Research Article

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STANDARDIZATION OF APAMARGAKSHARA TAILA

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ABSTRACT

Taila Kalpana (Medicated oil) are integral part of Ayurvedic treatment and Taila (Oil) can be used for both Bahya (Externally) and Abhyantara Chikitsa (Internally). Standardization of Ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile, or quality assurance for production. The Apamarga Ksharasootra is well proven to be an effective treatment for fistula-in-ano and has been standardized by the CCRAS, an apex research organization of Government of India in the field of Indian system of medicine. Mechanical introduction of the Ksharasootra into the tract is complex and painful procedure. More over it has limitation in the treatment of multiple fistulae. Injection of the liquid into the tract can minimize many of these drawbacks as it can diffuse throughout the fistulous tract and it is simple, painless, non-invasive and cost effective management. So Apamargakshara Taila was prepared as mentioned in classics for the purpose to evaluate the effects in Bhagandara (Fistula-in-ano). Before clinical trial, standardization of Apamargakshara Taila was performed to know its physical, chemical and HPTLC profile as per WHO and AYSUH testing guidelines and it was found that, specific gravity was 0.9431, rancidity was slightly oxidised, viscosity was 89.0120, refractive index was 1.4699, acid value was 2.20, saponification value was 161.90, unsaponification matter was 3.47, iodine value was 82.43 and ester value was 159.70. At 254 nm and 366 nm showed 5 and 12 spots respectively and after derivatisation also showed 12 spots.

Keywords: *Achyranthes aspera*, Kshara Taila, Quality control, Standardization

INTRODUCTION

Veda are the oldest writings available to mankind on this earth. Plenty of medicinal uses have been enumerated in these authentic texts. The drug Apamarga (*Achyranthes aspera* Linn.) is wild perennial herb which grows 30 to 90 cm in height with features such as branched tap root; stem is aerial erect, herbaceous, hairy and green; leaves are petiolate, opposite and semiorbicularis with suddenly pointed apex; reflexed flowers are arranged on long peduncle (spike); flowers are bisexual, tetracyclic, small, green and actinomorphic; fuit is indehiscent achene enclosed within persistent perianth and bracteole[1].

Kshara (Alkali) is derivative of plant drug ashes in the form of solutions, powder or crystals, all of which have the basic quality of being alkaline in nature. The prepared drug substance is called Kshara (Alkali), because it causes Ksharana (Destruction) to Mamsa (Tissue) and other Dhatu[2]. Acharya Sushruta defines the Kshara (Alkali) as the substance possessing Ksharana and Kshanan (Destruction) properties[3] and told that Kshara (Alkali) have Chedana (Excision), Bhedana and Lekhana (Scrapping) properties and also has Tridoshahara (Equilibrium of Vaata, Pitta and Kapha) properties[4].

Apamargakshara Taila (AKT) is mentioned in Chakradatta and Bhaishajya Ratnavali in the context of Karna Roga Adhikara which is prepared by Apamargakshariya Jala (alkali in liquid form) 4 parts, Tila Taila (Sesame Oil) 1 part and Apamarga Kshara Kalka (Paste) 1/4th part of Tila Taila (Sesame Oil), mixed together and heated on Mandagni (Low fire) till getting Taila Siddhi Lakshana[5].

Standardization[6] of Ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile, or quality assurance for production. AKT was prepared as mentioned in classics in Rasashashtra and Bhaishajya Kalpana for the purpose to evaluate the effects in Bhagandara (Fistula-in-ano). Before clinical trial the standardization of AKT was done to know its physical and chemical profile.

MATERIALS AND METHODS

Apamarga raw drug of about 25 kgs was procured from SDM Ayurveda Pharmacy, Udupi, Karnataka, India and AKT was prepared as mentioned in classics in Rasashashtra and Bhaishajya Kalpana laboratory of SDM College of Ayurveda, Udupi, Karnataka, India and physicochemical analysis like specific gravity, rancidity test, viscosity, refractive index, determination of acid value, saponification value, unsaponifiable matter, iodine value, ester value and HPTLC were carried out. The studies were done at SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India as per standard procedure for quality assurance specified by AYSUH[7] and WHO[8].

Method of preparation of AKT

Preparation of Kshariya Jala

Apamarga (*Achyranthes aspera*) raw drug of about 25 kg was procured from SDM Pharmacy,Kuthpady,Udupi. The drug was dried well for two days under sunrays and unwanted particles was removed from it. The drug was taken in an iron pan and burnt well until getting the ash. The ash was sieved and the fine

ash weighed .The quantity obtained was 3.5kgs in weight and 8 litres in volume. Then 6 times of water was added i.e.48 litres of water and the ash was soaked in the water for overnight. Next day filtration of the soaked solution of Kshara was done 21 times with a cotton cloth. 10 litres of Kshariya Jala was kept for Taila preparation and remaining 38 litres was used for preparation of Mrudu Kshara.

Preparation of Mrudu Kshara

The 38 litres of Kshariya Jala was taken in an iron pan and it was heated on Mandagni for the evaporation of water content until remaining of only Kshara part. 600 gms of Kshara Kalka was obtained.

Preparation of Kshara Taila

500 gms of Kshara Kalka + 2 litres of Murchita Tila Taila + 8 liters of Kshariya Jala were added together and kept on Mandagni and continuously stirring was done. The solution is heated up to remaining the Taila portion only and till getting Taila Siddhi Lakshana. When only Taila portion was left then heating was stopped and the Taila obtained is filtered in a dry vessel. The obtained Taila was measured which was 2.85 litres. To evaporate the water content which will remain even after heating, the Taila is kept under sunlight for Bhanupaka (From10am to 5pm for 6days).

High performance thin layer chromatography (HPTLC)

Unsaponifiable matter of AKT was dissolved in 10 ml of chloroform; 3, 6 and 9µl of the above was applied on a precoated silica gel F_{254} on aluminum plates to a band width of 8 mm using CAMAG (Muttenz, Germany) Linomat 5 TLC applicator. The plate was developed in CAMAG twin trough chamber using toluene – ethyl acetate (8: 0.5) as mobile phase, the developed plates were visualized under 254 and 366 nm and after derivatisation in vanillin-sulphuric acid spray reagent in CAMAG TLC Photodocumentation unit and scanned under UV 254 nm, 366 nm and 620 nm under CAMAG TLC Scanner 4. R_f, colour of the spots and densitometric scan were recorded using CAMAG winCATS software[9].

Determination of Saponification value

About 2g of the substance was weighed in tared 250 ml round bottom flask. 25ml of the alcoholic solution of KOH was added and a reflux condenser was attached. Kept it for boiling on water bath for 1hr, the contents of the flask was rotated frequently. The flask was cooled and 1ml phenolphthalein solution was added and excess of alkali titrated with 0.5N HCl. The number of ml (a) required was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml required (b) was noted. The experiment was repeated twice to get concordant values.

Saponification value = 56.1 x (b-a) x Strength of Hydrochloric acid / Weight of the sample taken

Determination of Acid value

Weighed 10g of sample in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 + 25ml) previously neutralised by the addition of 1 ml of Phenolphthalein solution and titrated against 0.1N potassium hydroxide solution. End point was the appearance of pale pink colour which persists for 15sec. Repeated the experiment twice to get concordant values.

Acid value = 56.1 x Titre x Strength of Potassium hydroxide / Weight of the Oil / Fat

Determination of Iodine value

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4 , 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

$$\text{Iodine value} = (b\text{-}a) \times 0.01269 \times 100 / w$$

RESULTS AND DISCUSSION

The prepared AKT is reddish brown viscous liquid with no characteristic odour (Figure 1). Refractive index indicates density of sample compared to air and liquid media; the value of AKT was found to be 1.4699. Specific gravity indicates the presence of solute content in the solvent; in AKT solvent being Tila Taila (Sesame Oil) and solute refers to the active principles extracted from Apamarga Kshara (Alkali) by oil medium; the value was found to be 0.9431. The amount of alkali needed to saponify a given quantity of oil will depend upon number of COOH group present; the saponification value also indicates the average molecular weight / chain length of all fatty acids present. Longer the chains, fatty acid have low saponification value and shorter chain fatty acid have high saponification value. Shorter chain fatty acid (high saponification value) have faster rate of absorption than longer chain fatty acid; saponification value of AKT was found to be 161.90. The acid value indicates presence of free fatty acid in the oil which is responsible of rancidity of compounds; higher the free fatty acid more is the rancidity, this helps to decide the shelf life of the oil; acid value for AKT was found to be 2.20. Iodine value indicates the degree of unsaturation of oil; greater the degree of unsaturation higher will be the possibility of absorption and atmospheric oxidation leading to rancidity. The more iodine number, the more unsaturated fatty acid bonds are present; unsaturated fatty acid better absorbed than saturated fatty acids, the iodine value of AKT was found to be 82.43. Viscosity is index of resistance offered by the surface to flow a liquid; higher the viscosity of a liquid, greater is the resistance to flow, if viscosity of the oil preparation is increases, the rate of absorption decreases. If oil is less viscous this means rate of absorption is very much high; viscosity of AKT is found to be 89.0120. The rancidity of AKT was slightly oxidized, unsaponification matter was found to be 3.47 and ester value was found to be 159.70. These constants can be used as standard values to derive quality parameter for AKT (Table 1).

On photo documented at 254 nm and 366 nm showed 5 and 12 spots respectively. Under white light, after derivatisation in vanillin – sulphuric acid spray reagent showed 12 spots. The spots with R_f 0.30 and 0.65 was commonly occurred in all the three visualisation methods (Figure 2, Table 2). On densitometric scan at 254 nm showed 8 peaks, peak with R_f 0.75 being the major spot contributing to 29.23%. Densitometric scan at 366 nm showed 3 peaks, peak with R_f 0.02 being the major spot contributing to 59.07%.

Table 1: Results of standardisation parameters for Apamrgakshara Taila

Parameters	Apamargakshara Taila
Specific gravity	0.9431
Rancidity	Slightly oxidised
Viscosity	89.0120
Refractive index	1.4699
Acid value	2.20
Saponification value	161.90
Unsaponifiable matter	3.47
Iodine value	82.43
Ester value	159.70

Table 2: R_f values of all the samples

At 254 nm	At 366 nm	After Derivatisation
0.04 (D. green)	0.04 (F. blue)	-
-	-	0.10 (purple)
-	-	0.15 (purple)
0.20 (D. green)	-	0.20 (D. violet)
-	0.22 (F. blue)	0.22 (D. violet)
-	0.25 (F. blue)	-
0.30 (L. green)	0.30 (F aqua. blue)	0.30 (D. violet)
0.36 (D. green)	-	-
-	0.39 (F. blue)	0.39 (D. violet)
-	0.47 (F. green)	0.47 (L. purple)
-	0.50 (F. green)	-
-	-	0.52 (L. purple)
-	0.54 (F. blue)	-
-	0.58 (F. blue)	0.58 (L. purple)
0.65 (D. green)	0.65 (DF. blue)	0.65 (L. purple)
-	-	0.69 (L. purple)
-	0.74 (F. blue)	-
-	-	0.80 (L. purple)
-	0.92 (DF. blue)	-

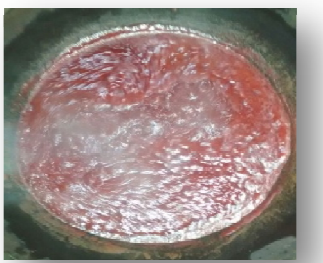
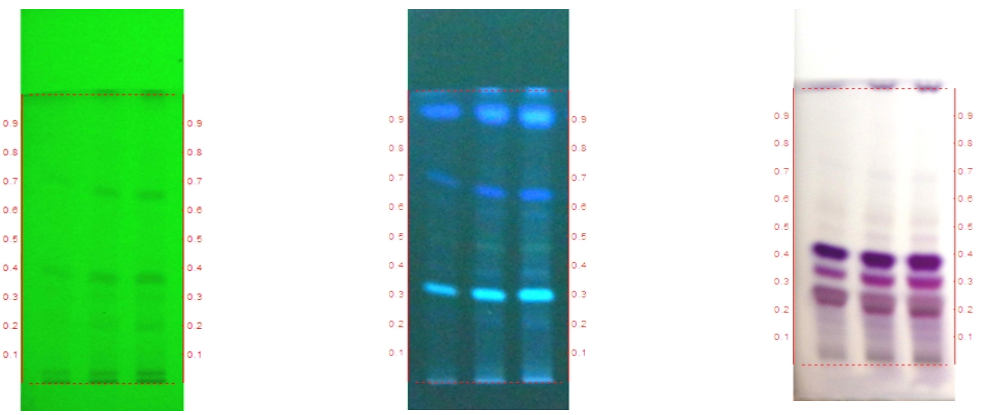
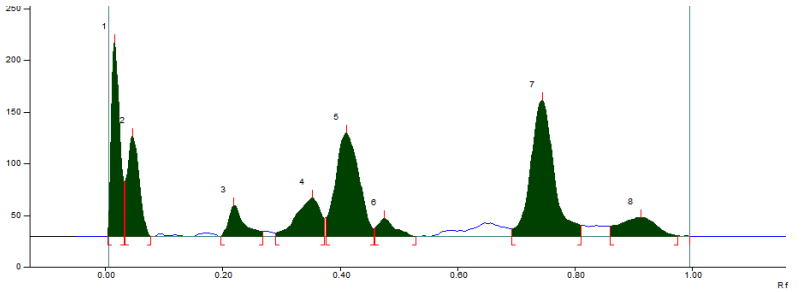


Figure 1: Apamargakshara Taila



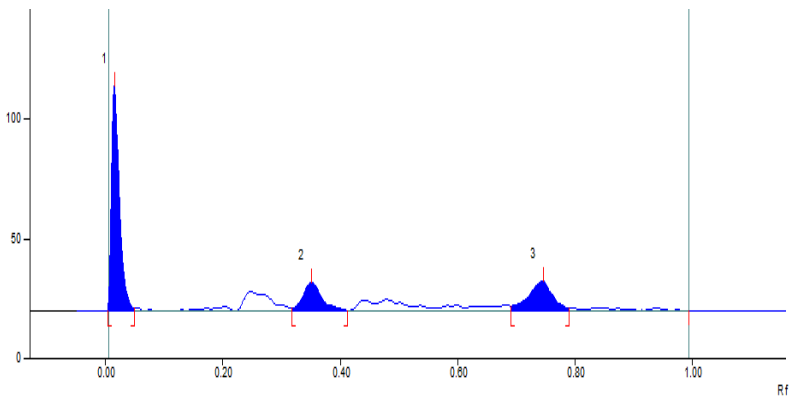
At 254 nm **At 366 nm** **After Derivatisation**

Figure 2: HPTLC photodocumentation of chloroform soluble portion of unsaponifiable matter of Apamargakshara Taila
Track 1- Apamargakshara Taila– 3µl; Track 2– 6µl; Track 3– 9µl
Solvent system: Toluene: Ethyl acetate (8:0.5)



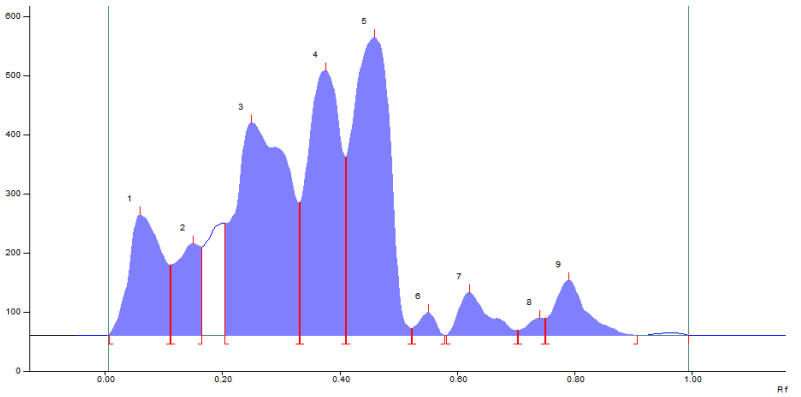
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	187.7 AU	30.33 %	0.03 Rf	52.5 AU	1928.5 AU	15.20 %
2	0.03 Rf	52.5 AU	0.05 Rf	97.1 AU	15.68 %	0.08 Rf	0.2 AU	1397.6 AU	11.01 %
3	0.20 Rf	0.4 AU	0.22 Rf	29.9 AU	4.84 %	0.27 Rf	5.0 AU	558.7 AU	4.40 %
4	0.29 Rf	3.5 AU	0.35 Rf	37.2 AU	6.02 %	0.37 Rf	17.7 AU	1074.0 AU	8.46 %
5	0.38 Rf	17.8 AU	0.41 Rf	100.0 AU	16.16 %	0.46 Rf	7.6 AU	2843.7 AU	22.41 %
6	0.46 Rf	8.1 AU	0.48 Rf	17.2 AU	2.78 %	0.53 Rf	0.1 AU	357.3 AU	2.82 %
7	0.69 Rf	7.2 AU	0.75 Rf	131.3 AU	21.21 %	0.81 Rf	11.1 AU	3709.8 AU	29.23 %
8	0.86 Rf	10.1 AU	0.91 Rf	18.5 AU	2.99 %	0.98 Rf	1.0 AU	820.1 AU	6.46 %

Figure 3: Densitometric scan of chloroform soluble portion of unsaponifiable matter of Apamargakshara Taila at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	93.9 AU	79.37 %	0.05 Rf	1.2 AU	933.4 AU	59.07 %
2	0.32 Rf	1.2 AU	0.35 Rf	11.9 AU	10.08 %	0.41 Rf	0.1 AU	276.6 AU	17.51 %
3	0.69 Rf	2.1 AU	0.75 Rf	12.5 AU	10.54 %	0.79 Rf	1.1 AU	370.2 AU	23.43 %

Figure 4: Densitometric scan of chloroform soluble portion of unsaponifiable matter of Apamargakshara Taila at 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.5 AU	0.06 Rf	203.4 AU	10.70 %	0.11 Rf	18.7 AU	8188.9 AU	9.78 %
2	0.11 Rf	118.8 AU	0.15 Rf	155.3 AU	8.18 %	0.16 Rf	49.6 AU	4622.2 AU	5.52 %
3	0.20 Rf	188.9 AU	0.25 Rf	358.7 AU	18.88 %	0.33 Rf	23.6 AU	22886.8 AU	27.32 %
4	0.33 Rf	224.5 AU	0.38 Rf	446.7 AU	23.52 %	0.41 Rf	00.4 AU	17941.9 AU	21.42 %
5	0.41 Rf	301.5 AU	0.46 Rf	503.0 AU	26.47 %	0.52 Rf	11.9 AU	22632.2 AU	27.02 %
6	0.52 Rf	12.1 AU	0.55 Rf	38.5 AU	2.03 %	0.58 Rf	0.1 AU	767.4 AU	0.92 %
7	0.58 Rf	0.2 AU	0.62 Rf	72.1 AU	3.79 %	0.70 Rf	8.4 AU	2617.8 AU	3.12 %
8	0.70 Rf	8.5 AU	0.74 Rf	29.4 AU	1.55 %	0.75 Rf	28.2 AU	606.7 AU	0.72 %
9	0.75 Rf	28.4 AU	0.79 Rf	92.6 AU	4.88 %	0.91 Rf	0.0 AU	3507.6 AU	4.19 %

Figure 5: Densitometric scan of chloroform soluble portion of unsaponifiable matter of Apamargakshara Taila at 620nm following derivatisation

Densitometric scan after derivatisation with vanillin sulphuric acid showed 9 peaks, peak with R_f 0.25 being the major peak with area % of 27.32 (Figure 3 to 5).

HPTLC technique is well appreciated and accepted all over the world. Many methods are being established to standardize the essay methods. HPTLC remains one step ahead when compared with other tools of chromatography[10] and one of the most flexible, reliable and cost – efficient separation technique ideally suited for the analysis of botanicals and herbal drugs. HPTLC is only chromatographic method offering the option of presenting the results as an image[11].

Kshara Taila (KT) is a rare type of Ayurvedic formulations mentioned in classical texts of Ayurveda. The normal types of Taila (Oil) are prepared from fresh drugs while KT is unique type of preparation prepared after ashing of plant materials. There are reports of standardisation of normal type of Taila (Oil),[12,13] while this study is the first time report of standardisation of AKT which can be important medication for fistula-in-ano.

CONCLUSION

The purpose of standardization of Ayurvedic medicine is to ensure therapeutic efficacy since the active constituents may vary according to geographical source of the drug. Thus it may not be easy to standardize drug chemically and hence maintaining the quality of these plant products is an essential factor. The quality indicating tests for Apamargakshara Taila reported from this study can be used as routine quality check parameter for this Taila (Oil) preparation.

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