ejpmr, 2022,9(11), 437-443

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYSICOCHEMICAL EVALUATION AND STANDARDIZATION OF KALINGADI TAILA- A POLYHERBAL PREPARATION

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Article Received on 08/09/2022 **Article Revised on 28/09/2022** **Article Accepted on 18/10/2022**

ABSTRACT

The purpose of drug standardization is to find the quality, efficacy and identity of products. *Kalingadi taila* is a polyherbal Ayurveda formulation is one of the important formulation used for Nasya Karma in rhinitis. The drug contains kalinga, hingu, maricha, laksha, tulasi, katphala, kushta, vacha, shigru and vidanga processed with Katu taila and Gomutra explained in chakradatta.[1] The drug was prepared at Teaching Pharmacy of Sri Dharmasthala Manjunatheshwara Institute of Ayurveda and Hospital, Bangalore as per the standard procedure of taila preparation. The prepared drug was subjected to various analytical methods of standardization to establish its quality and purity. There has been many such pharmaceutical preparation with different combination of drugs explained in classics for better action, penetration and absorption to reverse the pathological changes. Since the drugs used in this particular preparation possess ushna, teekshna, ruksha and lekhana in properties was chosen to conduct a clinical study on Kaphaja Pratishyaya (Chronic Rhinosinusitis) for a Research Project under RGUHS Research grants 2019-20 and before administration to subjects analysis was done for the standardization of the finished product and to establish the quality. In this analytical study prepared Kalingadi *taila* was analyzed for the physical and chemical parameters mentioned for oils viz. Organoleptic properties, Refractive index, Specific gravity, Viscosity, Acid Value, Saponification value, Determination of unsaponifiable matter, Rancidty, Iodine Value, Peroxide Value and HPTLC which were within the standard values.[3]

KEYWORDS: Standardization, *Kalingadi Taila.*

INTRODUCTION

Every drug mentioned for *Nasya karma* has its own characteristic features which determine the authenticity of that particular drug and properties. *Kalingadi Taila* is one such polyherbal Ayurvedic formulation explained by *Acharya Chakradatta* in the context of *Peenasa Chikitsa*.[2] The Properties of drugs used in *Kalingadi taila* are *Tridoshashamaka, Tikshana, Snigdha, Laghu, Ruksha guna, Ushana veerya, Kaphanisaraka, Vata kapha shamaka*.

Since our body is contained with lipids, fats, adipose tissue, thus any medicines given through this medium can be easily absorbed both internally and externally. *Sneha Kalpana* is one such group of formulations explained in *Ayurvediya Bhaishajya Kalpana* where *Taila* and *Ghritha formulations* are explained which is processed in a manner that both lipid soluble and water-soluble active principles of the drug are transferred into *Sneha*. Such formulations explained in classics need drug standardization to establish the quality, efficacy and identity of the product.

Aim and Objective

To study the organo leptic and physico chemical characters of Kalingadi *Taila*.

METHODOLOGY

- The formulation was evaluated for organoleptic characters such as colour, appearance and odour by means of examination using sensory organs.
- The physicochemical characters such as Refractive index, Specific gravity, Viscosity, Acid value, Saponification value, unsaponifiable matter, Rancidity test, Iodine value, Peroxide value, Loss on drying and HPTLC were done as per the standard testing protocol.

Physico chemical analysis

1. Refractive index[4]

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Note the reading. Distilled water has a refractive index of 1.3320 at 30˚C. The difference between the reading and 1.33194 gives the error of the instrument. If the reading is less than 1.3320, the error is minus (-) then the correction is

plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of the test sample was measured at 30˚C.

2. Specific gravity[5]

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

3. Viscosity[6]

The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula

$$\eta 1 = \frac{\rho 1 t 1 \; X \; \eta 2}{\rho 2 t 2}$$

η1 – Viscosity of sample
η2 - Viscosity of water
t1 and t 2- time taken for the sample and water to pass the meniscus
ρ1 and ρ2 – Density of sample and water
X= Specific gravity of sample x 0.9961/specific gravity of water
Π= X x Time for samplex1.004/specific gravity of waterx70sec

4. Acid value[7]

Weighed 2- 10g of Kalingadi Taila in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

5. Saponification value[8]

Weighed 2g of the Kalingadi taila into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

6. **Determination of unsaponifiable matter**[9]

Weighed 5g of the substance into the flask. Added 50ml alcoholic KOH into the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaked vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°c for about 1 hour to remove the last traces of ether. A few ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desiccator to remove last traces of moisture and then weighed.

7. Rancidity test[10]

1ml of *Kalingadi Tail*a was mixed with 1ml of conc. HCl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

8. Iodine value[11]

0.1g 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.

9. Peroxide value[12]

5g of the taila was weighed accurately into a conical flask, added 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking

with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5ml of starch indicator continued the titration until blue color disappears.
Peroxide value= 10(a-b)/W
Where W= weight in g of the substance

Sample preparation for HPTLC of *Kalingadi Taila*[13]
Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform.

HPTLC
3, 6 and 9µl of the above sample were applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: ethyl acetate (8:1) and the developed plates were visualized under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254nm, 366nm and 620nm (following derivatisation). Rf, colour of the spots and densitometric scan were recorded.

RESULTS
The results of organoleptic characters, standardization parameters, HPTLC photo documentation, Rf values and Densitometric scan are given in the following tables and figures.

Table 1: Results of organoleptic characters.

Sl/No		
1	Colour	Dark brown
2	Appearance	Greasy
3	Odour	Characteristic
4	Texture	Smooth

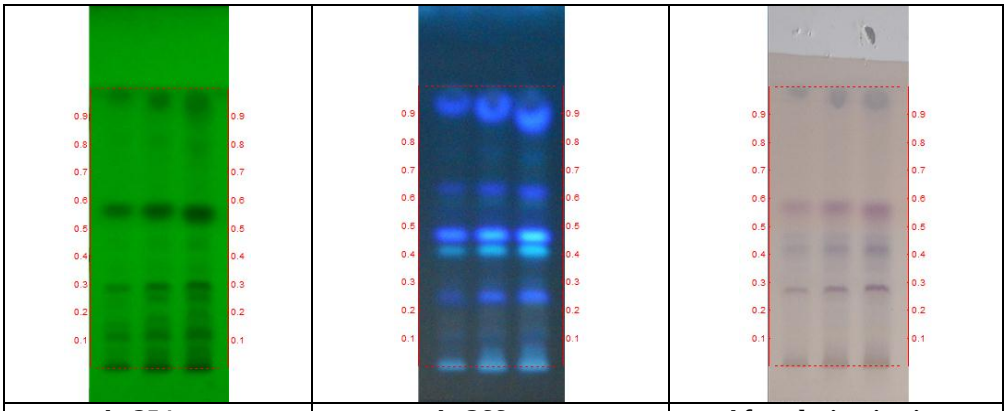
Table 2: Results of standardization parameters for *Vidanga Taila* and *Kalingadi taila.*

	Parameter	**Results *n = 3 %w/w***
		Kalinga taila
1	Refractive index	1.46646
2	Specific gravity	0.9114
3	Viscosity	52.38
4	Acid value	2.1
5	Saponification Value	167.4
6	Determination of Unsaponifiable matter (%)	2.43
7	Rancidity Test	Not detected
8	Iodine Value	98.5
9	Peroxide Value	Not detected
10	Loss on Drying	0.07

Table 3: Rf value of kalinga taila.

Short UV	**Long UV**	**Under white light (After derivatisation)**
0.07 (Green)	-	-
0.12 (Green)	0.12 (F. blue)	-
0.20 (Green)	-	-
0.25 (Green)	0.25 (F. blue)	-
-	-	0.28 (Purple)
0.31 (Green)		-
0.36 (Green)	-	-
-	0.42 (F. blue)	0.42 (Purple)
0.46 (Green)	0.46 (F. blue)	0.46 (Purple)
0.55 (Green)	-	-
-	0.58 (F. blue)	0.58 (Pink)
-	0.62 (F. blue)	-
-	0.75 (F. blue)	-
0.80 (Green)	-	-

***** F - fluorescent

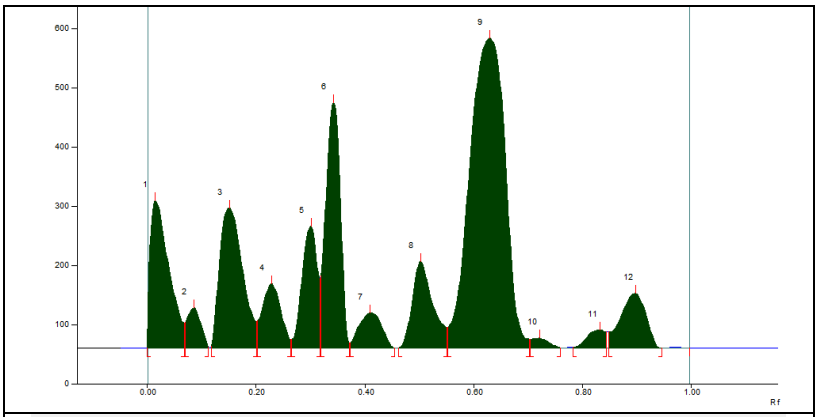


At 254 nm	At 366 nm	After derivatisation

Track 1- ***Kalinga taila***– 3µl Track 2- ***Kalinga taila***– 6µl Track 3- ***Kalinga taila***– 9µl

Solvent system – **Toluene: Ethyl Acetate (9:1)**

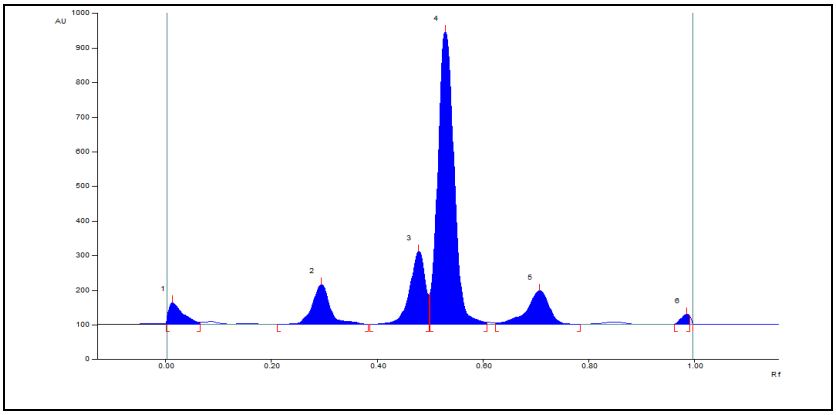
Figure 1: HPTLC photo documentation of chloroform extract of *kalinga taila.*



Track 12, ID: Kalinga taila

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	248.4 AU	11.57 %	0.07 Rf	42.3 AU	6162.4 AU	9.66 %
2	0.07 Rf	43.2 AU	0.09 Rf	67.9 AU	3.16 %	0.11 Rf	1.9 AU	1241.3 AU	1.95 %
3	0.12 Rf	2.8 AU	0.15 Rf	236.1 AU	10.99 %	0.20 Rf	45.1 AU	7193.3 AU	11.28 %
4	0.20 Rf	46.3 AU	0.23 Rf	108.6 AU	5.06 %	0.26 Rf	13.9 AU	2668.3 AU	4.18 %
5	0.27 Rf	14.2 AU	0.30 Rf	205.2 AU	9.55 %	0.32 Rf	17.0 AU	4127.2 AU	6.47 %
6	0.32 Rf	121.3 AU	0.34 Rf	413.6 AU	19.26 %	0.37 Rf	8.4 AU	7971.7 AU	12.50 %
7	0.37 Rf	9.0 AU	0.41 Rf	59.8 AU	2.79 %	0.45 Rf	0.0 AU	1793.7 AU	2.81 %
8	0.46 Rf	0.2 AU	0.50 Rf	146.1 AU	6.80 %	0.55 Rf	35.1 AU	3965.3 AU	6.22 %
9	0.55 Rf	35.4 AU	0.63 Rf	522.3 AU	24.32 %	0.70 Rf	15.1 AU	24436.9 AU	38.32 %
10	0.70 Rf	14.9 AU	0.72 Rf	16.8 AU	0.78 %	0.76 Rf	0.3 AU	383.0 AU	0.60 %
11	0.78 Rf	1.3 AU	0.83 Rf	30.8 AU	1.43 %	0.85 Rf	27.2 AU	745.8 AU	1.17 %
12	0.85 Rf	27.0 AU	0.90 Rf	91.9 AU	4.28 %	0.95 Rf	0.2 AU	3083.7 AU	4.84 %

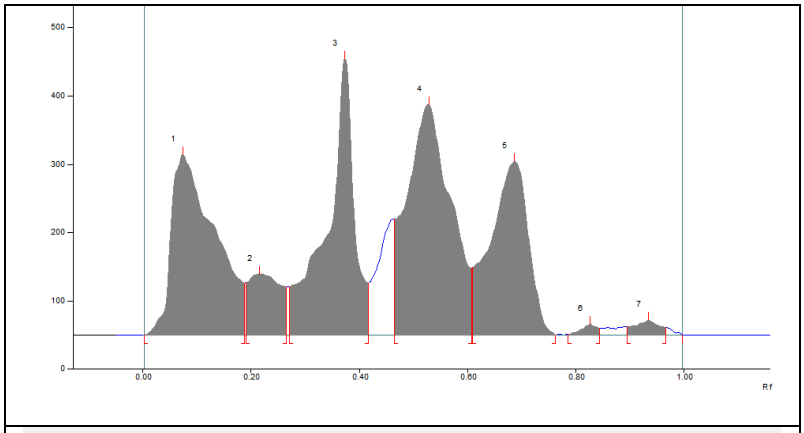
Figure 2: Densitometric scan of *kalinga taila at 254nm.*



Track 12, ID: Kalinga taila

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	63.3 AU	4.65 %	0.07 Rf	5.9 AU	1221.0 AU	4.10 %
2	0.21 Rf	0.0 AU	0.30 Rf	115.4 AU	8.48 %	0.38 Rf	0.0 AU	2727.8 AU	9.15 %
3	0.39 Rf	0.0 AU	0.48 Rf	210.7 AU	15.48 %	0.50 Rf	84.8 AU	4336.8 AU	14.55 %
4	0.50 Rf	87.2 AU	0.53 Rf	844.5 AU	62.03 %	0.61 Rf	6.1 AU	18273.2 AU	61.32 %
5	0.62 Rf	3.8 AU	0.71 Rf	98.1 AU	7.20 %	0.79 Rf	0.0 AU	2919.3 AU	9.80 %
6	0.96 Rf	0.4 AU	0.99 Rf	29.4 AU	2.16 %	0.99 Rf	24.7 AU	322.3 AU	1.08 %

Figure 3: Densitometric scan of *kalinga taila at 366 nm.*



Track 12, ID: Kalinga taila

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.1 AU	0.07 Rf	264.5 AU	19.07 %	0.19 Rf	76.1 AU	15400.5 AU	22.89 %
2	0.19 Rf	76.4 AU	0.22 Rf	89.2 AU	6.44 %	0.27 Rf	70.9 AU	3915.6 AU	5.82 %
3	0.27 Rf	70.2 AU	0.37 Rf	404.2 AU	29.15 %	0.42 Rf	76.2 AU	14433.0 AU	21.45 %
4	0.47 Rf	169.7 AU	0.53 Rf	337.2 AU	24.32 %	0.61 Rf	98.2 AU	19671.6 AU	29.24 %
5	0.61 Rf	98.8 AU	0.69 Rf	253.9 AU	18.31 %	0.76 Rf	0.0 AU	12821.3 AU	19.06 %
6	0.79 Rf	1.3 AU	0.83 Rf	15.9 AU	1.15 %	0.85 Rf	9.8 AU	332.0 AU	0.49 %
7	0.90 Rf	12.3 AU	0.94 Rf	21.7 AU	1.57 %	0.97 Rf	11.0 AU	701.6 AU	1.04 %

Figure 4: Densitometric scan of *kalinga taila at 620nm* (After derivatisation).

DISCUSSION

Refractive index depends on colour of the medium, Density of the medium, Temperature of the medium, No of solutes present in the medium More the Refractive Index, there will be more concentration of light, which facilitates rancidification of *Taila* and also due to presence of water content, the decomposition of *Taila* takes place at a faster rate.

Specific gravity is an important property of fluids being related to density and viscosity. It indicates the solid to liquid ratio in the *Taila*. Knowing the specific gravity will allow determination of a fluid's characteristics compared to a standard, usually water at a specified temperature. Here Specific gravity of Kalingadi taila is 0.9114. If the specific gravity is more than 0.9110 then it is considered as hydrogenated fat. It would be due to solid extractives that come from the herbs added during the process of *Taila Paka*.

The Acid value is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the oil. Acid value is the neutralization capacity of acid that is present in the *Kalingadi Taila* formulation by alkali (Potassium hydroxide). An increase in the amount of FFA in a sample of oil indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is and indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity). Here Acid value of Kalingadi taila is 2.1 and this indicates hydrolysis of triglycerides is lesser kalingadi taila. As the process hydrolysis of triglycerides is occurs

by lipase enzyme which intern indicator of inadequate processing and storage conditions. The free fatty acid is formed as a result of hydrolysis and it deteriorates the quality of the formulation making it unfit for further use. This was the first attempt to standardize one such formulation to be used in clinical practice. Less acid value denotes the less chance of decomposition of *Taila* thus increasing both life span and therapeutic value.

Saponification Value can be defined as the number of milligrams of KOH required to saponify one gram of oil. It is nothing but Alkaline Hydrolysis of pure oil giving soap & glycerol. It helps us to know the stability of the oil in aqueous/alkaline medium. It also signifies the composition of vegetable/animal oils, thereby helps to check the suitability of oils for lubrication purpose. Drying property of oils is harmful. Saponification is simply *the process of making soaps*. Soaps are just potassium or sodium salts of long-chain fatty acids. During saponification, ester reacts with an inorganic base to produce alcohol and soap. Saponification value of a fat or oil is inversely proportional to the molecular weight (or chain length). Lower value of saponification value indicates high molecular weight fatty acid residues in the given oil. High value of saponificaiton value indicates low molecular weight fatty acid residues in the given oil. Here Saponofication value of Kalingadi taila is 167.4. Comparatively sap value of kalingadi taila is at higher side means low molecular weight fatty acid residues in the given oil and indicates that it is good for permeability through the nasal mucosa and subsequently the absorption will be better.

Unsaponifiable matter is that fraction of the formulation which is not saponified by alkali but can be extracted by organic solvent. Unsaponifiable constituents are an important consideration when selecting oil mixtures for the manufacture of soaps. Unsaponifiables can be beneficial because they may have properties such as moisturization, conditioning, antioxidant, texturing etc. On the other hand, when proportion of unsaponifiable is too high (> 3%), or the specific unsaponifiables present do not provide significant benefits. In the present study the unsaponifiable matter in *Kalingadi Taila* is 2.43%

Rancidity testing determines the level of oxidation in a sample. When lipids (fats and oils) go rancid, its nutritional value is compromised, and the lipids will take on a rancid taste and odor. Proper rancidity testing is an essential component in determining the shelf life of the product. It is not detected in the sample *Kalingadi Taila*.

The application of the Iodine value is to determine the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. This parameter is frequently used to determine adulteration of commercial fats and oils. Iodine value is directly proportional to the degree of unsaturation and inversely proportional to the melting point of lipid. An increase in iodine value indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation. Here iodine value of Kalingadi taila is 98.5, means comparatively is at higher side which indicates the better absorption of drug. It has protective body mechanism.

Peroxide value is a useful method to determine the quality of oil. It is an index to measure the concentration of hydroperoxide, which is formed during lipid oxidation. Oxidation leading to rancidity in fats and oils is catalyzed by the presence of certain metallic salts. Here *in Kalingadi Taila* peroxide value not detected means it is not leading towards rancidity and stability is good.

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. More the LOD value indicates more moisture content this will lead to early spoilage of sample. Here LOD of *Kalingadi Taila* is 0.07.

HPTLC

Under short UV there were 9 bands observed in all the 3 concentrations applied, namely Rf- 0.07, 0.12, 0.20, 0.25, 0.31, 0.36, 0.46, 0.55 and 0.80 (all fluorescent green)

Under long UV there were 7 bands namely, Rf – 0.12, 0.25, 0.42, 0.46, 0.58, 0.62, 0.75, (all fluorescent blue)

After derivatization of the plate with VSA spraying reagent observed in white light there were 4 spots where 3 are purple namely – 0.28, 0.42, 0.46 and 1 pink namely 0.58

Densitometric scan at 254nm

Densitometric scan of the plate at 254nm showed the presence of 12 peaks at Rf 0.01, 0.09, 0.15, 0.23, 0.30, 0.34, 0.41, 0.50, 0.63, 0.72, 0.83, 0.90 with maximum absorption at 0.63 (38.32%) was the major peak.

Densitometric scan at 366nm

Densitometric scan at 366nm carried showed 6 peaks at Rf 0.01, 0.30, 0.48. 0.53, 0.71. 0.99 with maximum percentage area of 61.32 at 0.53 Rf.

Densitometric scan at 620nm (After derivatisation)

After derivatisaion when the plate was scan was subjected at 620nm total of 7 peaks were observed among which Rf of 0.07, 0.22, 0.37, 0.53, 0.69, 0.83 and 0.94 with maximum percentage of absorption being 29.24 percent at 0.53 Rf.

CONCLUSION

The given sample of ***Kalingadi taila*** has been standardized as per standard testing protocol. The results of standardization parameters and HPTLC Photodocumentation, R_f values and Densitometric scan are given in respective tables and figures. The

organoleptic characters and parameters of physico chemical analysis of *Kalingadi Taila* was within the normal reference range. Under Densitometric scan at 254nm 12 peaks and at 366nm 6 peaks were found. After derivatisation at 620nm 7 peaks were observed. Hence it is inferred that the *Kalingadi Taila* meets a maximum qualitative standard.

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