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Standard Manufacturing Procedure and Quality Control of Karpanpatru Roll-on Prepared with Coconut Oil

Sahara Shrestha*, Prashant Bedarkar, Biswajyoti Patgiri, Vinay Janaradan Shukla¹, Pradeep Kumar Prajapati²

Department of Rasa Shastra and Bhaishajya Kalpana, ¹Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar 361008, Gujarat. ²Department of Rasa Shastra and Bhaishajya Kalpana, All India Institute of Ayurveda, New Delhi 110076, India.

ABSTRACT

Introduction: *Karpanpatru* formulation is a formulation used since long in Gujarat Ayurved University for treating *Shvitra* (vitiligo). The aim of the present study was to develop standard manufacturing procedure and establish preliminary analytical profile of *Karpanpatru* roll on. As coconut oil is beneficial for skin and possesses emollient properties; it is used in conversion of the formulation into roll-on to assure uniform application with a target oriented approach and safe handling in view of arsenic content.

Methods: Total five batches of *Karpanpatru* roll-on were prepared from one litre of *Karpanpatru Taila* for each batch. The ingredients for the formulation after procurement from different sources were authenticated and was prepared following classical guidelines of *Sneha Paka* and *Siktha* (Bees wax) was added to prepare roll-on. Organoleptic and physico-chemical analysis of the finished product was carried out to develop preliminary quality parameters.

Results: For preparation of one litre of *Karpanpatru Taila*, an average of 5 days of intermittent heating (average 1.5 h/day) and 9.02 h duration for optimum *Sneha Siddhi* (completion of *Karpanpatru Taila*) was required. For preparation of *Karpanpatru* roll-on, *Siktha* was required in the proportion of 1/10th part of *Karpanpatru Taila*. Organoleptic and physico-chemical profiles of the roll-on form were established.

Conclusion: *Karpanpatru Taila* exhibited an average loss of 10.4%. Arsenic content was found to be 33.44 ppm in the roll-on. The adopted procedure, outcomes and test results of analysis of *Karpanpatru* roll-on may be useful in establishing the standardization of *Karpanpatru* roll-on and considered as standards for future studies.

KEYWORDS

Narikela, *Shvitra*, *Siktha*, Standardization, *Taila*

PICTORAL ABSTRACT



Heating of Coconut oil



Ingredients of Kalka



Addition of Gomutra in Taila



Sneha Siddhi Lakshana



Siktha addition



Roll-on bottle and TLC fingerprint

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CORRESPONDENCE

Dr. Sahara Shrestha

MD Scholar,

Department of Rasa Shastra and Bhaishajya Kalpana, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar 361008, India.

Email: saharestha@yahoo.com

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1. Introduction

Ayurveda embraces a holistic approach with its gist lying in curing the disease and promoting health of an individual. Ayurveda is unique in its sound foundation of documented theories and operational guidelines. Although Ayurvedic seers have scientifically documented outcomes of pharmaceutico-therapeutic aspects of regular practice and keen observations of medicinal formulations and established theories, still the validation of these theories in terms of modern scientific tools and parameters is needed to assure its credibility and global acceptability. *Karpanpatru* formulation in powder form has been used effectively since long in the hospitals of Gujarat Ayurved University, Jamnagar. ^[1] *Karpanpatru* formulation is derived from a compound name as Karpogi Paste. This is mentioned in Siddha pharmacopeia and has been utilized to treat vitiligo since mid 50's. ^[2] Studies were also attempted by modifying into *Lepa* (medicated paste) and *Taila* (medicated oil) forms to increase patient compliance and shelf life. ^[1] The comparative efficacy of

the media was assessed by replacing *Gomutra* (cow urine) with *Takra* (butter milk) and *Kanji* (sour gruel). ^[3] Later studies attempted on converting oil into gel for ease of application, transportation and better acceptability. ^[4] As the formulation contains *Haratala* (Arsenic trisulphide); its application using fingertip may pose a risk of dose variation and toxicity. Arsenic toxicity inactivates up to 200 enzymes, most notably those involved in cellular energy pathways and DNA replication and repair, and is substituted for phosphate in high energy compounds such as ATP. Unbound arsenic also exerts its toxicity by generating reactive oxygen intermediates during their redox cycling and metabolic activation processes that cause lipid peroxidation and DNA damage. ^[5] To minimize this; *Karpanpatru* formulation was modified into roll-on form that ensures site specific drug application, easy handling and increased stability than liquid dosage forms. Apart from commonly used *Taila* (*Sarshapa taila*) for *Sneha Paka* of *Karpanpatru Taila*, in present study, coconut oil was

used and converted into roll-on form by the addition of bee's wax and its in-process standards and quality parameters were established.

2. Materials and methods

2.1 Procurement of raw materials

Karpanpatru Taila is a herbo-mineral compound used for treating *Shvitra* (Vitiligo) (Table 1). *Bakuchi* (*Psoralea corylifolia* Linn.) and *Siktha* (bees wax) were procured from Pharmacy, Gujarat Ayurved University, Jamnagar. *Tuvaraka* (*Hydnocarpus laurifolia* Dennist.) was obtained from Mangaluru, Karnataka. *Krishna Jeeraka* (*Carum carvi* Linn.), *Ahiphena* (*Papaver somniferum* Linn.), *Karvi* (*Nigella sativa* Linn.), *Vatama* (*Prunus amygdales* Linn.), *Narikela* (*Cocos nucifera* Linn.) and coconut oil were obtained from local market, Jamnagar. Authentication of raw materials was done at Pharmacognosy Lab of Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar. Samples of the raw materials were then examined for probable adulterants and for foreign matters, which were removed.^[6-7] *Shodhita Haratala* (processed orpiment) was procured from the Department of Rasa Shastra and Bhaishajya Kalpana of Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar. Cow urine was procured from *Panjarapola Goshala* (Cow-shed), Jamnagar. Roll-on glass bottles (10 ml capacity) were purchased from Kerala. The specifications of the vessels are shown in Table 2.

Table 1. Formulation composition of *Karpanpatru Taila* for batch size of 1 litre

SN	Ingredients	Botanical name	Part used	Ratio	Quantity
Kalka Dravya(paste)					
1	<i>Bakuchi</i>	<i>Psoralea corylifolia</i> Linn.	Dried Seeds	1/8 th part	31.25gm
2	<i>Tuvaraka</i>	<i>Hydnocarpus laurifolia</i> Dennist.	Dried Seeds	1/8 th part	31.25gm
3	<i>Krishna Jeeraka</i>	<i>Carum carvi</i> Linn.	Dried Seeds	1/8 th part	31.25gm
4	<i>Karvi</i>	<i>Nigella sativa</i> Linn.	Dried Seeds	1/8 th part	31.25gm
5	<i>Vatama</i>	<i>Prunus amygdales</i> Linn.	Dried Seeds	1/8 th part	31.25gm
6	<i>Ahiphena</i>	<i>Papaver somniferum</i> Linn.	Dried Seeds	1/8 th part	31.25gm
7	<i>Narikela</i>	<i>Cocos nucifera</i> Linn.	Endocarp	1/8 th part	31.25gm
8	<i>Haratala</i>	Yellow arsenic sulphide	-	1/8 th part	31.25gm
Other ingredients					
1	Coconut oil	<i>Cocos nucifera</i> Linn.	Oil	4 parts	1 Litre
2	<i>Gomutra</i>	Cow's Urine	---	16 parts	4 Litres
Roll-on preparation					
1	<i>Karpanpatru Taila</i>		--	1 part	1 litre
2	<i>Siktha</i>	Bees Wax	--	1/10 th of <i>Taila</i>	100 gm

2.2 Preparation of *Karpanpatru Kalka*

The herbal ingredients were powdered and passed through sieve 40# (BSS). Powder of *Haratala* was mixed with other ingredients, mixed thoroughly to prepare a homogenous blend. This blend was taken in a stainless steel vessel and cow urine (125 ml per 250 gm of *Kalka* on an average) was added in sufficient quantity to prepare bolus of *Kalka*.

2.3 Preparation of *Karpanpatru Taila*

Coconut oil was taken in a steel vessel and heated over mild heat maintaining temperature around 90°C for 15 minutes. The vessel was removed from the heating source and allowed to cool slightly. *Kalka* was added to coconut oil followed by addition of cow urine. Heating was continued for duration of one and half hours per day maintaining temperature in between 100 ± 6°C. The contents were stirred continuously throughout the process to avoid the possibility of settling down of suspended contents and charring. The contents were left undisturbed through the night and heating was continued on the next day. Classical tests of *Taila* and *Kalka* for determination of *Sneha Paka* were conducted. At regular intervals; *Kalka* was rolled in between fingers to check the consistency. Heating was stopped after observing characteristic features of *Taila Paka*. It was filtered while hot through clean cotton cloth into sterile stainless steel container. Total five batches of *Karpanpatru Taila* were prepared [KN], the details of which are presented in Table 3 and 4.

2.4 Preparation of roll-on

The proportion of *Siktha* required for *Taila* was determined with 1/6th, 1/7th, 1/8th and 1/10th part of *Taila*. KN was taken into a stainless steel container and subjected to mild, controlled heating. When the temperature of *Taila* reached to 60°C, small pieces of *Siktha* were added and allowed to melt completely with continuous stirring. The contents were filtered while hot through a clean cotton cloth to separate insoluble particles, if any, possibly present in *Siktha*. After that, it was stirred continuously till the contents reached to room temperature and the blend became homogenous liquid in consistency. Then it was poured and stored in roll-on bottles. The details of the five batches of *Karpanpatru* roll-on prepared are presented in Table 5.

2.5 Analytical study

Karpanpatru roll-on was subjected to basic analytical tests. The organoleptic characters of *Kalka*, coconut oil, *Karpanpatru Taila* and *Karpanpatru* roll-on were assessed.

2.6 Physico-chemical characteristics

Physico-chemical parameters of cow's urine, coconut oil and *Karpanpatru Taila* were assessed.

Table 2. Equipment Specifications

Equipment	Specification
Type of Vessel	Stainless Steel Vessel
Depth	16 cm
Diameter	20 cm
Circumference	94.2 cm
Capacity	8 Litre
Size of Cotton Cloth	2x2 Feet
Length of Spatula	32 cm
Measuring Jar	2 Litre Plastic Jar
Weighing Machine	20 kg sensitivity ±0.2gm
Heating Device	LPG Cylinder
Thermometer	Mercury thermometer (0° C to 360°C)

Table 3. Observations and results of Karpanpatru Taila

Parameters	Batch					Average
	1	2	3	4	5	
Weight of powder of <i>Kalka Dravya</i> (g)	250	250	250	250	250	250
Quantity of Cow urine required for preparation of bolus (ml)	115	120	130	130	125	124
Weight of <i>bolus</i> (g)	349	353	368	372	363	361
Initial quantity of <i>Taila</i> (ml)	1000	1000	1000	1000	1000	1000
Final quantity of <i>Taila</i> (ml)	910	900	890	900	880	896
Residual <i>Bolus</i> after <i>Sneha Paka</i> (g)	574	578	584	585	582	580.6
Loss of <i>Taila</i> (ml)	90	100	110	100	120	104
Percent loss of <i>Taila</i>	9	10	11	10	12	10.4

Table 4. Time taken for preparation of Karpanpatru Taila

SN	Observations and temperature of mixture (at stage of observation and duration)	Duration required (Batches 1-5)					Average
		1	2	3	4	5	
On 1st day							
1	Duration of heating coconut oil to de- moisturize at 90°C (min)	17	15	15	16	17	16
2	Addition of <i>Bolus</i> at 80°C (min)	28	24	27	28	30	27.4
3	Addition of Cow urine at 80°C (min)	32	29	31	32	34	31.6
4	Boiling started at 100°C (min)	44	40	42	44	48	43.6
On last day (Duration required to get <i>Sneha Siddhi Lakshana</i>)							
5	<i>Phenodgama</i> at 104°C (hr)	7:55	7:49	7:52	8:00	8:07	7:56
6	<i>Mridupaka</i> at 104°C (hr)	8:20	8:15	8:17	8:24	8:35	8:22
7	<i>Madhyama Paka</i> at 106°C (hr)	9:00	8:55	9:00	9:04	9:15	9:02
8	Total Time (hr)	9:00	8:55	9:00	9:04	9:15	9:02

Table 5. Observations of pharmaceutical preparation of Roll-on from Karpanpatru Taila prepared with Coconut oil

Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Average
Quantity of Coconut oil (ml)	910	900	890	900	880	896
Quantity of Bee's wax (gm)	91	90	89	90	88	89.6
Duration of Stirring (min)	21	19	20	18	22	20
Yield (gm)	996	986	975	985	962	980.8
Percent yield (%)	99.5	99.59	99.59	99.49	99.38	99.51

3. Results and discussion

Standardization is an essential factor for medicinal formulations in order to assess their quality based on the concentration of chemical or bioactive marker.^[8] The use of fine powder in *Kalka* reduces the final yield of the *Taila*^[2] and also increases the chances of passing through the sieve during filtration, increasing turbidity of *Siddha Taila* leading to reduction of its shelf life. Hence ingredients were powdered and passed through sieve 40# BSS. Mild fragrance of coconut oil was perceived when it was heated to attain moisture free state. After being free from moisture, colour of *Taila* became darker. During this period, popping sound was perceived along with slight bubbling. The popping noise denotes the water leaving the *Sneha*, which quickly got subsided. Frothing was noted in initial hours of boiling after addition of cow urine in oil which may be due to partial emulsification and sudden release of ammonia from cow's urine. The bubbling in *Sneha*, on the verge of *Sneha Siddhi* was because of the moisture inside the *Kalka*. Cow urine is heavier than coconut oil so, it sank to the bottom and then boiled. The steam thus produced due to more quantity of hot oil and very little moisture fragmented into smaller compartments throughout the bolus eventually make bubbles rise suddenly to the surface. The contents were stirred well to allow equal dissipation of heat. *Sneha* was heated intermittently maintaining temperature in between 100±6°C for approximately 1.5 h/day for four days. On the fifth day, *Sneha* was heated till the *Paka lakshana* (completion test for chief

desired characteristics) were obtained like *Gandha-Varna-Rasotpatti* (desired smell, color and taste), *Shabdahinata* (no cracking sound on fire), *Phenodgama* (appearance of froth) and *Vartivat Kalka* (stickiness in bolus to get rolled in to a wick). An average duration of heating per day was decided from pilot batch of the same batch size so as to get *Sneha Siddhi Lakshana* in five days.^[9] The longer duration of interaction of the ingredients and the media was provided with a view to extract more chemical moieties. Repeated boiling causes several oxidative and thermal reactions bringing about a change in the physico-chemical and therapeutic properties in *Siddha Sneha*. *Phenodgama* occurred due to presence of unsaturated fatty acids in *Taila*.

Madhyama Paka of the *Siddha Sneha* was done so as to extract maximum active chemical constituents. The temperature during the *Paka Kala* (preparation time) was maintained near about 106°C. By this, reaction between the liquid and fat molecules occurs in a consistent manner over a specific time. This temperature facilitates easy evaporation of liquid molecules with the retention of water soluble extractives, which is slowly imbibed into the *Sneha* by loosening the bondage in between the fat molecules. The weight of the residual *Kalka* was comparatively more i.e. an average of 580.6 g than that of initial weight of bolus before heating. Gain in weight may be due to retention of oil by particles of bolus.

Table 6. Organoleptic characters

Character	Kalka	NT	KN	KN Roll-On
Color	Brownish Yellow	Whitish Yellow	Light Brownish yellow	Brownish Yellow
Smell	Gomutra	Mild	Characteristic	Characteristic
Touch	Slightly Rough	Creamy	Creamy	Creamy
Form	Bolus	Liquid	Smooth - oily	Semi-solid

NT= Coconut oil; KN =Karpanpatru Taila prepared with Coconut oil; KN Roll-On= Karpanpatru Roll-on prepared with coconut oil

Table 7. Physico-chemical parameters of Cow's urine and Coconut oil

Parameters	Cow's urine	Coconut oil
pH	7.46	-
Total Solid Content	6.51	-
Specific Gravity	1.029	0.931
Refractive Index	-	1.44
Saponification Value	-	248
Acid Value	-	1.76

Table 8. Physico-chemical parameters of Karpanpatru Taila

Parameters	Karpanpatru Taila
Moisture Content	0.184 % w/w
Refractive index	1.457
Specific gravity	0.9233
Viscosity	24.652 Cp
Acid value	8.477
Saponification value	255.53
Peroxide value	0.796
Iodine Value	155.84
Lead (Pb)	ND
Cadmium (Cd)	ND
Arsenic (As)	33.44ppm
Mercury (Hg)	ND
Total microbial plate count	02 cfu/mL
Total yeast & mould	Absent
<i>Staphylococcus aureus</i>	Absent
<i>Escherichia coli</i>	Absent
<i>Salmonella Spp.</i>	Absent
<i>Pseudomonas aeruginosa</i>	Absent

ppm - parts per million; ND - Not Detected; cfu/mL - Colony forming unit per millilitre; cP - Centipoise

The roll-on was prepared with the addition of *Siktha* in 1/10th parts of *Karpanpatru Taila* as it was found to be the most appropriate ratio to get the desired consistency from the results of the pilot batch made by incorporating different proportions of *Siktha*. The homogenous liquid blend was poured into the roll-on bottles and the cap was properly fitted. The organoleptic characters were assessed as shown in Table 6.

The physico-chemical parameters of cow's urine, coconut oil and *Karpanpatru Taila* were assessed as shown in Table 7 and 8. An acid value of coconut oil has been significantly increased after *Sneha Paka* suggesting chemical breakdown of glycerides of *Sneha* during process of *Sneha Paka*. The viscosity of prepared oil was found 36800 cP which may also influence rate of absorption of topical applicant. Free fatty acids are nascent and hence susceptible for formation of newer compounds in an attempt to get stabilized which has been seen in the form of changes in saponification value after *Sneha Paka*. Saponification value of *Karpanpatru Taila* was 255.53. The long chain fatty acids found in fats have a low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids.^[10] High saponification values of fats and oils are due to predominantly high proportion of shorter carbon chain

lengths of the fatty acids and more number of low molecular weight fatty acids.^[11-12] Shorter chain fatty acids (high saponification value) have faster rate of absorption than longer chain fatty acids. Comparatively higher saponification value of KN points out that the absorption of KN may be quicker than coconut oil itself. Increase in saponification value of KN than coconut oil may be due to different reactions during *Sneha Paka* including oxidation, hydrolysis, polymerization due to repetitive heating of fats^[13] saturation due to heating with aqueous media etc.

All these physico-chemical parameters such as viscosity, specific gravity, peroxide value, iodine value and saponification values are qualitative properties of oils and do not indicate the position of the double bonds or the amount of olefinic carbon but rather it provides an overall status of unsaturation of the oils so it is not possible to point out the position of double bond(s) which are more susceptible to oxidation.^[14]

Table 9. Physico-chemical parameters of Karpanpatru Roll on

Parameters	Result
Moisture content (%w/w)	0.148
Viscosity (cP)	36800 cP
Spreadability (mm/10g)	22

cP - Centipoise

Table 10. HPTLC profile of samples of Roll-on at 254nm, 366nm and 540nm

Spot No. (10µL)	Short UV	Long UV	White light
1	0.11	0.14	0.05
2	0.29	0.24	0.23
3	0.52	0.35	0.33
4	0.60	0.43	0.54
5	0.67	0.52	0.79
6	0.92	0.75	0.91

Solvent System- Ethyl Acetate: Methanol: Ammonia (8:2:0.2)

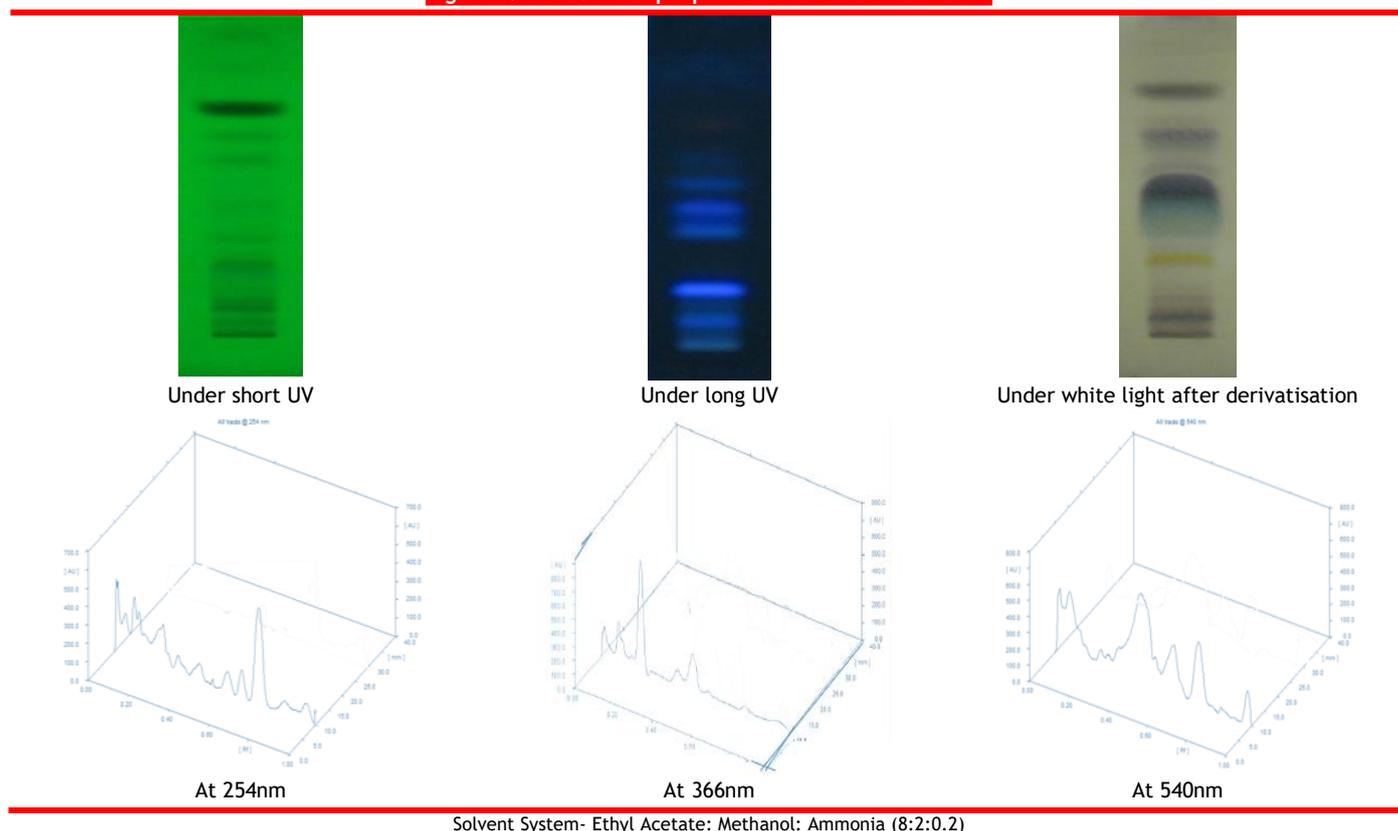
The spreadability of the roll-on form was found to be 22mm/10g as shown in Table 9. This indicates that the formulation has a good flow on skin. Ultimate acceptability and clinical efficacy of topical preparations require them to possess optimal mechanical properties (ease of removal from the container, spreadability on the substrate), rheological properties (viscosity, elasticity, thixotropy, flowability) and other desired properties such as bio-adhesion, desired drug release, and absorption.^[15] KN was found to be free from fungal and bacterial growth suggesting that KN is free from microbial contamination and contain moisture in negligible limits. Arsenic content was found to be 33.44 ppm. HPTLC study showed same number of spots in the visualizing conditions as shown in Table 10 and Figure 1.

Karpanpatru roll-on prepared with coconut oil solidifies in winter. Coconut oil melts at 24°C (75°F)^[16] and is solidified below that temperature. The roll-on had *Siktha* added to it, which further helped it to solidify in winter. Hence the flow of the roll-on prepared with coconut oil was not identical in all seasons. The roll-on needed to be placed in hot water bath to attain a good flow during winter season. Appropriate excipient may be added to maintain the identical consistency of roll-on.

4. Conclusion

Karpanpatru Taila prepared with coconut oil was light brownish yellow with a characteristic smell and had an average loss of 10.4% on an average and required 9:02 h for preparation. It took 20 min for the preparation of roll-on from *Karpanpatru Taila* and the yield was 99.51% with addition of 1/10th part of *Siktha*. The evaluated parameters i.e. moisture content, viscosity and spreadability were 0.148% w/w, 36800cP and 22 respectively of roll-on (Table 9) and 6 spots of HPTLC of KN Roll-on were at 254 nm, 366 nm and 540 nm.

Figure 1. HPTLC of Karpanpatru Roll-on with coconut oil



Solvent System- Ethyl Acetate: Methanol: Ammonia (8:2:0.2)

The standard operating procedures for preparation along with quality control parameters of *Karpanpatru* roll-on with coconut oil observed in the current study may be considered as standards for future studies.

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