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Pharmacognostical and Pharmaceutical evaluation of Herbo mineral Formulation: *Aragvadhadi taila*

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ABSTRACT

Background: *Aragvadhadi taila* is a *Sneha Kalpana*, indicated in *Kushtha Roga* specially in *Shvitra* (Vitiligo) *Roga*. In present study, it has been used for external application in *Shvitra* (Vitiligo) *Roga*. **Objective:** Present study is aimed to look out on herbal drugs used in the preparation of *Aragvadhadi taila* and standardization of Pharmacognostical, Physicochemical parameters and HPTLC evaluation. **Methods:** Intermediate product sample (*Kalka*) identification and authentication was done by pharmacognostical study i.e. morphological features, organoleptic characters and powder microscopy. Physicochemical evaluation and HPTLC was carried out of final product. **Results:** Pharmacognostical Study of Intermediate product sample (*Kalka*) showed presence of Scleroids, Spoon cells, Tannin containing cells, Cluster crystal, Rosette crystal, Oil globules, Lignified fibres etc. Pharmaceutical evaluation showed results specific gravity 0.91, Refractive Index 1.4870, Acid Value 7.12, Saponification Value 316, Iodine Value 7.58. High Performance Thin Layer Chromatography at 254nm and 366 nm results in to 8,5 and 6 spots before and after spray respectively. **Conclusion:** Identification, Authentication of Herbal drugs used in the preparation and Intermediate product sample (*Kalka*) has been done. Physicochemical evaluation has been carried out of prepared drug which is further useful for standardization of *Aragvadhadi taila* and another researches.

Keywords: *Aragvadhadi taila*, Herbo mineral, *Kushtha*, Pharmacognosy, *Shvitra*, Standardization.

INTRODUCTION

Medicated oils occupy an important section of Ayurveda pharmaceuticals described under heading of *Sneha Kalpana*. *Aragvadhadi taila* is one of the Herbo mineral formulation prescribed in Ayurvedic text Chakradatta in *Kushtha Chikitsa*^[1]. This preparation contains five herbal drugs and two arsenicals that are *Manahshila* (Realgar), *Haratala* (Orpiment). *Gomutra* is used as *Drava dravya* and *Sarshapa Taila* as *Sneha dravya*. This formulation contains *Gomutra* so, five days are needed for proper *paka* of medicated oil as per classics^[2]. It is specially indicated as in *Shvitra* (vitiligo) *Roga*. *Aragvadhadi taila* is one of the Herbo mineral medicated oil easily prepared oil which can be used externally to treat *Shvitra* (vitiligo) *Roga*. Present study is focus on first attempt to develops quality parameters of *Aragvadhadi taila* on the basis of pharmacognostical, physicochemical parameters and chromatographic evaluation. Hence, there is need to scientific proof for standardization of quality parameters. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of drug. Therefore, the present study was designed to evaluate the physicochemical, pharmacognostical parameters and develop the TLC fingerprint profiles of *Aragvadhadi taila*.

OBJECTIVE OF STUDY

Present study, is aimed to look out on herbal drugs used in the preparation of *Aragvadhadi taila* and Standardization of Pharmacognostical, Physicochemical parameters and HPTLC evaluation. The purpose of Standardization of raw drugs and final product is to ensure therapeutic efficacy. Therefore, maintaining the quality of this product is an essential factor.

MATERIALS & METHODS

Collection, identification, authentication of raw drugs

Collection of raw materials

Herbal *Kalka Dravya* like *Kushtha*, *Haridra*, *Daruharidra* were procured from the pharmacy of Gujarat Ayurveda University, Jamnagar. *Aragvadhadi* fruit was collected from campus of I.P.G.T & R.A, Jamnagar. *Dhava* (*Anogeissus Latifolia*) *Tvaka* (bark) was collected from kevadiya colony (Gujarat).

The ingredients of *Aragvadhadi taila* and its part used are given at Table No 1. The raw drugs were identified and authenticated by Pharmacognosy Laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar. Identification was done on basis of organoleptic characters [Table No 2,3,4], morphological features and powder microscopy of Intermediate product sample (*Kalka*) as per API standards for authentication. Powder of *Kalka* (60#) and *Aragvadhadi taila* was stored in well filled closed glass containers away from the light. [Figure No 1,2]



Figure 1: Powder of *Kalka* (60#)



Figure 2: *Aragvadhadi Taila*

Table 1: Formulation composition: *Aragvadhadi taila*

No. Ingredients	Latin / English name	Part used	Proportion
Herbal Drugs (<i>Kalka</i>)			
1. <i>Aragvadhā</i>	<i>Cassia fistula</i> Linn.	Fruit pulp	1/7 part
2. <i>Dhava</i>	<i>Anogeissus latifolia</i>	Bark	1/7 part
3. <i>Kushtha</i>	<i>Saursurealappa</i> C.B. Clerk	Root	1/7 part
4. <i>Haridra</i>	<i>Curcuma longa</i> Linn.	Rhizome	1/7 part
5. <i>Daruharidra</i>	<i>Berberis aristata</i> D.C	Rhizome	1/7 part
Arsenicals (<i>Kalka</i>)			
6. <i>Shodhita Manahshila</i>	Processed Red arsenic sulphide	Whole	1/7 part
7. <i>Shodhita Haratala</i>	Processed Yellow arsenic sulphide	Whole	1/7 part
Kwatha Dravya			
8. <i>Gomutra</i>	Cow's urine	-	16 part
Sneha Dravya			
9. <i>Sarshap Taila</i>	Mustard oil	-	4 part

Preparation of *Aragvadhadi taila* in Bhaishajya Kalpana Laboratory of IPGT & RA.

Preparation of *Kalka*

Shudhha Manahshila [3] and *Shudhdha Haratala* [4] was powdered in porcelain *kharala*. *Aragvadhā* fruit pulp was collected from *Aragvadhā* fruit. Then, it was dissolved in sufficient quantity of *Gomutra* and it was boiled for 10 minutes. Remained *dravya* from *Kalka Dravya* were taken in *yavakuta* form. Then, subjected into electrical mixer grinder and sufficient quantity of *Gomutra* was added in it and grinded till a paste (*Kalka*) was prepared. Properly made bolus kept in stainless steel vessel for further process.

Preparation of *Aragvadhadi taila*

Sarshapa Taila in the mentioned quantity was taken in a stainless steel vessel and heated over mild flame (80°C for 5 min) till complete evaporation of moisture and then bolus of *Kalka* were added in it. After mixing of *Kalka*, the specified quantity of *Gomutra* was added and the mixture was subjected to heat. Heating was continued maintaining the temperature in between 95-100°C with continuous stirring. The mixture was left undisturbed through the night and heating was given for 5 days. Contents were stirred continuously to avoid the possibility of settling down. Heating was continued on 5th day till *Sneha Siddhi Lakshana* were obtained. After obtaining desired *Sneha Siddhi Lakshana*, the vessel was taken out from heat and oil was filtered through two folded cotton cloth in its hot stage. The prepared oil was stored in a properly labelled air tight bottle after cooling.

Pharmacognostical Study

Herbal Drugs used in Intermediate product sample (*Kalka*) was identified and authenticated by pharmacognosy department, IPGT & RA, Gujarat Ayurved University, Jamnagar. The identification was carried out on the basis of organoleptic features, morphological features and powder microscopy of Intermediate product sample (*Kalka*) [5,6].

Pharmaceutical Evaluation

Physicochemical Parameters

Aragvadhadi taila was analyzed by using qualitative and quantitative parameters at Pharmaceutical Laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar. The common parameters mentioned in Ayurvedic Pharmacopeia of India [7] and CCRAS guidelines [8] i.e. Refractive index [9], Specific gravity [10], Acid value [11], Iodine value [12], Saponification value [13] were taken.

High Performance Thin Layer Chromatography (HPTLC)

Sample preparation

0.1 ml of oil was taken and 1 ml of hexane was added. The solution was prepared used for chromatography. Thereafter pre chromatographic derivatization was done. Alcoholic KOH (base) and thereby heated for 10-15 minutes in CAMAG TLC plate heater. Sample application was done using CAMAG linomat 5.

HPTLC of *Aragvadhadi taila* was carried out using the solvent system petroleum ether: Diethyl ether: Acetic Acid (9:1:0.1v/v). HPTLC study was performed for the normal phase separation of components of product. Post chromatographic derivatization was done with vanillin sulphuric acid spray reagents [14].

OBSERVATIONS AND RESULTS

Organoleptic characters: Organoleptic characters like Taste, Colour, Odour, Touch and Texture were scientifically studied as per detailed in Table 2,3,4.

Table 2: Organoleptic characters of raw herbal materials used in formulation

Sr. No.	Ingredient	Colour	Taste	Odour	Touch
1	<i>Aragvadha</i> fruit pulp	Dark Blackish	Sweet	Strong aromatic	Slight rough
2	<i>Dhava</i>	Off-white	Sweet	Aromatic	Rough
3	<i>Kushtha</i>	Brownish	Pungent	Aromatic	Slight rough
4	<i>Haridra</i>	Yellowish	Pungent, Bitter	Characteristic	Rough
5	<i>Daruharidra</i>	Yellowish	Astringent, Bitter	Characteristic	Rough

Table 3: Organoleptic characters of Intermediate product sample except arsenicals (*Kalka*)

Sr no.	Various parameters	Results
1	Colour	Yellowish brown
2	Odor	Aromatic
3	Taste	<i>Katu, Tikta, Madhura</i>
4	Touch	Slightly rough
5	Texture	Paste

Table 4: Organoleptic characters of prepared Drug (*Aragvadhadi taila*)

Sr no.	Various parameters	Results
1	Colour	Yellowish brown
2	Odor	Smell of <i>Gomutra</i>
3	Taste	Not applicable
4	Touch	Viscous
5	Texture	Liquid

Powder Microscopy of Intermediate product sample except arsenicals (*Kalka*)

Powder microscopy of Intermediate product sample (*Kalka*) was done with powder (60#) by studying under the Carl Zeiss Trinocular Microscope before and after staining with Phluroglucinol and concentrated HCL to study the characters of drug. The microphotographs were taken by a camera attached with the microscope as given below.

Microscopic Characters of intermediate product sample (*Kalka*)

The diagnostic characters under microscope showed scleroids, spoon

Table 6: Results of *Aragvadhadi taila*

Before/After spray	Wavelengths	No of Spots	Rf value	AUC (Area Under Curve)
Before spray	254 nm	8	0.03,0.07,0.11,0.14,0.20,0.28,0.69,0.97	13925.6 Total: 15100.4
	366 nm	5	0.03,0.06,0.10,0.14,0.16	
After spray	600 nm	6	0.02,0.07,0.11,0.18,0.78,0.92	-

cells in surface view, tannin containing cells of *Aragvadha* fruit [Figure 9,10,11], Presence of cluster crystal, cork cells, rosette crystal and stone cells of *Dhava* bark [Figure 1 to 6], Presence of cork cells, oil globules, silica deposition of *Kushtha moola* [Figure 7,8,20], Presence of oil globules, parenchymal cells of *Haridra* [Figure12,13], Presence of lignified fibres, lignified pitted vessels, stone cells of *Daruharidra* [Figure 14 to 19].

Pharmaceutical Analysis

Comparative Physicochemical Analysis of *Sarshapa Taila* and *Aragvadhadi taila* i.e. Refractive index, Specific gravity, Acid value, Iodine value, Saponification value were scientifically studied and results were detailed in respectively Table 5, 6.

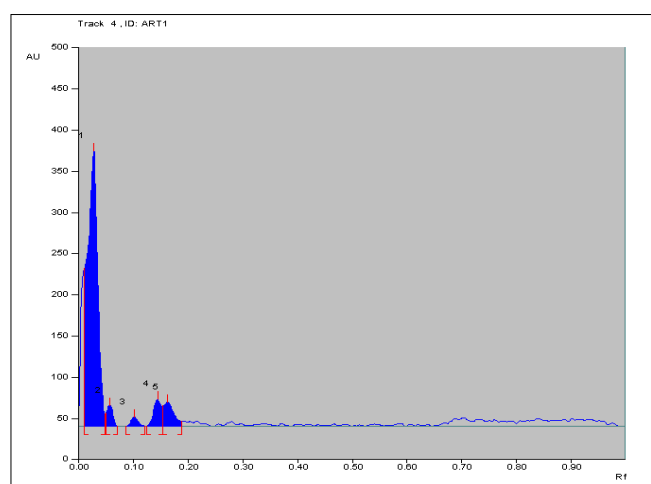
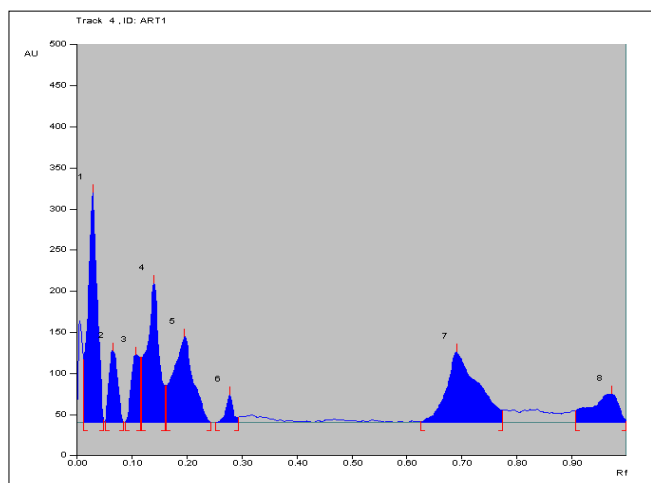
Table 5: Comparative Physicochemical Parameters of *Sarshapa Taila* and *Aragvadhadi taila*

Sr No.	Analytical Parameters	Result of <i>Sarshapa Taila</i>	Result of <i>Aragvadhadi taila</i>
1	Refractive Index	1.4860	1.4870
2	Specific gravity @ 25° C (g/ml)	0.90	0.91
3	Acid Value	5.34	7.12
4	Iodine Value	8.00	7.58
5	Saponification Value	288	316

HPTLC Study

Chromatographic study (HPTLC) was carried out under 254nm and 366nm to establish fingerprinting profile. It showed spots at 254 nm, spots at 366 nm before spray and spots at 600nm after spray.

DENSITOGRAM OF ARAGVADHADI TAILA (Before Spray)

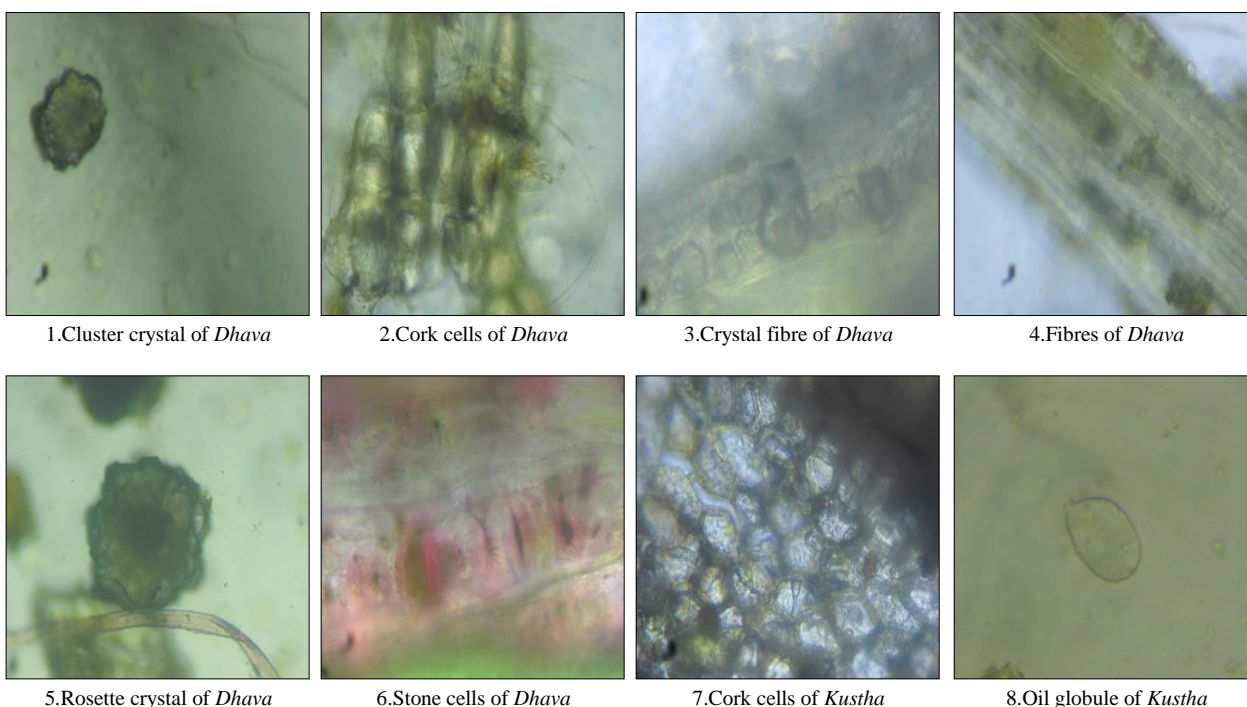


DISCUSSION

Normally oils give different characteristics like colour and odor relative to ingredients which were used to prepare the medicated oil. In this Herbo mineral oil, yellowish brown colour is given due to mainly *Gomutra* and *Aragvadha* fruit pulp. The characteristic odor is due to *Gomutra*, *Sarshapa Taila* which were used in preparation. Authentication of used drugs was done by morphological and histological. This can prevent misuses of drug adulteration. The pharmacognostical evaluation shows that the intermediate product sample (*Kalka*) contains all ingredients which were observed in the microscopical characters. This shows the purity and quality of product.

According to present study, Saponification value of *Aragvadhadi taila* was 316mg/g as compared to normal value of *Sarshapa Taila* (288mg/g). It is the measure of average molecular weight of all fatty acids present in it. The short chain fatty acids found in fats have more saponification value. Relatively, more numbers of carboxylic functional group per unit mass of the fat. Acid values are used to measure the extent to which glycerides in the oil has been decompose by lipase and other physical factors like heat and light. Minor changes was observe in acid value suggests that medicated *Taila* is very saturated. The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in oil which reflects the susceptibility of oil to oxidation. This medicated oil was taken continuously 5 days of heating. However, in present study, slightly deviation was observed in Iodine value, Saponification value and Acid value as compared to normal values of *Sarshapa Taila*. Refractive index is an important parameter to assess quality of oil as it is change according to its compounds. Specific gravity is varying according to density of liquid which was more as compared to *Sarshapa Taila*. So, it suggests that the more heating gives more Saponification value, Acid value and Specific gravity. TLC finger print profile consists of 8,5 prominent spots under UV light at 254nm and 366nm respectively before spray and 6 were after spraying. Total Area under curve was occupy 15100.4 under UV light at 254nm and 366nm HPTLC fingerprint profile helps in identification of various phytochemical constituent present in the crude drug thereby substantiating and authenticating of product, This profile helps in identify and isolate the important phytoconstituents. These findings could be helpful in identification and authentication.

MICROPHOTOGRAPHS OF ARAGVADHADI TAILA (PLATE-1)

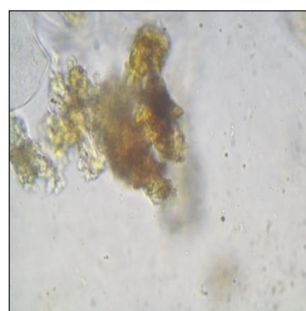




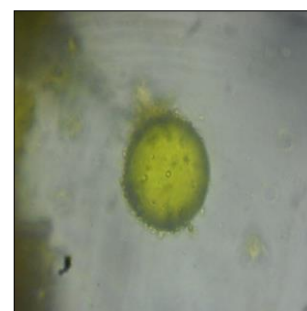
9.Scleroids of *Aragvadha majja*



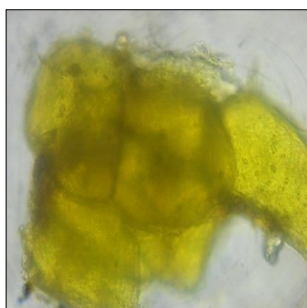
10.Spool cells in *Aragwadha majja*



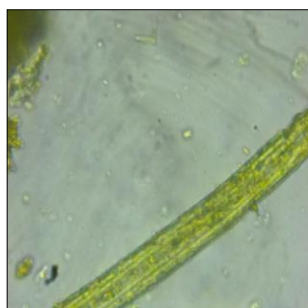
11.Tannin containing cells of *Aragvadha majja*



12.Oil globule of *Haridra*



13.Parenchyma cells of *Haridra*



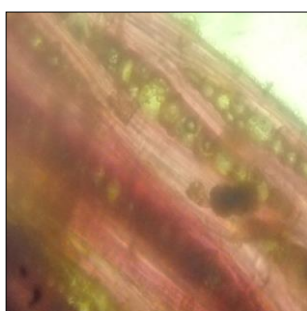
14.Fibres of *Daruharidra*(1)



15.Fibres of *Daruharidra*(2)



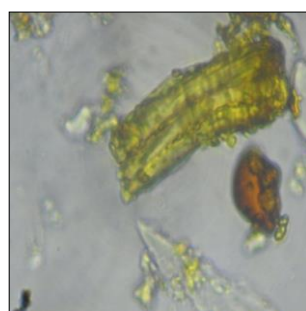
16.Lignified fiber of *Daruharidra*



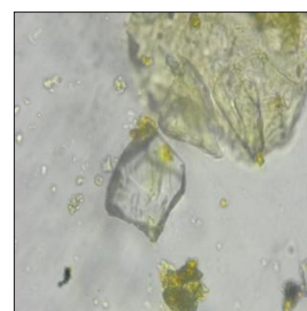
17.Phloem fibres of *Dharuharidra*



18.Pitted vessels of *Daruharidra*



19.Stone cells of *Daruharidra*



20.Silica deposition of *Kustha*

CONCLUSION

Present study reveals that quality of *Aragvadhadi taila* as per pharmacognostical and physico chemical parameters, which helps in justifying the quality of formulation and meet the desired quality. In the present work, the obtained results were found within normal prescribed limits. For first time, this profile of *Aragvadhadi taila* was established. On the basis of observations and experimental result, the evaluation of research of *Aragvadhadi taila* may be used as standard reference for further quality control research works and clinical studies.

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