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PHYSIOCHEMICAL STANDARDIZATION AND HPTLC OF *ARTEMISIA ABSINTHUM*, LINN COLLECTED FROM KHARI BAOLI, DELHI

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ABSTRACT

Wormwood (*Artemisia absinthium*) has been used for various medical ailments with an age old tradition. Apart from therapeutic usage it has also been used as an ingredient in the liquor absinthe. *Wormwood* is a native to temperate regions of Europe, Asia and northern Africa. *Artemisia absinthium* of Asteraceae family is a perennial shrubby plant and extensively used in Indian system of medicine such as Unani, Ayurveda, Sidha etc. since the time of Greek. In Unani system of medicine, infusion or decoction of the herb has been used since centuries as hepatoprotective, antipyretic, anti-inflammatory, anthelmintic, diuretic, purgative of bile, insecticidal, appetizer etc. Therefore in this study, we have provided chromatographic fingerprinting of decoction of Artemisia to evaluate concentration of pharmacologically active constituents. Physiochemical characterization of drug material procured from Khari Baoli Old Delhi has been done. In addition, difference in extractive value of *Artemisia absinthium* through decoction based classical method versus through reflux distillation has also been reported in this paper.

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INTRODUCTION

Artemisia absinthium Linn, commonly known as wormwood or 'afsanteen' is a member of Asteraceae family and grows in North Asia- Kashmir, Nepal and Mountainous district of India 5000-7000 ft where locally known as 'Tethwen'. [1-3] It is an aromatic, pubescent, shrubby and perennial plant, 100 cm in height with intensely bitter astringent [1, 4-6] unpleasant odor and occur throughout the year. Flowering and fruiting takes place from July to September. So collection is usually done in these months. The species is main source of 'Afsanteen' used in India. [2, 7-11]

Whole plant of *Artemisia absinthium* Linn (*A. absinthium*) consist of stems, twigs, leaves and flowers heads have been used in the Unani system of medicine, Ayurveda, Sidha etc. since centuries in the treatment of various medical ailments. [7-11] Chemical constituent of flowers and leaves include volatile essential oil, absinthin- a bitter glycoside (active principle) very soluble in alcohol but less so in ether and slightly in water, anabsinthin- bitter substance, absinthic acid identical as succinic acid, artemetin- crystalline compound, artabsin, azulin- best sources (40-70 mg), [2, 7-12] tannins/ phenols, resins, succinic acid, maltes, nitrates of potassium, lead, aluminum, iron, calcium, magnesium, sodium, and ash. The bitter taste of wormwood is from sesquiterpene lactones (0.15-0.4%) [13]- absinthin and artabsin being the main ones [13-15] and guainolides. [13] Volatile oil obtained through wormwood is known as 'Absinthe' or 'Wormwood Oil' is having a camphoraceous odor and dark brown or yellow in color is obtained by distillation. Oil yield varies from 0.12 to 0.52% (fresh basis). It contains thujone or absinthol, thugyl alcohol, cadinene, phellandrene, pinene, S-guaiazulene, turpenes 2 p. c., and a deep blue oil. [2, 3, 7, 9-11, 16-18]

In Unani system of medicine, whole plant is used for medicinal purposes after boiling with water in certain proportions which is known as decoction. In clinical practice, decoction of wormwood has been used widely to treat various diseases such hepatitis, hepatomegaly, jaundice, splenomegaly, cirrhosis, obstruction in hepato-billiary system, anorexia, fever, flatulence, [3, 4, 10, 11, 17, 19] worm infestation, [3, 6, 11, 17, 21, 22] paralysis, skin disease, [12, 17] insect bite [6, 20] etc.

In the present paper, physio-chemical standardization of the powder of *Artemisia absinthium*, Linn (*A. absinthium*) with chromatographic analysis of decoction of *A. absinthium*, prepared as per the instructions of Unani pharmacopeia of India have been done. In addition, it also provides difference in extractive value of decoction based classical method and reflux distillation.

MATERIAL AND METHODS

Aim

To insure the quality of 'afsanteen' (*Artemisia absinthium*) procured from Khari Baoli, Delhi. In addition, an attempt has been made to compare the difference in quantity of extract matter, obtained after using decoction based classical method versus reflux distillation of 'afsanteen'.

Plant material collection and identification

'Afsanteen' (*Artemisia absinthium*) was purchased from a local market of Khari Baoli, Old Delhi, India. Plant materials were authenticated as afsanteen (*A. absinthium*) by Prof. (Dr.) M.P Sharma of Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India.

Physio-chemical parameters

Physiochemical parameters were determined for *A. absinthium* according to the methods described in the WHO guidelines. [23]

Morphological features

Macroscopic observation of plant material of *A. absinthium* was done. It comprised of shape, size, surface, consistency, color, odour, taste etc. Transverse sections of *A. absinthium* stems were taken by using a microtome. Permanent mount of stem was prepared using by double staining technique. [24]

Loss on drying /Moisture content (M_c)

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

$$\mathbf{M_c\,(\%) = (W_0 - W_3/W_0) \times 100}$$

$W_{0=}$ Weight of the moist sample = 5 g
Weight of empty china dish=W_1
Weight obtained after successive drying= W_2
Weight of dried sample (W_3) = W_2-W_1

Ash value

5 g of powdered drug was placed in a crucible. It was ignited up to a constant weight by gradually increasing the heat to 500-600 °C until it became white.
The content of total ash (in mg/g) of air-dried material was calculated as follows:

a) Total ash value

% Ash (dry basis) = W_{Ash} / W_{Dry}×100

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

b) Acid insoluble ash content

HCl (2 N; 25 mL) was added to the crucible containing the total ash. It was then 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. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

Percentage of acid insoluble ash = W_{HCl} / W_{Dry}×100

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

c) Water soluble ash

25 mL of water 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 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.

Percentage of water soluble ash = W_{H2O} / W_{Dry}×100

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

pH of crude drugs

a) pH of 5% solution

5 g of drug was dissolved in 100ml of distilled water. Filtered solution was pH was measured with a standard glass electrode.

b) pH of 10% solution

10g of drug instead of 5g was taken and similarly pH was measured.

Successive hot extractive value

25 g of coarse powder of the drug 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 40°C. The liquid extracts thus obtained was evaporated to dryness. After keeping dried extracts for 5 min in hot oven and their constant successive extractive values were recorded.

Percentage hot extractive value was calculated using following formula.

% hot extractive value = (extract obtained / plant material taken) X100

Powder drug reaction with different reagents

Coarse powder of drug was treated with different reagents and the color of the solution appeared in the test tubes were noted.

Extractive matter of *A. absinthium* using decoction based classical method versus reflux distillation

Decoction of 100g of coarse powder of *A. absinthium* was prepared as per the method given below. Decoction obtained was further dried in the hot oven till a constant extractive value was obtained. Extract obtained was weighed and recorded. Similarly, 100 g of the coarse powder was refluxed with 800 ml of water at a constant temperature of 40°C on mental continuously for 5 hr. Drug solution thus obtained was strained, filtered and dried up to a constant weight. Extractive value thus obtained through reflux distillation was weighed and recorded.

Preparation of sample solution (decoction)

Step 1: Preparation of powder:

Sample of *A. absinthium* was minced (ground) into small pieces. The plant material was then pulverized to powder form with the help of a mixer grinder.

Step 2: Decoction:

100 g of drug was mixed with 1600 ml water (16 times of the drug) in the 5000 ml round bottom flask. Drug was kept in the water overnight. Next day, drug solution was heated at 40^{o}C on mental till it contracted to 1/4th of the original volume (400 ml) as per advised by Unani scholars and documented in Unani pharmacopeia of India for the preparation of the decoction. The drug solution took approximately 5 h to get shrink to 400 ml. Once drug solution cool down, it was strained & filtered with the help of muslin cloth to separate the solid residue. The liquid extract was then dried up to 200ml (as per advised by Unani scholars) and preservative was added and marked as Decoction (*Joshanda*) Artemisia.

Preparation of solvent system

Solvent systems were developed to establish the HPTLC pattern of decoction (aqueous based extract) of afsanteen (*A. absinthium).*

Mobile phase development: Initially, various combination were tried such as chloroform: petroleum ether: ethyl acetate (2:2:1), Hexane: chloroform (5:5), butenol: acetic acid: water, and other solvents in various ratios. Finally toluene: ethyl acetate: formic acid: methanol (4:3:0.5:1) was used as mobile phase for decoction (water based) of *A. absinthium.*

Thin layer chromatography analysis

TLC pre-coated Alumina silica gel of 60F254 (20x10 cm) which was pre-heated at 60 degree for 10 min in hot air oven to evaporate the moisture. Then, a fine drop of decoction in a concentration of 20mg/ mL was applied on TLC plate at a distance of approx. 1cm from the base of the plate. TLC plate was kept in straight upright position with its base partially dipped in prepared mobile phase. When solvent covered almost 80% of TLC plate then removed from TLC chamber. After drying in air, TLC plate was heated for 10 min in hot oven at 100^{o}C after anisaldehyde spray to visualize spots of different colors. TLC plate was visualized in white light, UV 254 (short wavelength), UV 366, UV 400, UV 500 (long wavelength) etc.

High performance thin layer chromatographic analysis (HPTLC)

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 aluminum plate (20cm x 10cm) using a Camag Linomat- Vsample applicator (Switzerland). The plates were activated at 60^{0} for 0.5 h prior to chromatography. The mobile phase consisted of toluene: ethyl acetate: formic acid: methanol (4:3:0.5:1). 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 performed on Camag TLC scanner III (Switzerland) using deuterium lamp in absorbance mode at wavelength of 440 & 540 nm.

RESULTS

Morphological features

Dried sample of the plant consisted of broken stem, twigs, leaves and flower heads. The stem and twigs had prominent ridges and furrows covered by white hairs. Leaves and twigs were silvery hoary on both surfaces. Flowers head showed the receptacle with long white hair.

Microscopically, stem in transverse section showed a prominent wavy outline. The young stem and twigs showed outer single layer of epidermis which consisted of cubical cells. Many of the epidermal cells are extended outwards to form trichomes. When ground, powder of plant appeared brownish green in color.

Moisture content

Sample of *A. Absinthium* had moisture content of 9.6%.

Ash value

Ash value is given in the table 1:

Table 1: Ash value of *A. absinthium*.

Total ash	5 %
Acid soluble ash	2.4%
Water soluble ash	1.4%

pH value

pH of 5% solution and 10% solution of powdered drug was 7.40 and 7.67 respectively.

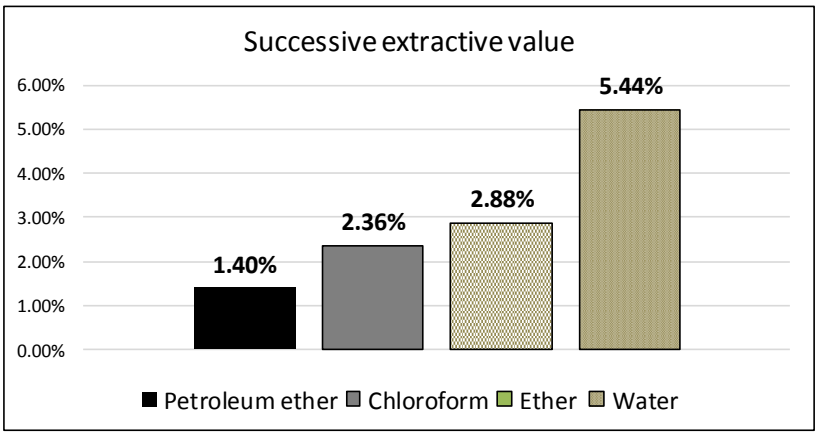


Figure 1: Successive extraction of powder of A. Absinthium.

Extractive matter of *A. absinthium* using decoction based classical method versus reflux distillation
Extract obtained from decoction of 100g of *A. absinthium* was 14.38 g while through reflux distillation, it was 14.43 g.

Successive hot extraction value of plant material
Successive extractive value in different solvents, given in the figure 1.

Powdered *A. absinthium* reaction with different reagents
Reaction of powdered plant material with different reagents are given in the table 2.

Table 2: Powder drug reaction with different reagents.

Reagent	Observations
Conc. HCl	Black
Conc. HNO_3	Dark brown
Conc. H_2SO_4	Black
Glacial acetic acid	Light green
Powder	Dark brownish green

TLC analysis
5 spots of different color were visualized in the TLC plate. [Figure 2] R_f values are given in the table 3.

Figure 2: TLC plate.

Table 3: TLC of decoction of A. *Absinthium*.

Drug	Extract	Solvent system	R_f value in iodine chamber	No. of spots
Artemisia absinthium	Decoction (aqueous)	Toluene : Ethyl acetate : formic acid : methanol 4 : 3 : 0.5 : 1.0	0.90, 0.80 0.52, 0.76, 0.15	5

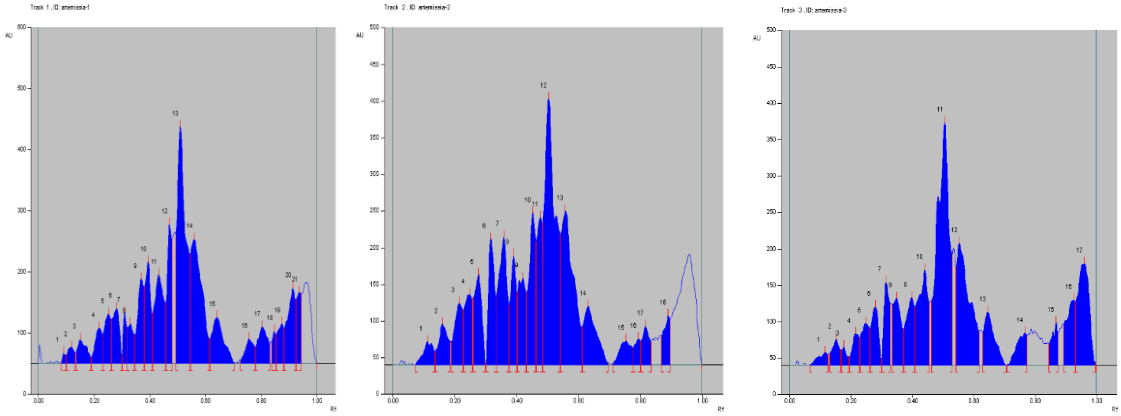
HPTLC analysis

Many spots were visualized at UV 440 and 540 nm.

R_f value of decoction *A. Absinthium* at 440 nm (Table 4; Figure 3)

Table 4: R_f value of decoction of *Artemisia absinthium* at 440 nm.

S. No.	R_f	Area (AU)		
		Track 1	Track 2	Track 3
1.	0.15	1057.2	1414.6	693.5
2.	0.22	1244.3	1870.4	812.8
3.	0.25	1541.4	1861.3	1223.9
4.	0.28	1908.3	2455.3	1558.9
5.	0.31	845.7	2818.8	1722.3
6.	0.33	1087.4	--------	---------
7.	0.37	2543.6	3896.6	---------
8.	0.39	2852.0	2216.2	2043.0
9.	0.43	4064.2	2403.6	3507.2
10.	0.47	3147.6	2842.9	---------
11.	0.51	10195.8	10515.6	10564.5
12.	0.56	6921.9	7079.1	6003.2
13.	0.64	2675.7	2741.0	2108.4
14.	0.76	1025.1	1038.2	1221.9
15.	0.80	1715.2	515.6	--------
16.	0.85	544.7	--------	--------
17.	0.88	1200.7	1174.5	976.4
18.	0.92	2854.0	---------	2159.7
19.	0.94	1678.9	---------	4408.2



Track 1 **Track 2** **Track 3**

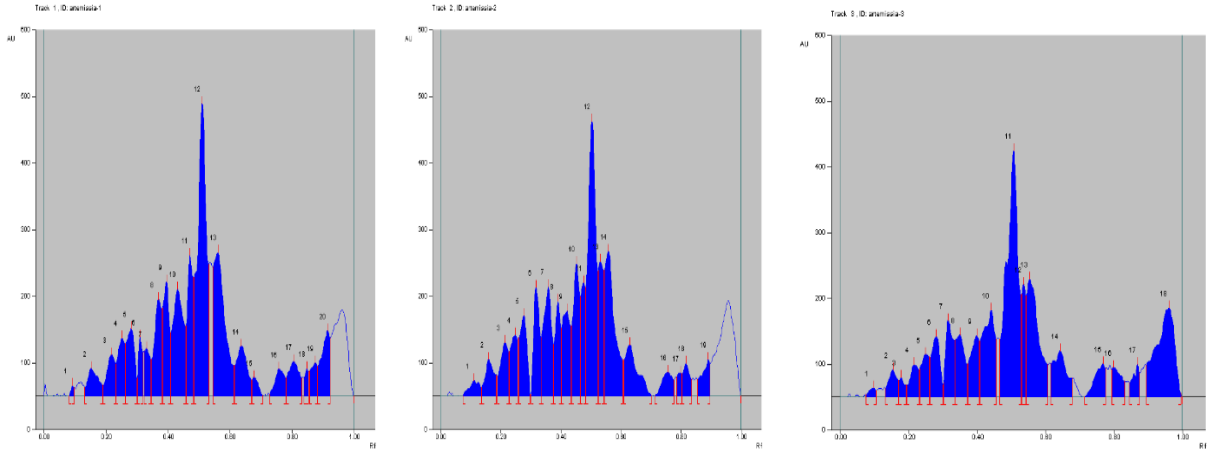
Figure 3: Decoction of artemisia at 440 *nm.*

R_f value of decoction *A. Absinthium* at 540 nm (Table 5; Figure 4).

DISCUSSION AND CONCLUSION

Standardization and adherence to reliable protocol to assess quality of the Unani drug material using standard techniques are extremely important. Quality control profile of any plant drug is the macroscopic and microscopic evaluation, physiochemical characterization and HPTLC finger printing to ensure their authenticity, stability and reproducibility in results. In the present study we have procured a sample of plant material of *A. absinthium* from Khari Baoli Old Delhi. Physiochemical characterization of the drug revealed that the plant material was good in quality when compared with standards, given in Unani pharmacopeia of India. [11]

Plant material had a moisture content of 9.6%. Upon ignition, ash content was 5% which was less than standard 8% as per the Unani pharmacopeia of India, for a good quality sample of *A. absinthium*. A high ash value above standard implies of contamination, substitution and adulteration. [25].



Track 1 Track 2 Track 3

Figure 4: Decoction of artemisia at 540 nm.

In this study, we have prepared decoction of the *A. absinthium* and used it for TLC and HPTLC analysis. Decoction is an age old technique used to prepare liquor of desirable drug obtained after heating or boiling a herb of medicinal value in the water. [26] We have compared the extractive value of *A. absinthum* obtained through the method documented in the classical text and through reflux distillation. There was no significant difference in extractive value thus decoction can be used as a synonym to aqueous extract but prepared through classical method.

HPTLC is a low cost, simple and speedy procedure and now a routine analytical technique for providing fingerprints of drug material used in a particular study to ensure accuracy, reliability, robustness and reproducibility in further studies. [27]

HPTLC fingerprinting of water based decoction of *A. absinthium* showed various peaks which demonstrates the concentration of chemical constituents in the plant material sample. Many peaks were obtained indeed justified the high pharmacological and medicinal value of the decoction since ages.

Table 5: R_f Value of decoction of* Artemisia *absinthium at 540 nm.

S. No.	R_f	Area (AU)		
		Track 1	Track 2	Track 3
1.	0.15	1189.9	1345.6	838.7
2.	0.22	1366.0	1749.5	1009.2
3.	0.25	1619.5	1736.4	1262.0
4.	0.28	2201.7	2400.3	1916.5
5.	0.31	941.7	2661.6	2164.0
6.	0.33	1111.0	-------	-------
7.	0.37	2859.3	3682.9	2105.3
8.	0.40	2797.3	2132.9	1971.5
9.	0.43	4590.9	2789.1	3801.8
10.	0.47	3123.2	2118.0	------
11.	0.51	10170.4	8706.6	10303.0
12.	0.56	6781.1	6018.1	5215.4
13.	0.64	2187.5	2705.1	2318.7
14.	0.68	464.6	--------	-------
15.	0.76	1032.6	1046.4	1258.7
16.	0.81	1488.9	462.3	1051.5
17.	0.85	417.2	911.6	-------
18.	0.88	887.4	1217.8	769.7
19.	0.92	2210.2	--------	6117.1

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Conflicts of interest
None declared

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