Evaluation of Hydroalcoholic Extract of *Convolvulus pluriculis* (*Shankapushpi*) for Standardization by Colorimetric Method: A Preliminary Report

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Abstract

Aim This study was aimed to evaluate the hydroalcoholic extract of *Convolvulus pluricaulis* (HACP) for standardization using colorimetric method.

Materials and Methods Establishment of standardization for the Ayurvedic formulations is most important for its chemical compounds, biological action, and its quality reassurance in production and manufacturing of traditional herbal medicines. As most of the drugs are standardized, drug companies are using substitute drugs instead of true drugs. So to make finest superiority drugs, it is necessary to validate the raw drugs. Observing the existing trend in mind, HACP was subjected to standardize procedures for the phytochemical tests. The separation of the bioactive substances from the HACP was performed using both manual methods and high-profile thin-layer chromatography (HPTLC).

Results From this study, it is revealed that the seed contains alkaloids, carbohydrates, steroids, tannins, terpenoid, and phenol which gave the medicine numerous therapeutic properties.

Conclusion The study was rapid, reproducible, and could be used for routine monitoring of various biological properties of HACP.

Keywords
- hydroalcoholic
- Convolvulus pluriculis
- phytochemical analysis
- HPTLC

Introduction

The 80% rural population of a country is more tending toward traditional ways of treatment due to easy availability and cheaper cost.[1] In the world, approximately 35,000 to 70,000 species of plant have been used at one time or another for medicinal, pharmaceuticals, and cosmetic.[2] *Convolvulus pluricaulis* (*C. pluricaulis*) popularly known as *Shankapushpi* is one such herb that has been extensively investigated for its pharmacological and therapeutic effects. Its branches spread on the ground which are more than 30-cm long. The flowers are blue/white in color and the leaves which are elliptic in shape are located at alternate positions with branches or flowers. The herb is commonly found throughout India. All the parts of the herb are known to possess therapeutic benefits.[3] The plant contains alkaloid (shankhapushpine), volatile oil, flavonoids (kampferol derivatives), a phytosterol (β-sitosterol), carbohydrates (glucose, rhamnose, and starch), ceryl alcohol, and scopoletin.[4] The fresh plant contains volatile oils, fatty acids, fatty alcohols, and hydrocarbons, that is, myristic acid (30.9%), palmitic acid (66.8%), linoleic acid (2.3%), and straight-chain hydrocarbon hexatriacontane.[5]

It is reported that it is traditionally used to treat nervous debility, insomnia, fatigue, fever, nervous debility, and loss of memory. It is a good remedy in bowel complaints like dysentery. *C. pluricaulis* is used as a one of the main ingredients in the brain tonics. The plant is reported to be a prominent memory enhancer and also a psychostimulant, tranquilizer and reduce mental tension.[6,7] A phytochemical investigations on *C. pluricaulis* have been reported that an

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alkaloid (shankhpushpine), flavonoids, and inorganic salts (e.g., potassium chloride) were present and also two varieties of bases had been isolated viz base A ($C_5H_{11}N0_2$) which depressed the blood pressure in an anesthetized dog and had temporary inhibitory action on pithed frog's heart. Base B ($C_5H_9NO_2$) had no significant pharmacological action.[8]

As the drug has numerous therapeutic properties and its uses in the field of medicine, one should be aware of its photochemistry also. So, to prepare the best quality *C. pluricaulis* drug, it is necessary to authenticate the raw drugs. Keeping the current trend in mind, *C. pluricaulis* was subjected to standardizing procedures. For the current study, genuinity indicating parameters for *C. pluricaulis* were derived.

Materials and Methods

Plant Material

The *C. pluricaulis* whole plant was collected from the Sri Dharmastala Ayurveda Medical College and Research Centre at Udupi in Karnataka, India. The plant material was stored in ambient conditions for further study.

Preparation of Extracts

The *C. pluricaulis* whole plant was dried in the shade and powdered in our research laboratory with the help of pulverizer. The hydroalcoholic extract of *Convolvulus pluricaulis* (HACP) was prepared by soaking 500 g of powder in 2 L of 50% ethanol and 50% cold distilled water for 24 hours, filtered, and concentrated by evaporating on water bath till free from water. The extract has been stored in an airtight container under normal temperature.[9]

Phytochemical tests like tests for alkaloids, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin, quinine, and high-profile thin-layer chromatography (HPTLC) were performed as per the WHO (World Health Organization) guidelines,[10] Ayurvedic Pharmacopoeia,[11] and Indian Pharmacopoeia.[12]

Preliminary Phytochemical Tests

Tests for Alkaloids

a. Dragendroff's test: To a few mg of HACP extract dissolved in alcohol and few drops of acetic acid and Dragendroff's reagent was added, then shake well. An orange–red precipitate formed indicates the presence of alkaloids.[13]
b. Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish-brown precipitate formed indicates the presence of alkaloids.[14]
c. Mayer's test: To a few mg of HACP extract dissolved in acetic acid and few drops of Mayer's reagent was added. A dull-white precipitate will be formed if the alkaloids are present.[15]
d. Hager's test: To a few mg of extract dissolved in acetic acid, 3 mL of Hager's reagent was added, the formation of a yellow precipitate indicates the presence of alkaloids.[16]

Tests for Carbohydrates

a. Molisch's test: To the HACP, along the sides of the test tube, 1 mL of α-naphthol solution and concentrated (conc.) sulfuric acids were added along the sides of the test tube. If carbohydrates are present then a violet color formed at the junction of the two liquids.[17]
b. Fehling's test: Few mg of HACP was mixed with equal quantities of Fehling's solutions A and B. The mixture was warmed in a water bath. If carbohydrates are present, then the formation of a brick-red precipitate is seen.[18]
c. Benedict's test: To 5 mL of Benedict's reagent, a few mg of the extract was added, and boiled for 2 minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.[19]

Test for Steroids

a. Libermann–Burchard test: To the extract was dissolved in chloroform, 1 mL of acetic acid and 1 mL of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. sulfuric acid was added along the sides of the test tube. The appearance of a bluish-green color indicates the presence of steroids.[20]
b. Salkowski's test: The HACP was dissolved in chloroform and equal volume of conc. sulfuric acid was added. Formation of bluish-red to a cherry-red color in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.[21]

Test for Saponins

In a test tube containing 0.5 mL of extract, 5 to 10 drops of dilute HCl and ZnCl were added the solution was boiled for a few minutes. Presence of reddish-pink or dirty-brown color confirms flavonoid.[22]

Test for tannins

To the extract, a drops of dilute solution of ferric chloride ($FeCl_3$) were added, formation of dark-blue color shows the presence of tannins.[23]

Test for Flavonoids

Shinoda's test: in a test tube containing 0.5 mL of extract, 5 to 10 drops of dilute HCl and ZnCl were added to the solution and was boiled for a few minutes. Presence of reddish-pink or dirty-brown color confirms flavonoid.[24]

Test for Phenol

To HACP in alcohol, add two drops of alcoholic ferric chloride. Formation of blue to black indicates the presence of phenol.[25]

Test for Coumarins

To the extract in alcohol, a few drops of 2-N (normal) sodium hydroxide (NaOH) solutions were added. Dark yellow color formation indicates the presence of coumarins.[23]

Test for Triterpenoids
The extract was warmed with tiny bits and a few drops of vinyl chloride. Formation of pink color indicates the presence of triterpenoids.[26]

Test for Carboxylic Acid
Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.[27]

Test for Resin
Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resin.[28]

Test for Quinine
Few mg of HACP was treated with 0.5% of NaOH. If quinine is present it gives deep coloration like pink, purple or red.[26]

High-Profile Thin-Layer Chromatography

Powdered sample of 1 g was dissolved in 10 mL ethanol and kept for cold percolation for 24 hours and filtered. Of the sample, 4, 8, and 12 µL were applied to a precoated silica gel F254 on aluminum plates to a bandwidth of 7 mm using Linomat 5 Thin Layer Chromatography (TLC) applicator. The plate was developed in *n*-butanol:acetic acid:water (4:1:1). The developed plates were visualized in short ultraviolet (UV), long UV, and then derivatised with vanillin sulfuric acid reagent and scanned under 254, 366 nm, and white light at 620 nm. Retention factor ($R_{f)}$, the color of the spots and densitometric scan were recorded.[29]

Results and Discussion

In the present experiment, it has been found that qualitative analysis of phytochemical compounds obtained in HACP by colorimetry (►**Tables 1** and **2**), photo documentation (►**Fig. 1**), the unique R_f values (►**Tables 3**), densitometric scan, and densitogram (►**Figs. 2**–**4**) obtained at different wavelengths from the HPTLC demonstrate that tests for alkaloids, carbohydrates, tannins, terpenoid, and phenol are positive for HACP. Photo documentation, the unique R_f values, densitometric scan, and densitogram obtained at different wavelengths from the HPTLC can be used as a fingerprint to identify the herbal drugs HACP powder. The phytochemical tests performed to serve as a preliminary test for the standardization of the HACP formulation.

Similar findings about the presence of the compounds in the *C. pluricaulis* has been reported by several authors.[30,31] Several reports on *C. pluricaulis* about central nervous system depression, anxiolytic, tranquillizing, antidepressant, antistress, neurodegenerative, antiamnesic, antioxidant, hypolipidemic, immunomodulatory, analgesic, antifungal, antibacterial, antidiabetic, antiulcer, anticatatonic, and cardiovascular activity[32] have been documented. A Study on dry leaves of *C. pluricaulis* by TLC methods was found that phytochemicals like flavonoids and phenol are found.[33] Dry leaf and its ash by colorimetric study was done and it was found the phytochemicals like alkaloids, carbohydrates, phenols, tannins, flavonoids, and resin were found.[34] Our present phytochemical analysis of HACP also confirms similar types of compounds found in other studies. Thus, it can be used for further various clinical trials. By preserving the fundamental aspect of the Ayurvedic drug, the standardization requires a rational approach. The main obstacle in

Table 1 Results of preliminary phytochemical tests

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

Note: (+) denotes present.

Table 2 Preliminary phytochemical test results

Sl No	Tests	Color if positive	*Shankapushpi*
1.	Alkaloids		
	Dragendrof's test	Orange precipitate	Orange precipitate
	Wagner's test	Red precipitate	Red precipitate
	Mayer's test	Dull-white precipitate	Dull-white precipitate
	Hager's test	Yellow precipitate	
2.	Steroids		
	Liebermann–Buchard test	Bluish green	Light red color
	Salkowski's test	Bluish red to cherry red	Cherry-red color in the chloroform and colorless in the acid form
3.	Carbohydrate		
	Molish's test	Violet ring	Violet ring

(continued)

Table 2 *(continued)*

Sl No	Tests	Color if positive	*Shankapushpi*
	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.	Flavanoids		
	Shinoda's test	Red to pink	Colorless solution
6.	Saponins		
	With $NaHCO_3$	Stable froth	No froth
7.	Triterpenoids		
	Tin and thionyl chloride test	Red	Light pink
8.	Coumarins		
	With 2-N NaOH	Yellow	Light brown
9.	Phenols		
	With alcoholic ferric chloride	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		
	Ninhydrine reagent	Purple color	Colorless

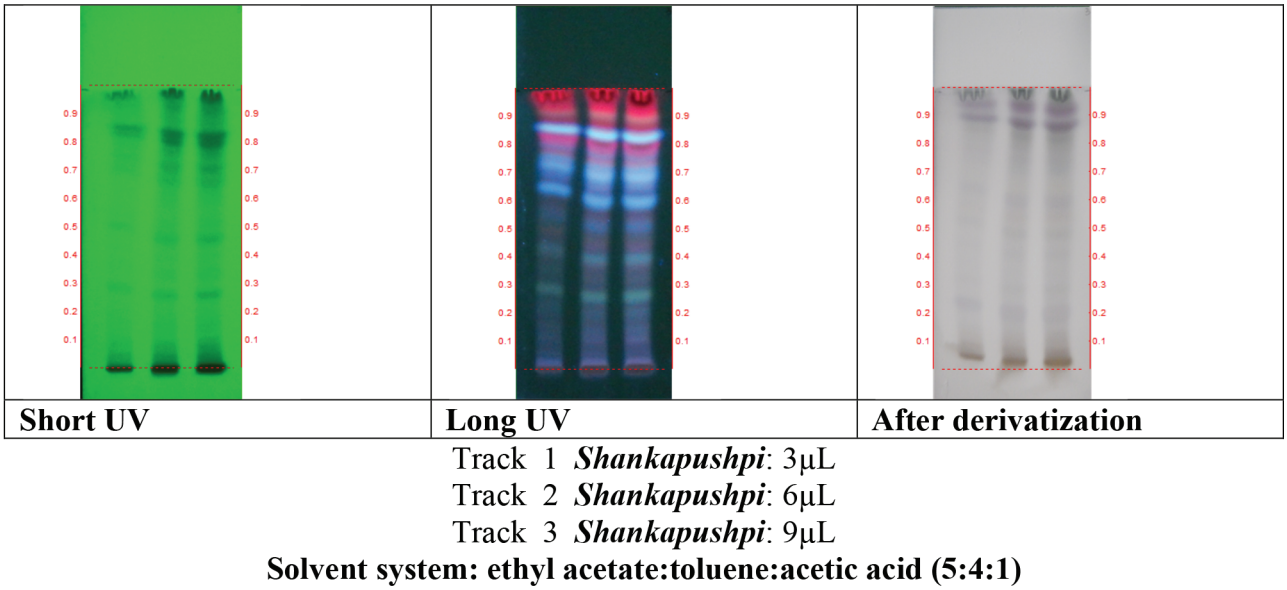


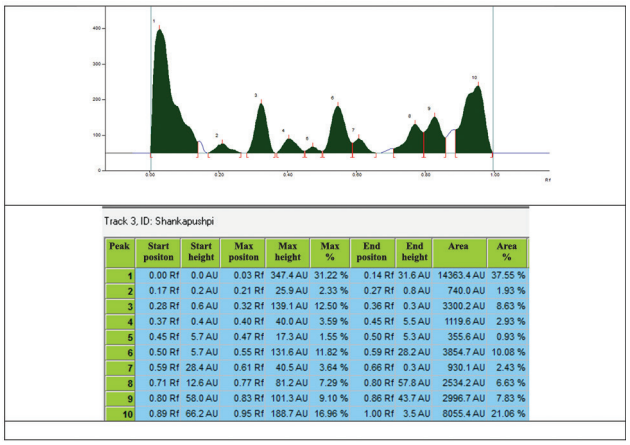
Fig. 1 HPTLC photo documentation of ethanolic extract of *Shankapushpi*. HPTLC, high-profile thin-layer chromatography; UV, ultraviolet.

the standardization of the Ayurvedic drug is the identification of its biological source. Drugs from the different geographical source may vary with its active constituent and it may not be feasible to standardize drug chemically

Table 3 Rf values of samples

Short UV	Long UV	After derivatisation
–	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 (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)

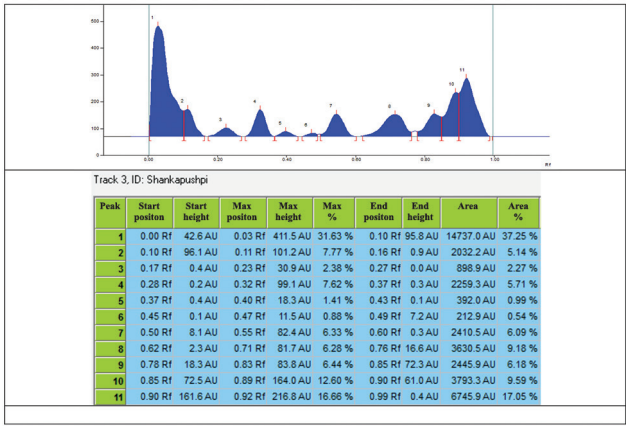
Abbreviations: D, dark; F, fluorescent; L, light; UV, ultraviolet.



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 %

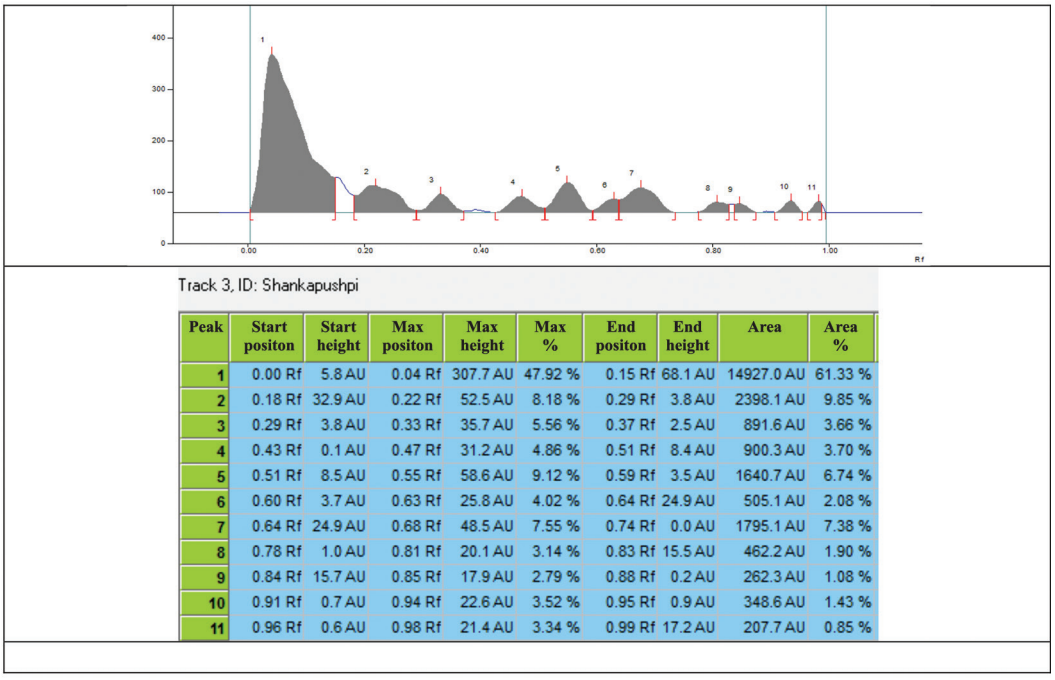
Fig. 2 Densitometric scan at 254 nm.



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 derivatisation at 620 nm.

Conclusion

The parameters used in this work ensure the quality control of HACP. The results found through this study were rapid, reproducible, and could be used for routine monitoring of HACP. HACP is endowed with various biological properties and hence efforts have been made here to provide scientific data on the same.

Conflict of Interest
None declared.

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