photo documentation of ethanolic extract ofshankhpushpi

Table 14. Result For HPTLC

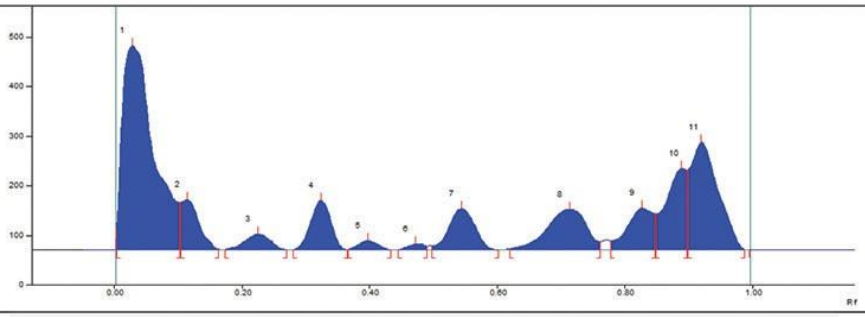
Short UV	Long UV	After Derivatization
-	0.06 (Violet)	0.06 (L Purple)
-	0.11 (FL Violet)	0.11 (L Purple)
-	0.20 (FL Blue)	0.20 (L Purple)
0.26 (D Green)	0.26 (FL Green)	-
-	-	0.29 (L Purple)
0.33 (L Green)	0.33 (Violet)	-
0.38 (L Green)	0.38 (FL Blue)	-
-	-	0.40 (L Purple)
0.46 (D Green)	0.46 (FL Pink)	-
		0.48 (L Purple)
0.51 (L Green)	0.51 (L FL Blue)	-
-	-	0.54 (L Purple)
-	0.60 (FL Blue)	0.60 (L Purple)
0.64 (D Green)	-	-
0.69 (D Green)	0.69 (FL Blue)	-
-	0.72 (F Orange)	-
-	0.74 (Violet)	-
-	0.78 (FD Pink)	-
0.82 (D Green)	0.82(FL Blue)	0.82 (L Purple)
-	0.86 (FD Pink)	0.86 (D Purple)
-	0.91 (F Red)	0.91 (D Purple)



Track 3, ID: Shankapushpi

Peak	Start positon	Start height	Max positon	Max height	Max %	End positon	End height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	347.4 AU	31.22 %	0.14 Rf	31.6 AU	14363.4 AU	37.55 %
2	0.17 Rf	0.2 AU	0.21 Rf	25.9 AU	2.33 %	0.27 Rf	0.8 AU	740.0 AU	1.93 %
3	0.28 Rf	0.6 AU	0.32 Rf	139.1 AU	12.50 %	0.36 Rf	0.3 AU	3300.2 AU	8.63 %
4	0.37 Rf	0.4 AU	0.40 Rf	40.0 AU	3.59 %	0.45 Rf	5.5 AU	1119.6 AU	2.93 %
5	0.45 Rf	5.7 AU	0.47 Rf	17.3 AU	1.55 %	0.50 Rf	5.3 AU	355.6 AU	0.93 %
6	0.50 Rf	5.7 AU	0.55 Rf	131.6 AU	11.82 %	0.59 Rf	28.2 AU	3854.7 AU	10.08 %
7	0.59 Rf	28.4 AU	0.61 Rf	40.5 AU	3.64 %	0.66 Rf	0.3 AU	930.1 AU	2.43 %
8	0.71 Rf	12.6 AU	0.77 Rf	81.2 AU	7.29 %	0.80 Rf	57.8 AU	2534.2 AU	6.63 %
9	0.80 Rf	58.0 AU	0.83 Rf	101.3 AU	9.10 %	0.86 Rf	43.7 AU	2996.7 AU	7.83 %
10	0.89 Rf	66.2 AU	0.95 Rf	188.7 AU	16.96 %	1.00 Rf	3.5 AU	8055.4 AU	21.06 %

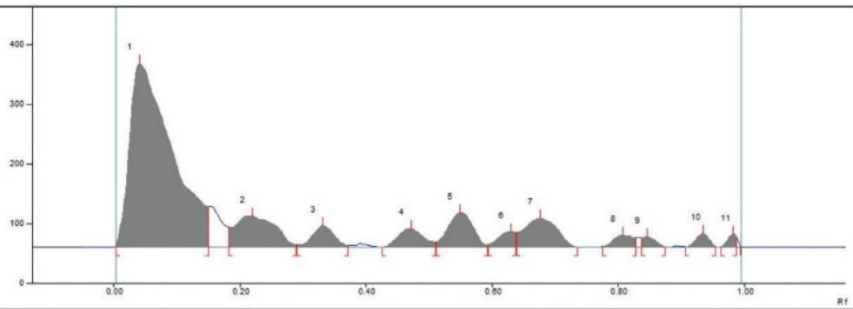
Fig. 2. Densitometric scan AT 254 nm



Track 3, ID: Shankapushpi

Peak	Start positon	Start height	Max positon	Max height	Max %	End positon	End height	Area	Area %
1	0.00 Rf	42.6 AU	0.03 Rf	411.5 AU	31.63 %	0.10 Rf	95.8 AU	14737.0 AU	37.25 %
2	0.10 Rf	96.1 AU	0.11 Rf	101.2 AU	7.77 %	0.16 Rf	0.9 AU	2032.2 AU	5.14 %
3	0.17 Rf	0.4 AU	0.23 Rf	30.9 AU	2.38 %	0.27 Rf	0.0 AU	898.9 AU	2.27 %
4	0.28 Rf	0.2 AU	0.32 Rf	99.1 AU	7.62 %	0.37 Rf	0.3 AU	2259.3 AU	5.71 %
5	0.37 Rf	0.4 AU	0.40 Rf	18.3 AU	1.41 %	0.43 Rf	0.1 AU	392.0 AU	0.99 %
6	0.45 Rf	0.1 AU	0.47 Rf	11.5 AU	0.88 %	0.49 Rf	7.2 AU	212.9 AU	0.54 %
7	0.50 Rf	8.1 AU	0.55 Rf	82.4 AU	6.33 %	0.60 Rf	0.3 AU	2410.5 AU	6.09 %
8	0.62 Rf	2.3 AU	0.71 Rf	81.7 AU	6.28 %	0.76 Rf	16.6 AU	3630.5 AU	9.18 %
9	0.78 Rf	18.3 AU	0.83 Rf	83.8 AU	6.44 %	0.85 Rf	72.3 AU	2445.9 AU	6.18 %
10	0.85 Rf	72.5 AU	0.89 Rf	164.0 AU	12.60 %	0.90 Rf	61.0 AU	3793.3 AU	9.59 %
11	0.90 Rf	161.6 AU	0.92 Rf	216.8 AU	16.66 %	0.99 Rf	0.4 AU	6745.9 AU	17.05 %

Fig. 3. Densitometric scan AT 366 nm



Track 3, ID: Shankapushpi

Peak	Start positon	Start height	Max positon	Max height	Max %	End positon	End height	Area	Area %
1	0.00 Rf	5.8 AU	0.04 Rf	307.7 AU	47.92 %	0.15 Rf	68.1 AU	14927.0 AU	61.33 %
2	0.18 Rf	32.9 AU	0.22 Rf	52.5 AU	8.18 %	0.29 Rf	3.8 AU	2398.1 AU	9.85 %
3	0.29 Rf	3.8 AU	0.33 Rf	35.7 AU	5.56 %	0.37 Rf	2.5 AU	891.6 AU	3.66 %
4	0.43 Rf	0.1 AU	0.47 Rf	31.2 AU	4.86 %	0.51 Rf	8.4 AU	900.3 AU	3.70 %
5	0.51 Rf	8.5 AU	0.55 Rf	58.6 AU	9.12 %	0.59 Rf	3.5 AU	1640.7 AU	6.74 %
6	0.60 Rf	3.7 AU	0.63 Rf	25.8 AU	4.02 %	0.64 Rf	24.9 AU	505.1 AU	2.08 %
7	0.64 Rf	24.9 AU	0.68 Rf	48.5 AU	7.55 %	0.74 Rf	0.0 AU	1795.1 AU	7.38 %
8	0.78 Rf	1.0 AU	0.81 Rf	20.1 AU	3.14 %	0.83 Rf	15.5 AU	462.2 AU	1.90 %
9	0.84 Rf	15.7 AU	0.85 Rf	17.9 AU	2.79 %	0.88 Rf	0.2 AU	262.3 AU	1.08 %
10	0.91 Rf	0.7 AU	0.94 Rf	22.6 AU	3.52 %	0.95 Rf	0.9 AU	348.6 AU	1.43 %
11	0.96 Rf	0.6 AU	0.98 Rf	21.4 AU	3.34 %	0.99 Rf	17.2 AU	207.7 AU	0.85 %

Fig. 4. Densitometric scan after Derivatization AT 620 nm

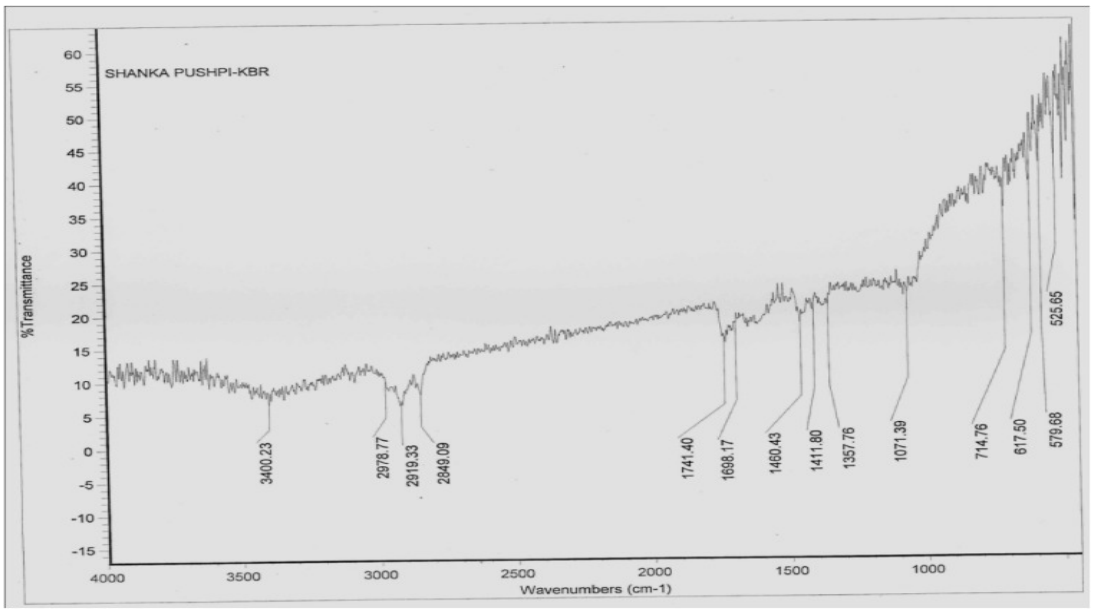


Fig. 5. FTIR spectrum of convolvuluspluricaulis 70% ethanolic extract

2.5 Fourier-Transform Infrared Spectroscopy

Plant leaves were taken from the herb, properly washed before being dried in the shade and powdered.

The powdered leaves were macerated in a shaker for two days with 70% ethanol. The extract was filtered and concentrated with a flash evaporator before being lyophilized to remove the water.

2.6 RESULT FOR FTIR

"Based on the peak's values in the IR radiation region, the functional groups of active compounds of C. pluricaulis were studied using FTIR. The plant extract was passed into the FTIR, the functional groups of the compounds were separated based on its peak's ratio" [12,13]. The absorption range for the FTIR spectra was between 4000 and 500 cm^{-1}. The results revealed the presence of alcohols, carboxylic acids, acid anhydrides, alkanes, phenols, alkynes, alkyl halides, aromatics, halogens, esters.

3. CONCLUSION

In order to increase efficiency, any product with similar therapeutic/ chemical properties, low grade drugs are usually added in products. Phytochemical screening (qualitative method) along with analytical methods i.e. TLC, HP-TLC, GC etc. are used to determine purity of products, quantity of heavy metals and presence of contaminants. HP-TLC analysis of the sarpagandha medicine shown that, sarpagandha churna exhibits 11 peaks while sarpagandha Ghana vati has 17 peaks that indicate fingerprint of test drugs [6]. Chromatogram usually depends on distinct components and concentration of these chemically active components which are not only responsible for therapeutic properties but also different symptomatic/ non-symptomatic side effects. In Shankhpushpi Churna, vanillin sulfuric- acid reagent were scanned under 254, 366 nm and white light at 620 nm which revealed presence of alcohols, carboxylic acids, acid anhydrides, phenols, alkyl halides, aromatics, halogens, esters etc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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