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STANDARDIZATION AND QUALITY CONTROL EVALUATION OF *KRIMIMUDGARA RASA* USING MICROSCOPIC STUDIES AND HPTLC

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ABSTRACT

Herbs and minerals are the integral parts of traditional systems of medicine in many countries. Herbo-Mineral medicinal preparations called *Rasa* are unique to the ayurvedic system of Indian Traditional Medicine. These preparations have been used since long and are claimed to be the very effective and potent dosage form. However, there is dearth of scientific analytical studies carried out on these products, and even the existing ones suffer from incomplete analysis. *Krmimudgara rasa* is a unique preparation belonging to this class. This particular preparation has been successfully used by traditional practitioners for the treatment of digestive impairment and ascariasis. This work presents an attempt to evaluate *Krmimudgara rasa*. The formulation was prepared in the laboratory as per the traditional procedure reported in standard text. A marketed sample was also procured and both the formulations were subjected to microscopic studies and HPTLC fingerprinting, and compared with the authentic ingredients as reference. It was observed that the microscopic and chromatographic analyses compliment each other in their findings and can be used effectively for the identification of the raw materials in the compound formulation(s).

KEYWORDS: Indian traditional medicine, Ayurvedic formulation, Chromatographic fingerprinting studies, Herbal drugs.

INTRODUCTION

Ayurveda is an intricate system of healing that originated in India thousands of years ago. Therapeutic effectiveness of the Ayurvedic drugs has been established and well documented in the form of classics

attributed to them.[1] Medicinal preparations called *Rasa* are unique to the Ayurvedic system of medicine which incorporates natural substances such as herbs, minerals, metals, as therapeutic agents.[2-3]

In former days the traditional medicinal formulations were prepared by Vaidyas and delivered to the patients in the fresh form where quality assurance was not needed in the present way. Quality control of traditional medicines is a critical and essential issue to be considered in assuring the therapeutic efficacy, safety and to rationalize their use in the health care.[4]

Although the acceptance of herbal therapies is increasing in developed and developing countries, there is a lack of standardization and quality control methods which produce resistance in the usage of these therapies. Standardization of an ayurvedic formulation assures the quality, quantity, safety and therapeutic efficacy and this evaluation is needed for reliable beneficial therapeutic use. Factors which make standardization important are adulteration, biological and geographical variations, variability of plant constituents and environmental factors. Further due to lack of quality control profile, there is batch to batch variation amongst same product as well as variations amongst same product from different sources.[5]

In the present study, the consistency of an ayurvedic formulation, *krmimudgra rasa* is evaluated using microscopic and chromatographic (HPTLC) parameters of standardization.

Krmimudgara rasa, is an ayurvedic formulation, mentioned in the Ayurvedic formulary of India (Part-1), which is claimed to be have therapeutic effect in treatment digestive impairment and ascariasis. The *rasa* contains Ajmoda fruits (*Apium graveolens*), Vidang fruits (*Embellia ribes*), Shudh visamusti seeds (*Strychnous nux vomica)*[6] and Palasa seeds (*Butea monosperma*). *Rasa* (Mercury) and *gandhaka* (Sulphur) are used to prepare *kajjali*[6].

Literature survey reveals that microscopic studies on the ingredients of *Krmimudgara rasa* have been performed but their evaluation in the formulation has not been done yet. The estimation of Strychnine and Embelin by HPTLC has also been reported.[7-9] However, the formulation also contains a number of pharmacologically active crude drugs which needs to be studied. Thus in the present study, evaluation of the formulation was done by performing microscopic studies and HPTLC studies on the plant ingredients present in the laboratory prepared formulation as well as marketed formulation.

MATERIALS AND METHODS

Plant material

Ajmoda fruits (*Apium graveolens*), Vidang fruits (*Embellia ribes*), Visamusti seeds (*Strychnous nux vomica)* and Palasa seeds (*Butea monosperma*) were purchased from a local store and authentified at the Shivaji Science College, Amravati. Their voucher specimens are maintained in the herbarium at the Government College of Pharmacy, Amravati.

Chemicals

Mercury and sulphur were purchased from S.D fine Chemicals of A.R. grade.

All other solvents used in the study were of A.R. grade.

Formulation

Krmimudgara rasa of one batch, manufactured by Ayurvedic rasashala, Pune, was procured from a local store.

Preparation of Laboratory formulation

Nux vomica seeds were purified by *shodhan* process as per reported method before incorporation in the formulation. The purification of Nux vomica seeds was done by keeping the seeds in cow urine for 7 days and on each day, fresh cow urine was replaced. On the 8th day, seeds were removed and boiled in cow milk for 3 h in *Dola yantra*. The testa and embryo were then removed and seeds were roasted in cow ghee and powdered. [10-12] Other crude drugs were also powdered and sieved through a sieve of Mesh Size. 80. Kajjali was prepared by triturating Mercury and Sulphur together in mortal and pestle till black color was obtained. [13] In Kajjali, other powdered drug material was added and mixed. [14] Prepared formulation was stored in a well closed container.

Microscopic studies

The plant materials were treated with water and glycerine and mounted on a glass plate and observed under the microscope. Powder material was also stained with Phloroglucinol : HCl (1:1) and iodine solution. The microscopic studies in both the formulations was also performed using the same method. [15]

HPTLC studies - Chromatographic Conditions: A Camag microlitre sample (Hamilton, Bonaduz, Switzerland) syringe was used for sample application on pre-coated silica gel aluminium plate 60F-254, (10

cm x 10 cm with 0.2 mm thickness, (E. Merck, Darmstadt, Germany) using a Camag Linomat-V (Switzerland). Samples were then separated using different solvent systems as shown in Table.1. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode for all measurements and operated by CATS software (V1.4.3 Camag). The plate was scanned at 254 and 366 nm. The plate for Nux vomica sample was sprayed with Dragendorffs reagent and immediately scanned at 500 nm and Rf values of all the bands were recorded. [16-18, 24]

Table 1: Mobile phases used for HPTLC Fingerprinting Studies

Drug	**Mobile phase**
Ajmoda	Chloroform : Methanol (9 :1)
Vidang	n-Propanol : n-Butanol : 4N Ammonia (7:1:2)
Nuxvmica	Toluene : Ethyl acetate : Di-ethylamine (7 : 2 :1)
Palasa	Toluene : Ethyl acetate (9:1)

Sample Preparation

For HPTLC studies, 2 gm of each crude drug material and sample of formulations were extracted with 25ml of ethanol and concentrated. The extracts were weighed and dissolved in ethanol. The volume was then made up with ethanol.

Extractive values

Extractive values of laboratory prepared formulation and marketed formulation was evaluated for petroleum ether, alcohol and water. About 1g of both formulations were placed in respective solvents and kept for 24 hrs for cold maceration. After this the samples were filtered and dried under vacuum. Resulting residue was weighed and percentage of extractive value was calculated.[19]

RESULTS

Krimimudgara rasa was prepared successfully in laboratory. The laboratory prepared formulation and marketed formulation were subjected to analysis as above. Both the samples were black in color, smooth with very fine granules.

Microscopic characters

Powder microscopic examinations were carried out to confirm the presence of individual crude drug in laboratory as well as marketed formulations. A number of identifying microscopic characters of individual crude drug powder were observed in laboratory prepared formulation and marketed formulation.

Nux vomica seeds

Powder microscopy of *Shudh nux vomica* seeds showed only the presence of endosperm with yellowish content and not the trichomes with epidermis. The same observation was made in both the formulations. (Figure 1)

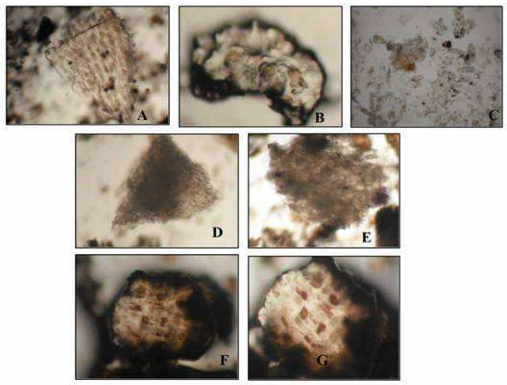

Figure 1: Microscopic characters of powdered Nux vomica seeds(shudh)
A, B and C- Crude drug (endosperm with yellowish content), D and E –Laboratory prepared formulation (endosperm with yellowish content), F and G- Marketed formulation (endosperm with yellowish content).

Vidang fruits

Epicarp consisting of tabular cells of epidermis, obliterate in surface view cells rounded with wrinkled cuticle, reddish brown coloured cells and stone cells were the characters of vidang fruits. Similar characters of *Vidang* fruits were observed in both the formulations as shown in Figure 2. [20]

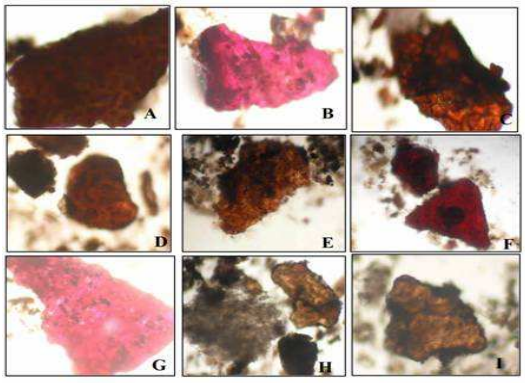

Figure 2: Microscopic characters of powdered *Vidang* fruits

A and D-Crude drug (obliterate in surface view cells rounded with wrinkled cuticle), B-Crude drug- stone cells stained with phloroglucinol : HCL, C- Crude drug (tabular cells of epidermis), E and F- Laboratory prepared formulation (obliterate in surface view cells rounded with wrinkled cuticle), G- Laboratory prepared formulation (obliterate in surface view cells rounded with wrinkled cuticle with staining), H and I- Marketed formulation (obliterate in surface view cells rounded with wrinkled cuticle).

Palasa seeds

Parenchymatous tissues with reddish content, gray and cream colored fragments of testa and starch grains were observed in the seeds. Similar cellular contents were observed in laboratory prepared formulation and the marketed formulation. [21] (Figure 3)

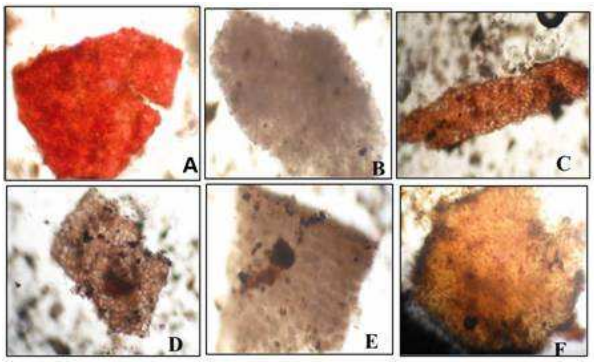

Figure 3: Microscopic characters of *Palasa* seeds

A - Crude drug (Parenchymatous tissues with reddish content), B- Crude drug (cream colored fragments of testa), C and D- Laboratory Prepared formulation (Parenchymatous tissues with reddish content), E - Laboratory Prepared formulation (cream colored fragments of testa), F- Marketed formulation (Parenchymatous tissues with reddish content)

Ajmoda fruits

Thick walled cells of epicarp, trichomes and glandular hairs, yellowish brown vittae, fragments of endosperm were the characters observed for Ajmoda fruits and found to be present in both formulations. [22] (Figure 4)

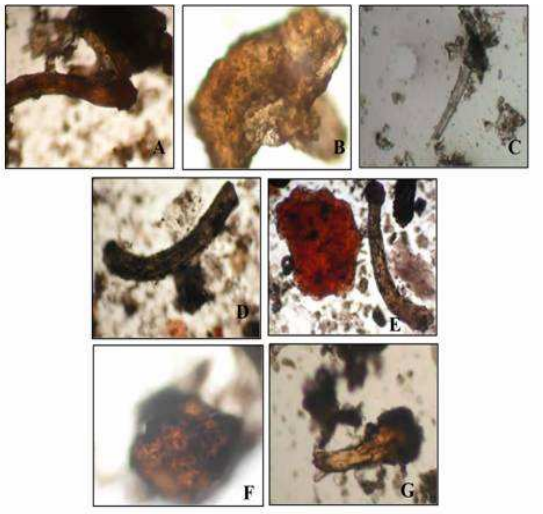

Figure 4: Microscopic characters of powdered Ajmoda fruits.
A- Crude drug (glandular hairs), B- Crude drug (yellowish vittea), C- Crude drug (trichomes), D and E- Laboratory prepared formulation (glandular trichomes), F-Marketed formulation (vittea), G-Marketed formulation (glandular trichomes).

Results of HPTLC fingerprinting Studies

HPTLC fingerprinting studies were performed for individual drugs, laboratory prepared formulation and marketed formulations.

The results obtained are depicted in Figure.5 for the different drugs. The TLC profiles of laboratory and marketed formulations were found to be similar to the TLC profiles of each drug. Thus, the presence of similar chemical constituents was confirmed in the marketed formulation depicting the usage of standard traditional method of formulation preparation. Table 2 shows the comparative Rf values observed in crude drug extract which match with that of both the formulations.

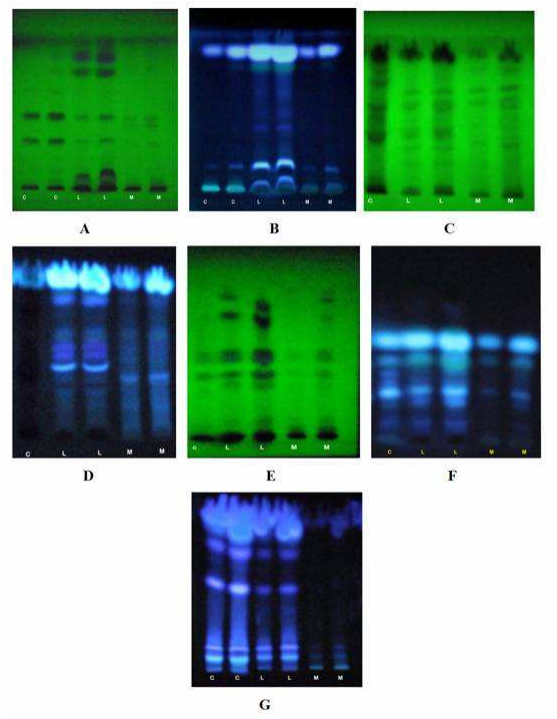

Figure 5: HPTLC fingerprinting profile of Methanol extracts of Crude Drugs, Laboratory Formulation and Marketed Formulation.
A- Nux vomica at 254 nm , B-Nux vomica at 366 nm, C-Vidang at 254 nm, D-Vidang at 366 nm, E-Palasa at 254, F-Palasa at 366 nm, G- Ajmoda at 366 nm.
C- Crude drug (3 µl and 6 µl), L- Laboratory Prepared Formulation (3 µl and 6 µl), M
- Marketed formulation (3 µl and 6 µl).

Table-2: HPTLC Fingerprinting results for Crude drugs (C.D), Laboratory Prepared formulation (LAB) and Marketed formulation (MAR).

Spot No.	Nux Vomica						Vidang					
	C.D	LAB	MAR	C.D	LAB	MAR	C.D	LAB	MAR	C.D	LAB	MAR
	Rf values at 254nm			Rf values at 366nm			Rf values at 254nm			Rf values at 366nm		
1	0.03	0.03	0.02	0.03	0.03	0.2	0.04	0.05	---	0.06	0.06	0.05
2	0.07	0.06	0.05	0.07	0.06	0.07	0.06	---	---	0.1	---	0.13
3	0.13	0.12	---	0.13	0.12	---	0.1	0.1	0.1	0.19	---	0.21
4	0.2	---	0.2	0.74	0.73	0.79	0.19	0.19	---	0.33	0.35	0.35
5	0.31	0.31	0.28	0.83	0.82	0.84	0.33	---	---	0.47	0.46	0.48
6	0.46	0.46	0.41	0.92	0.93	0.94	0.36	0.35	0.35	0.51	0.51	0.53
7	0.53	0.52	---	---	---	---	0.4	0.39	---	0.66	0.65	---
8	0.61	0.61	0.61	---	---	---	0.47	0.45	0.47	0.83	0.84	0.83
9	0.74	0.73	0.76	---	---	---	0.51	0.51	0.53	---	---	---
10	0.84	0.83	0.86	---	---	---	0.66	0.7	0.64	---	---	---
11	0.92	0.92	0.94	---	---	---	0.83	0.84	0.83	---	---	---
Spot No.	**Palasa**						**Ajmoda**					
	C.D	LAB	MAR	C.D	LAB	MAR	C.D	LAB	MAR			
	Rf values at 254nm			Rf values at 366nm			Rf values at 366nm					
1	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	---			
2	0.05	---	0.05	0.05	---	0.06	0.07	0.08	---			
3	0.19	0.19	---	0.1	---	---	0.12	0.13	---			
4	0.21	0.21	---	0.25	0.27	---	0.2	0.21	---			
5	0.33	0.34	0.34	0.33	0.34	0.34	0.27	0.28	0.29			
6	0.4	0.4	0.4	0.39	0.4	0.41	0.34	0.35	---			
7	0.49	0.5	0.49	0.49	0.5	0.5	0.48	_---	---			
8	0.59	---	0.58	0.72	0.71	0.72	0.67	_---	---			
9	0.72	0.71	0.73	0.85	---	0.83	0.8	0.79	0.79			
10	0.85		0.82	---	---	---	0.86	---	0.86			
11	0.93	0.92		---	---	---	0.97	---	---			

Extractive values

The extractive values of the formulations have been given in Table 3. It was observed that the extractive value of laboratory prepared formulation was less than the marketed formulation. Thus the organic content in the laboratory formulation was less than that in the marketed formulation. [23]

Table-3: Extractive values of Laboratory prepared formulation and marketed formulation.

Sr. no.	Solvent	Extracive value	
		Marketed formulation	Lab formulation
1.	Petroleum Ether	14.8 %	17.0%
2.	Alcohol	31.8%	22.0%
3.	Water	44.0%	19.61%

DISCUSSION

In the present study, an ayurvedic formulation, *Krmimudgara rasa*, was prepared as per the standard traditional procedure. This formulation was then evaluated by performing microscopic studies and HPTLC fingerprinting studies. The results obtained were then compared with the results of a marketed formulation. It was observed that all the microscopic structures of each individual crude drug was present in the laboratory prepared formulation and marketed formulation. The fingerprinting pattern obtained by HPTLC also showed similar results in both the formulations. All the bands in the crude drugs were observed in both the formulations.

The extractive value of both the formulations was found to vary, the reason being variation in the organic and inorganic content. Thus, further study needs to be performed for evaluating the quantity of the inorganic content in such formulations. Thus, the above study provides with certain simple methods of preliminary quality control evaluation of *Krmimudgara rasa*. The results reported in the present paper can be used as a standard data for the comparison by manufacturers in order to ascertain the quality of the formulation.

CONCLUSION

The various quality control methods which have been used in the present studies can be used as quality control methods for evaluation of samples in industry to check the uniformity of the samples marketed by manufacturer. The routine use of such scientific techniques will lead to standardization of the product to a certain extent and would definitely help in building confidence in use of such products for medication. However, further analytical and pharmacological study is necessary to explore and justify the biological claims of *Krmimudgara rasa.*

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