



Pharmaceutical Standardization

Quality control evaluation of *Keshamasi*, *Keshanjana* and *Keshamasi* eye ointment

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Abstract

Background: *Keshanjana* (collyrium) is a well known Ayurvedic preparation prepared out of *Keshamasi* (ash prepared by scalp hairs) mixed with *Goghrita* (cow's ghee). This medicine is indicated for the treatment of *Shushkakshipaka* (dry eye syndrome) in the classical literature of Ayurveda; hence, it was under taken for standardization and clinical evaluation in an extra-mural research project from Central Council for Research in Ayurvedic Sciences, Department of AYUSH, New Delhi. **Aim:** To develop standard quality parameters for the *Keshamasi*, *Keshanjana* and *Keshamasi* ointment. **Materials and Methods:** Scalp hairs of male and females collected from saloons were converted to classical *Masi Kalpana* and mixed with cow ghee and petrolatum in the ratio of 1:5 to prepare the *Keshanjana* and *Keshamasi* ointment respectively. Standard Operation Procedure (SOP) were adopted and recorded accordingly. The raw material, furnished products and plain *Goghrita* were subjected for quality control parameters i.e., physico-chemical evaluation, anti-microbial study, particle size analysis, heavy metal analysis through inductive couple plasma spectroscopy with high performance thin layer liquid chromatography fingerprints. **Results:** Rancidity was negative in all the samples, indicating that the physico-chemical parameters are in acceptable range. Lead and zinc were present in most of the samples; while all samples were free from microbial contamination. **Conclusion:** As no standards are available to compare the results of the current study, the observations cannot be compared. Thus the profile generated in the current study can be considered as standard to refer in future studies.

Key words: *Keshamasi*, *Keshanjana*, ointment, quality control profile

Introduction

Keshanjana (collyrium) is an Ayurvedic formulation prepared out of *Keshamasi* (ash prepared by scalp hairs) mixed with *Goghrita* (cow ghee). This particular preparation is indicated for treating *Shushkakshipaka* (dry eye syndrome) in *Vagbhata Samhitas* and classical *Ayurvedic* treatises.^[1] *Keshanjana* has tried clinically as PG thesis work with control of artificial tears and better results were found in comparison to control group.^[2] Encouraged by this outcome an EMR project on the "standardization and clinical evaluation of *Keshanjana*-an Ayurvedic formulation" has been granted by Central Council

for Research in Ayurvedic Sciences, Department of AYUSH, New Delhi and in the present paper analytical/quality parameters for the standardization process are presented.

Keshmasi prepared by using *Putapaka Vidhi* (incineration method). Hairs were smeared with *Goghrita* (cow ghee) on glass slab. After proper mixing, mixture was equally divided in four parts and kept in four earthen saucers. Later, it was subjected for *Putapaka* in the conventional *Puti* (30 *Angulas* = 57 cm in length, breadth and height). After complete burning, it was allowed for self-cooling. *Masi* (ash) was collected from the inner surface of earthen saucers recovered from *Puti* after self-cooling and then collected *Masi* (ash) was packed in air tight bags and kept for further processing. Same method was adopted for the preparation of both male and female scalp hairs *Keshamasi*.

The *Keshanjana* (collyrium prepared by ash of scalp hairs) prepared by using male and female *Keshamasi* (ash of scalp hairs) by adopting same method. During this process, *Masi* (ash) was transferred into a mixer grinder and sieved through 400#.

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Afterward, initially *Keshamasi* (ash of scalp hairs) and *Goghrita* was taken in the ratio of 1:2 and triturated for 6 hours. Then gradually 100 g of *Goghrita* was added and triturated again up to the formation of uniform mixture. After complete attrition, the *Keshanjana* (collyrium prepared by ash of scalp hairs) was weighed and transferred in to an air tight container for further processing. The complete process was repeated for preparation of ointments but instead of *Goghrita*, petrolatum was used as the base.

For the preparation of the *Keshamasi*, male and female hairs were taken as starting material.^[3] This reference is indicated in the treatment of dry eye syndrome. Although no direct indication regarding the gender based nature of raw drug is indicated; however, here an attempt made to modify the *Keshamasi* into *Keshanjana* an *Raskriya Anjana* (ointment) from male and female hair. Modified preparations found more feasible with later for pharmacy scale as well as far as modishness of the finished product is concerned.^[4]

Objectives of the study

Present research work provides physico-chemical parameters for its quality control of raw and finished product which includes its physico-chemical evaluation, anti-microbial study, particle size analysis, Heavy metal analysis through (Inductive Couple Plasma [ICP] spectroscopy) with high performance thin layer liquid chromatography (HPTLC) fingerprint of finished products. These qualitative parameters can be used for good manufacturing practices of the Drug preparation.

Materials and Methods

Procurement of material

Finished product *Keshamasi*, *Keshanjana* and *Keshamasi* ointment prepared out of male and female scalp hair. All samples were subjected for the quality control process and coded as follows:

- *Keshamasi* (prepared out from the male's scalp hair) - KMM
- *Keshamasi* (prepared from female's scalp hair) - KMF
- *Keshanjana* (prepared from male's scalp hair) - KSJM
- *Keshanjana* (prepared from female's scalp hair) - KSJF
- Ointment (prepared from male's scalp hair) - OM
- Ointment (prepared from female's scalp hair) - OF
- *Goghrita* – cow ghee
- *Keshamasi* is considered as raw material in this study for preparation of *Keshanjana* and *Keshamasi* ointment.

Physico-chemical evaluation

In physical evaluation prescribed standardization parameters of ghee such as rancidity test, saponification value, acid value, iodine value, ester value, peroxide value, specific gravity, refractive index etc., studied as per standards.^[5]

The analysis of raw materials included organoleptic parameters such as consistency, color, taste and odor, physico-chemical parameters such as loss on drying, total ash, acid-insoluble ash, water soluble ash, alcohol soluble extractive values, water soluble extractive values and petroleum ether soluble extractive values were also carried out. Particle size analysis has been done.

The analysis of finished products included the organoleptic

parameters such as consistency, color, taste and odor, determination of pH, acid value, iodine value, saponification value, ester value, peroxide value, estimation of unsaponifiable matter. The determinations were performed in triplicate and results are expressed as mean \pm standard deviation.

Microbiology study

In this study total microbial count, total bacterial count, total fungal count with pathogens-*Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* in both aerobic and anaerobic conditions for KMM, KMF, KSJM, KSJF, OM and OF were carried out.

Particle size analysis

The KMM, KMF, KSJM, KSJF, OM and OF were analyzed in Symantec HELOS (H1004) instrument, by taking water as dispersion medium and measured at 0.5/4.5-875 μ m range. It has three type Fourier lances: R1-0.1 μ m to 35 μ m, R2-0.5 μ m to 175 μ m, R3-0.5 μ m to 875 μ m.^[6]

Heavy metal analysis

ICP spectroscopy study

The ICP analyses of the samples were carried out. All samples KSJM, KSJF, OM and OF were tested for the presence of heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and zinc (Zn).^[7,8] Microbiological study, particle size analysis and heavy meal analysis were carried out at SICART laboratory Vallabh Vidyanagar, Gujarat.

HPTLC study

For HPTLC investigation, the chromatogram of unsaponifiable matter of cow ghee, raw materials and formulated finished products obtained. A Camag HPTLC equipped with a sample applicator Linomat V, twin trough plate development chamber, thin layer liquid chromatography Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland) were used.^[9]

Experimental condition

- Stationary phase: Precoated silica gel Gf 254.
- Mobile phase: Hexane:diethyl ether:acetic acid (7:3:0.5).
- Spray reagents: Anisaldehyde sulphuric acid.

Table 1: Results of physico-chemical parameters for cow's ghee

Parameters	Values
Specific gravity at 40°C	0.9097
Refractive index at 40°C	1.4620
Rancidity test	Nil

Table 2: Organoleptic characters of raw materials

Observation	KMM	KMF
Color	Black	Black
Odor	Disagreeable	Disagreeable
Taste	Bitter	Bitter
Texture	Fine powder	Fine powder

KMM: *Keshamasi* (prepared out from male's scalp hair), KMF: *Keshamasi* (prepared out from female's scalp hair)

Table 3: Organoleptic characters of finished products

Observation	KSJM	KSJF	OM	OF
Color	Black	Black	Black	Black
Odor	Disagreeable	Disagreeable	Disagreeable	Disagreeable
Appearance	Black oily	Black oily	Black paste like	Black paste like
Clarity	NA	NA	NA	NA

KSJM: *Keshanjana* (prepared from male's scalp hair), KSJF: *Keshanjana* (prepared from female's scalp hair), OM: Ointment (prepared from male's scalp hair), OF: Ointment (prepared from female's scalp hair), NA: Not applicable

Observations and Results

Cow ghee shows specific gravity value of 0.9097 at 40°C and refractive index value of 1.4620 at 40°C, which are preferable under as given in application programming interfaces (Ayurvedic Pharmacopoeia of India) references for cow ghee samples [Table 1].

Organoleptic characters of raw materials both the KMM and KMF show black color, disagreeable in odor, bitter in taste and show fine powder texture [Table 2].

Organoleptic characters of finished products show that both KSJM and KSJF show black color, disagreeable in odor, smooth in touch and shows black oily appearance. While both OM and OF shows black color, disagreeable in odor, smooth in touch and shows black paste like appearance [Table 3].

Physico-chemical characters of KMM show more percentage of acid insoluble ash in comparison to KMF while KMF shows more percentage of loss on drying and ash value in comparison to KMM. Both raw materials show nearly 7 pH values. KMM shows more alcohol soluble and petroleum Ether soluble extractive values than KMF. KMF shows more water soluble extractive values than KMM [Table 4].

Physico-chemical parameters of cow's ghee and finished products (KSJM and KSJF) were performed, where KSJM shows more acid value, saponification value, iodine value and ester value in comparison to KSJF. While KSJF shows more peroxide value in comparison to KSJM. OM shows more acid value, saponification value, iodine value and ester value in comparison to OF and OF shows more peroxide value than OM to similar *Keshanjana* drugs. Cow ghee shows all values, which are preferable under as given in Ayurvedic pharmacopoeia of India references for cow's ghee samples. Cow ghee shows negative result to rancidity test. Hence, it is considerable for analysis [Table 5].

Microbial study

Determinations of microorganism of the samples were carried out and the result of the same has been presented. Cow ghee shows all the results of total microbial count and pathogens in aerobic and anaerobic culture are under as limit as per World Health Organization (WHO) safety guideline^[8] [Table 6].

Determination of microorganism and the bacteriological examination of the samples were carried out and it is found that finished products of OM, OF, KSJM and KSJF show total microbial count and pathogens in culture are under the limit mentioned in WHO safety guideline^[8] [Table 7].

Particle size analysis

The distribution curve of particle seemed near to skewness as observed in KMM is 60 µm to 300 µm and in KMF is 60 µm to

Table 4: Results of physico-chemical parameters for *Kesha masi* samples

Parameters (% w/w)	KMM	KMF
Loss on drying at 105°C	5.89	7.31
Ash value	32.55	39.88
Acid insoluble ash	17.17	1.88
pH value	7.74	7.24
Water soluble extractive	43.51	45.21
Alcohol soluble extractive	27.68	23.72
Petroleum ether soluble extractive	7.64	7.39

KMM: *Keshamasi* (prepared from male's scalp hair), KMF: *Keshamasi* (prepared from female's scalp hair)

Table 5: Results of physico-chemical parameters for cow ghee and finished products

Parameters	Cow's ghee	KSJM	KSJF	OM	OF
Acid value	0.888	3.294	2.629	1.579	1.187
Saponification value	255.78	274.63	108.28	15.49	11.15
Iodine value	32.858	42.511	40.726	7.49	7.158
Peroxide value	1.988	3.500	5.472	9.500	10.447
Ester value	254.892	271.336	105.651	13.911	9.963
Rancidity test	-ve	-	-	-	-

KSJM: *Keshanjana* (prepared from male's scalp hair), KSJF: *Keshanjana* (prepared from female's scalp hair), OM: Ointment (prepared from male's scalp hair), OF: Ointment (prepared from female's scalp hair)

Table 6: Results of microbial study of cow ghee in aerobic and anaerobic culture

Test parameters	Results for aerobic culture	Results for anaerobic culture	Limit
Total microbial count	40 CFU/g	40 CFU/g	100 CFU/g
Total bacterial count	30 CFU/g	30 CFU/g	
Total fungal count	10 CFU/g	10 CFU/g	
Pathogens			Should be absent/10 g
<i>E. coli</i>	Absent	Absent	
<i>Salmonella spp.</i>	Absent	Absent	
<i>Pseudomonas aeruginosa</i>	Absent	Absent	
<i>S. aureus</i>	Absent	Absent	

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, CFU: Colony forming unit

400 µm range. The operational confirmation and force may be modified during method validation stage [Table 8].

Table 7: Results of microbial study

Test parameters	Limit	Result for KSJM	Result for KSJF	Result for OM	Result for OF
Total microbial count	100 CFU/g	20 CFU/g	30 CFU/g	40 CFU/g	35 CFU/g
Total bacterial count		15 CFU/g	20 CFU/g	30 CFU/g	30 CFU/g
Total fungal count		5 CFU/g	10 CFU/g	10 CFU/g	05 CFU/g
Pathogens	Should be absent/10 g				
<i>E. coli</i>		Absent	Absent	Absent	Absent
<i>Salmonella spp.</i>		Absent	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i>		Absent	Absent	Absent	Absent
<i>S. aureus</i>		Absent	Absent	Absent	Absent

KSJM: Keshanjana (prepared from male's scalp hair), KSJF: Keshanjana (prepared from female's scalp hair), OM: Ointment (prepared from male's scalp hair), OF: Ointment (prepared from female's scalp hair), *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, CFU: Colony forming unit

Table 8: Observations of particle size distribution test

Sample	X ₁₀ (in µm)	X ₁₆ (in µm)	X ₅₀ (in µm)	X ₈₄ (in µm)	X ₉₀ (in µm)	X ₉₉ (in µm)	SMD (in µm)	VMD (in µm)
KMM	14.05	23.51	83.19	168.79	198.88	301.77	28.34	96.07
KMF	39.87	58.07	147.28	271.12	308.83	451.34	63.07	163.46

KMM: Keshamasi (prepared from male's scalp hair), KMF: Keshamasi (prepared from female's scalp hair), SMD: Sauter Mean diameter, VMD: Visual Molecular Diameter

Table 9: ICP analysis of samples

Element	Wavelength	Instrument detection limit (ppm)	Sample results mg/kg (ppm) OM	Sample results mg/kg (ppm) OF	Sample results mg/kg (ppm) KSJM	Sample results mg/kg (ppm) KSJF
Cadmium	228.802	0.0027	Not detected	Not detected	Not detected	Not detected
Lead	220.353	0.0420	4.386	4.914	14.515	25.706
Mercury	253.652	0.610	Not detected	Not detected	Not detected	Not detected
Arsenic	193.696	0.0530	Not detected	Not detected	Not detected	37.082
Zinc	206.200	0.0059	29.195	1.039	5.666	Not detected

OM: Ointment (prepared from male's scalp hair), OF: Ointment (prepared out from female's scalp hair), KSJM: Keshanjana (prepared out from male's scalp hair), KSJF: Keshanjana (prepared from female's scalp hair), ICP: Inductive Couple Plasma

ICP spectroscopy study

ICP analysis show the presence of lead in all the samples, OM and OF shows lead in prescribed limits. While KSJM and KSJF show lead above prescribed limits. Zinc is present in OM, OF and KSJM while it is absent in KSJF. In OM zinc is present more than prescribed limits while in OF and KSJM shows zinc in prescribed limits. Arsenic is present in KSJF above the prescribed limits. Cadmium and mercury are not detected in any samples. So, finished products are free from these elements [Table 9].

HPTLC study

In the chromatogram of unsaponifiable matter of cow ghee and formulated finished products; R_f values of spots were noted under different conditions. Then all tracks were scanned under 254 nm and 366 nm and also post chromatographic derivation through spray methanolic sulphuric acid and R_f are mentioned in the table. Different tracks are represented at the wavelength of 254 nm and 366 nm [Table 10 and Figure 1].

Discussion

The parameters studied such as saponification value, iodine value, acid value, ester value, peroxide value, specific gravity, refractive index can be considered as standard parameters. Rancidity is an important qualitative parameter to check the quality of ghee sample. Since *Bhaisajya* of ghee always

Table 10: R_f values of unsaponifiable matter of cow's ghee, raw materials and finished products

Sample	Track no.	λ max 254 nm		λ max 366 nm	
		No. of spots	R _f	No. of spots	R _f
KMM	1	3	0.09, 0.34, 0.95	2	0.12, 0.94
KMF	2	5	0.11, 0.14, 0.15, 0.34, 0.41	2	0.14, 0.16
KSJM	3	7	0.12, 0.14, 0.17, 0.33, 0.39, 0.46, 0.61	3	0.14, 0.17, 0.35
KSJF	4	3	0.12, 0.34, 0.39	2	0.15, 0.18
OM	5	6	0.19, 0.30, 0.35, 0.40, 0.76, 0.80	2	0.16, 0.18
OF	6	9	0.19, 0.28, 0.34, 0.56, 0.58, 0.64, 0.77, 0.80, 0.83	5	0.13, 0.18, 0.56, 0.77, 0.83
Cow ghee	7	6	0.30, 0.35, 0.41, 0.49, 0.52, 0.83	5	0.13, 0.18, 0.31, 0.35, 0.90

KMM: Keshamasi (prepared from male's scalp hair), KMF: Keshamasi (prepared from female's scalp hair), KSJM: Keshanjana (prepared from male's scalp hair), KSJF: Keshanjana (prepared from female's scalp hair), OM: Ointment (prepared out from male's scalp hair), OF: Ointment (prepared from female's scalp hair), R_f: Retention value

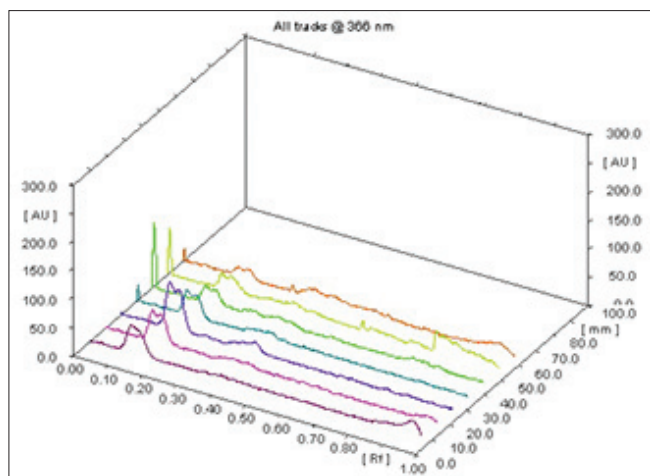


Figure 1: 3D image of all tracks at 366 nm

categorized in to three level of heat *Mridu*, *Madhyama* and *Khara* in short *Ghrta* preparations when prepared is heated as suggested in the drug regime in case of KSJ no other drug added in preparation. During the process of preparation, the analytical values of raw ghee are expected to undergo alterations. Hence, a detailed analytical study was carried out. Further to rule out the influence of extraneous factors on the expression of pharmacological activity and for safety concerns WHO quality control criteria included heavy metal. Hence in this study, the test samples were analyzed for the presence of heavy meals also.

The analysis of raw materials included organoleptic parameters such as consistency, color, taste and odor, physico-chemical parameters such as loss on drying, total ash, acid-insoluble ash, water soluble ash, alcohol soluble extractive values, water soluble extractive values and petroleum ether soluble extractive values were also carried out. Particle size analysis has done to check the uniformity of raw materials.

The analysis of finished products included the organoleptic parameters such as consistency, color, taste and odor, determination of pH, acid value, iodine value, saponification value, ester value, peroxide value, estimation of unsaponifiable

matter. In addition the HPTLC fingerprinting was also carried out, to check the presence of more unsaponifiable matter and separation of more components in the sample with HPTLC fingerprinting. All samples were tested for the presence of heavy metals such as mercury, lead, cadmium, zinc and arsenic. Microbial study has also done to check the absence of pathogens, which are harmful in finished products.

All these qualitative parameters can be taken for good manufacturing of these preparations for APIs and other standard reference.

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हिन्दी सारांश

केश मषी एवं केशाञ्जन का गुणात्मक विश्लेषण

करतारसिंग धीमान, विनय जे. शुक्ला, नयन आर. भालोडिया, विनय आर. शर्मा

केशाञ्जन – केश मषी एवं गो घृत से निर्मित महत्वपूर्ण योग है। यह योग शुष्काक्षिपाक की चिकित्सा में बताया गया है। इस योग का मानकीकरण पूर्व में किया गया। प्रस्तुत अध्ययन में भविष्य की सम्भावनाओं एवं शोध कार्य को बल प्रदान करने के लिये उपयुक्त विश्लेषण हेतु मानकों एवं तथ्यों को समावेशित किया गया है।