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PHYSICO-CHEMICAL STANDARDIZATION OF A POTENT UNANI DRUG "SANKHAHOLI" (*EVOLVULUS ALSINOIDES* LINN.)

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ABSTRACT

Sankhaholi (*Evolvulus alsinoides* Linn.) of family Convolvulaceae which is available in Indian herb and spices market with the name of *Shankhpushpi*, widely used in the traditional system of medicine including Unani medicine since ages. It is a perennial herb with a small woody branched rootstock which contains alkaloids: shankhapushpine and evolvine. Fresh plant of *sankhaholi* contains volatile oil. It also contains a yellow neutral fat, an organic acid, and saline substances. Therapeutic uses of *Sankhaholi*, mentioned in the Unani text are elexiteric (*Mufarreh*), cardiac tonic (*Muqawwi-e Qalb*), brain tonic (*Muqawwi-e Dimag*), blood purifier (*Musaffi-e-Khoon*), general tonic (*Muqawwi-e-aam*), diuretic (*Mudirr-e-Baul*), anti-inflammatory (*Muhallil-e-waram*), hypoglycemic (*Dafa-e-Ziabitus*). It has been used in various ailments such as headache, asthma, hyperlipidemia, etc. In this article, we have provided standardized value of a specimen of *sankhaholi*, assessed on physico-chemical and analytical parameters viz (i) Macroscopic and microscopic features (ii) extractive values (iii), moisture contents (iv), Ash values (v), loss of weight on drying, (vi), pH of 1 % and 10 % solution (vii) TLC and (viii) HPTLC.

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INTRODUCTION

Standardization is a system that ensures a predefined amount of therapeutic effect, quantity and quality of the herbal drugs and now it is considered a prerequisite for the assessment of biological activity of the plant materials. [1] The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health and to ensure reproducible quality of herbal medicine. [2] Since, the efficacy of a drug mainly depends upon its physical and chemical properties therefore, the determination of physico-chemical characters to ascertain the authenticity of a drug is necessary before subjecting it for pharmacological screening. A little deviation from the normal in terms of quality and quantity of the constituents may alter the effect of a drug. In view of these facts, physico-chemical standardization of *sankhaholi [Evolvulus alsinoides* Linn. (EA)] was carried out to characterize the drug sample and set a standard of its quality. The drug *sankhaholi* (EA) of family Convolvulaceae which is commonly known as *shankhpushpi*, in the traditional system of medicine including Unani medicine. [3, 4] It is a perennial herb with a small woody branched rootstock which contains alkaloids: shankhapushpine and evolvine. Fresh plant of *sankhaholi* contains volatile oil. It also contains a yellow neutral fat, an organic acid, and saline substances. [5, 6, 7] Therapeutic uses of *sankhaholi*, mentioned in the Unani medicine are alexiteric (*mufarreh*), cardiac tonic (*muqawwi-e qalb*), brain tonic (*muqawwi-e dimag*), digestive (*hazim*), blood purifier (*musaffi-e-khoon*), general tonic (*muqawwi-e-aam*), diuretic (*mudirr-e-baul*), anti-inflammatory (*muhallil-e-waram*), hypoglycemic (*dafa-e-ziabitus*), and antihypertensive (*dafye imtela*). It has been used in the headache, asthma, hyperlipidemia etc. since centuries and recent studies have substantiated its beneficial therapeutic effects. [8, 9, 10, 11]. In the present paper, *sankhaholi* was subjected to physico-chemical and chromatographic analysis to ensure the quality of herb, available in Indian market.

Materials and methods

Plant material identification

Whole plant of *sankhaholi* (*Evolvulus alsinoides* Linn.) were purchased from the local market at Khari Baoli, Delhi, India. Specimens was identified and authenticated by Prof. (Dr.) M.P Sharma of Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India as *sankhaholi*.

Macroscopic and microscopic features: were recorded.

Moisture content (M_c)

The powdered material (5g) was placed in a moisture dish and dried to a constant weight in hot oven at 100-105^{0}C. [12] The loss of weight (in mg/g) of air dried was calculated as follows:

$$Mc\ (\%) = (W0 - W3/W0) \times 100$$

W0= Weight of the moist sample = 5 g
Weight of empty china dish=W1
Weight obtained after successive drying= W2
Weight of dried sample (W3) = W2-W1

pH of *sankhaholi*

pH of 5% solution

5 g of *sankhaholi* was dissolved in 100ml of distilled water. The resulting solution/ mixture was filtered and pH was measured with a standard glass electrode.

pH of 10% solution

The experimentation was performed in the same manner as above taking 10g of *sankhaholi* instead of 5g.

Ash value

The powdered material (5 g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600 °C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. [12]
The content of total ash (in mg/g) of air-dried material was calculated as follows:

Total ash value

$$\%\ Ash\ (dry\ basis) = W_{Ash} / W_{Dry} \times 100$$

W_{Ash} = Weight of the ashed sample
W_{Dry} = Weight of the dried sample
Percentage of total ash = $W_{Ash} / W_{Dry} \times 100$

Acid insoluble ash content

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. [12] The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\textbf{Percentage of acid insoluble ash} = W_{HCl} / W_{Dry} \times 100$$

W_{HCl} = Weight of HCl insoluble ash
W_{Dry} = Weight of the dried sample

Water soluble ash

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight. [12].

$$\textbf{Percentage of water soluble ash} = W_{H2O} / W_{Dry} \times 100$$

W_{H2O} = Wt. of water soluble ash = Total ash wt. – wt. of water insoluble matter

Successive hot extractive value

25 g of powdered *sankhaholi* was extracted successively with different solvent system like petroleum ether, then chloroform, then ethanol and at last in water through soxhlet apparatus for 6 h for each solvent at constant temperature of 40oC. The liquid extracts thus obtained were put over water bath and evaporated to dryness. The dried extracts were afterward kept for 5 min in hot oven and their constant successive extractive values were recorded.

Reaction of Sankhaholi with different reagents

Sankhaholi was treated with various reagents and color in the test tube was noted.

Thin Layer Chromatography (TLC Profile)

Sample solution preparation:

Methanolic extract of *sankhaholi* was used for TLC and HPTLC analysis. Methanolic extract was prepared by dissolving 25g of herb in 100 ml of methanol. Drug solution thereafter filtered and filtrate was concentrated under reduced pressure. This methanolic extract was used in HPTLC and TLC.

Mobile phase development (solvent system):

Initially, various combination were tried such as chloroform: petroleum ether: ethyl acetate (2:2:1), and other solvents in various ratios. Finally upper layer of Toulin: Ether: 10% Acetic acid: (1:1) was used as mobile phase for methanolic extract of *sankhaholi*.

TLC procedure:

TLC pre-coated Almina silica gel of 60F254 (20x10 cm), pre-heated at 60 degree for 15 min in hot air oven to evaporate the moisture. Then, a drop of methanolic extract of 20mg/dl concentration was applied on TLC plate at a distance of approximately 1cm from the base of the plate. When solvent covered almost 80% of TLC plate then removed from TLC chamber. TLC plate was dried in air. TLC plate was visualized in white light, UV 254 (short wavelength), UV 366, UV 400, UV 500 (long wavelength) with suitable developing agent (anisaldehyde spray). R_f values of spots were measured. [12]

HPTLC (high performance thin layer chromatography)

HPTLC Procedure:

The sample was spotted in the form of bands of width 4 mm using Camag 100 µL sample. (Hamilton, Switzerland) syringe on pre-coated silica gel 60F254 aluminium plate (20cm x 10cm) using a Camag Linomat- Vsample applicator (Switzerland). The plates were activated at 60° for 0.5 h prior to chromatography. The mobile phase consisted of toulin: ether (1:1:10% acetic acid). Linear ascending development was carried out in 20cm x 10cm twin through glass chamber, previously saturated with mobile phase for 15 min. The length of the chromatogram run was 80 mm. After the development, plates were dried in air. Densitometric scanning was done with Camag TLC scanner III using deuterium lamp in absorbance mode at wavelength of 450, 540 nm. [12]

RESULTS

Macroscopic and microscopic features

Colour : Greenish.
Odour : Aromatic and fragrant penetrating.
Taste : Test less slightly toward sweetness.
Consistency : small woody branched rootstock, stem membranous,
Leaves are small numerous, Flowers are light blue or deep blue.

Sankhaholi is a perennial herb with a small woody branched rootstock, stem membranous, more than 30 cm long, prostrate, spreading, slender or rounded wiry usually covered with long spreading hairs, but sometime quite glabrous. Leaves were small numerous 6-20 by 4-8 mm, alternate, elliptic-oblong, obtuse, strongly petioles very short, sometimes almost absent. Flowers were light blue or deep blue very small solitary or sometime in pairs. Peduncles were very long, filiform and axillary. Calyx densely silky, sepals 4 mm long, lanceolate. The powdered drug was greenish in color.

Moisture content (M_c)

Sankhaholi had moisture content of 9.8%.

pH of the solution

pH of 5 and 10% solution was 6.51 and 6.29 respectively.

Ash value [Table 1].

Table 1: Ash value of *sankhaholi.*

S. No.	Ash value	Value
1.	Total ash value	9%
2.	Acid insoluble ash	5.8%
3.	Water soluble ash	1.8%

Successive hot extractive value [Figure 1].

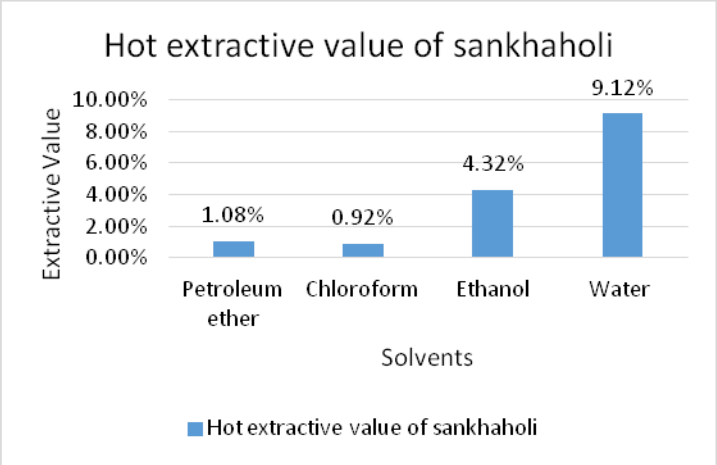

FIGURE 1: HOT EXTRACTIVE VALUE OF *SANKHAHOLI.*

Reaction of *sankhaholi* with different reagents [Table 2].

Table 2: Reaction of *sankhaholi* with different reagents.

Reagents	Observation
	Sankhaholi
Conc. HCL	Dark green
Conc. HNO_3	Black
Conc. H_2SO_4	Dark brown
Glacial acetic acid	Light green
Powder	Greenish

TLC analysis

Spots of different color were visible in the TLC. R_f values of spots are mentioned in the table 3 and figure 2.

Table 3: Rf value of TLC of *sankhaholi.*

Drug	Extract	Solvent system	Rf value in iodine chamber	No. of spots
Sankhaholi	Methanolic	Toulin Ether 1 : 1 10% dilute acetic acid	0.81 (Green) 0.63 (Brown) 0.42 (Green) 0.24 (Green) 0.15 (Green)	5

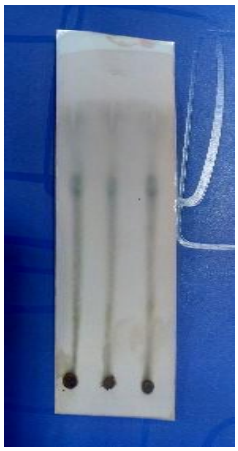

FIGURE 2: TLC OF *SANKHAHOLI.*

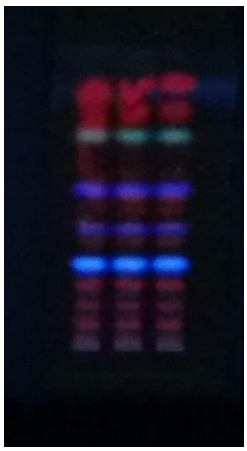

FIGURE 3: HPTLC OF *SANKHAHOLI.*

HPTLC analysis

Many spots were visualized in HPTLC, which indicated many chemical components and pharmacological actions. The peak obtained was pure. [Figure 3, 4, 5; Table 1, 2].

Table 4: R_f Value of methanolic extract of *Sankhaholi* at 254 nm.

S. NO	R_f	Track 1	Track 2	Track 3
1.	0.06	151.3 AU	304.0	202.8
2.	0.13	182.1 AU	340.9	336.0
3.	0.21	330.8 AU	266.6	576.3
4.	0.27	247.4 AU	149.4	417.7
5.	0.40	503.2 AU	----------	3071.2
6.	0.42	385.6 AU	213.3	2096.3
7.	0.50	6624.9 AU	5035.0	----------
8.	0.57	914.1 AU	5184.6	----------
9.	0.62	6445.0 AU	7266.1	26554.7
10.	0.77	52437.8 AU	45077.9	78708.9

Table 5: R_f Value of *methanolic extract of Sankhaholi* at 366 nm.

S. NO	R_f	Track 1	Track 2	Track 3
1.	0.06	824.3 AU	559.6 AU	1404.7 AU
2.	0.13	644.4 AU	492.8 AU	1231.7 AU
3.	0.16	1030.9 AU	798.9 AU	1816.2 AU
4.	0.21	1617.7 AU	1160.2 AU	3076.6 AU
5.	0.27	1551.6 AU	1520.7 AU	2825.7 AU
6.	0.36	374.6 AU	937.9 AU	1087.0 AU
7.	0.41	639.6 AU	1379.9 AU	3023.6 AU
9.	0.50	928.4 AU	1850.8 AU	3105.1 AU
10	0.58	703.1 AU	1015.3 AU	1472.7 AU
11	0.63	292.7 AU	175.8 AU	----------
12	0.70	754.2 AU	928.4 AU	1789.3 AU
13	0.83	4117.5 AU	8518.9 AU	10582.6 AU
14	0.85	3380.2 AU	7407.9 AU	8425.0
15	0.93	727.2 AU		----------

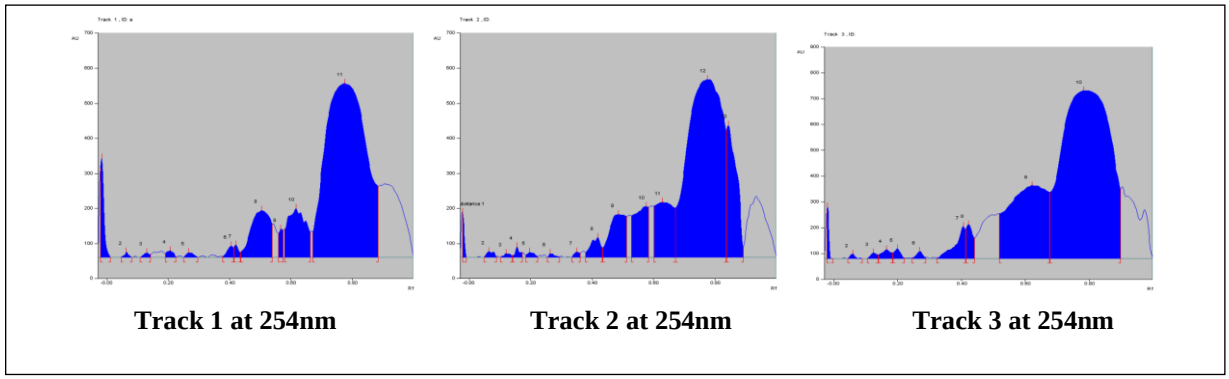

FIGURE 4: METHANOLIC EXTRACT OF SANKHAHOLI AT 254NM

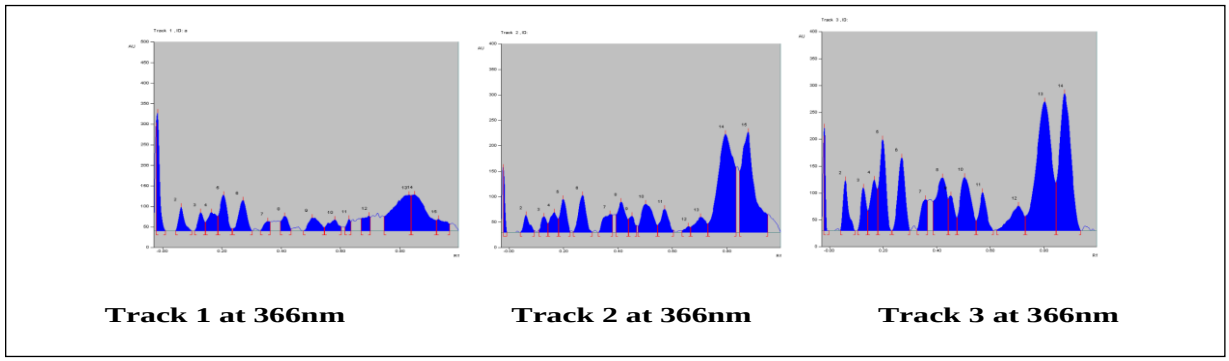

FIGURE 5: METHANOLIC EXTRACT OF SANKHAHOLI AT 366NM

DISCUSSION AND CONCLUSION

Morphological characterization of a drug helps in identification of the native plant as well as detection of adulteration. In some cases, quality of crude drug can be checked on the basis of morphology only. In our study, we have observed that test drug had a moisture content less than 10%. Low moisture content is always desirable for stability of drug. [12] High ash value of 9% suggests presence of high inorganic matter. Lower value of the acid insoluble ash suggests the greater physiological availability of drug. Extractive value gives information about availability of soluble phytoconstituents in particular solvent. [13] Water soluble extractive is more as compared to ethanol, petroleum ether and chloroform extractive value suggesting that aqueous extract would be more beneficial as compared to methanolic extract for therapeutic purposes. In HPTLC, many spots were seen, which indicated many chemical components and pharmacological actions. Thus, sample of *sankhaholi* was good in quality on physiochemical and chromatographic parameters.

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