ISSN: 2320-2882

IJCRT.ORG



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Role of Mardan with respect to Comparative Pharmaceutical and physicochemical analysis of Samaguna Gandhak Kajjali prepared in two days and seven days

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Abstr<mark>act</mark> :

The first reference of Kupipakwa Rasayana is found in Rasaprakash Sudhakar of Acharya Yashodhara in 12th century where the formulation is named as Udayabhaskara Rasa in the context of Rasa bhasma. Kupipakwa Rasayanas are the unique Murchana yogas of Parada. The formulation are prepared in glass bottle with the aid of Valukayantra. Mercury has a great affinity to the sulfhydryls or thiols. The mercury atom or molecule will tend to bind with any molecule present that has sulfur or a sulfur-hydrogen combination in its structure. Hence, globules of *Parada* molecules disintegrated rapidly in sulfur during the preparation of *Kajjali*. There are also procedures and samskaras mentioned to potentiate the formulations or drugs. One such samskara (process) is mardana samskara that is used as a purification procedure of Parada as well as a bhavana for preparing Khalviya yogas (preparation made in mortar and pestle).

SEM study suggests more fine nature of kajjali prepared in 7days(micron to nano size) than prepared in 2 days(1 to 10 micron size). 7)For the effective role in therapeutic bio-availability of metallic and mineral preparation, particle size is one of the important factor as it decides the permeability of drug through cells, tissues and blood capillaries.(Goldberg, M., Langer, R. and Xinqiao, J., Nanostructured materials for applications in drug delivery and tissue engineering. J. Biomater. Sci. Polym. Ed., 2007, 18(3), 241–268).

Keywords: kupipakva gandhak. kajjali, valukayantra, khalviya yoga, murchana yogas, mardana, samskara

1. Introduction : Ayurveda, though devoted primarly to the maintenance of health and long life of mankind, is related to other animals also like, Asvayurveda, Gajayurveda, Vrksayurveda are also parts of Ayurveda. The tradition of Ayurveda started from Brahma(the creator) himself and camedown in entirely through Daksa prajapti and Ashvins to indra.

The initial concise form of Ayurveda revealed to Brahma consisted of three broad divisions(trisutra)of medical sciences as Hetu(etiology), Linga(symptoms) and aushadha(remedy).In order to understand the vedic period history is divided in to three periods; Pre vedic, Vedic and Post vedic periods.

Prevedic period covers history before the emergence of Vedas, includes Indus valley civilization. Vedic period includes; Samhitas, brahamanas, upnisads and kalpa sutras. Postvedic period is when Ayurved emerged in its crystalized form as a result of discussions and serious thinking in the preceding age.

Rasasastra is the science of minerals ores, metals and precious stones. Rudra is considered as the creator (updeshta) of Rasashastra. Among the samhita texts Charaka enumerated the following as bhauma dravyas- Suvarna, panchaloha(silver,copper,iron, lead,bronze),sikata,(sand),Sudha(lime),etc.(C.S. SU. 1\71-72)

We also came across references about utilization of Gandhaka and Makshika under different formulations. Sushruta mention mineral drugs under two ganas(therapeutic groups) while classifying various drugs like Trapvadi ganas, Usakadi ganas(S.S.SU.38)

In Sushruta external usage of Parada is mentioned. We come across internal usage of Parad in Ashtang sangraha, Rasayana Prakarana.In this text mercury,double pyrite of copper, iron and black bitumen are indicated for internal usage along with honey. It is interesting to note that mercury and honey is used but not mercury and sulphur. Therefore, it may be concluded that mercury and sulphur combination is a much later concept.

In Ashtang Sangraha, two varities of iron Krsna and Tiksna are mentioned. Different Paka procedures Sthali paka(A.S. Ci. 10\10) and Aditya paka(A.S. UT. 28\32)were also found in this text. He also used the term Bhasma in his work. The evolution of Rasashastra mainly based upon mercury. In Charaka we come across reference in the context of Kusthachikitsa where Rasa(mercury) is mentioned(C.S.Ci.7\71)

The evolution of Rasashastra may be arranged in to 4 periods- Prarambhika kala- 5-8A.D.

Madhyama kala- 9-12A.D.

Proudhi kala- 13-15A.D.

Adhunika kala- 16-20A.D.

The Rasaushadhis are well known for their rapid action and are supposed to be superior to other drugs,hence Rasashastra has a unique importance in the field of Ayurveda

Rasa preparation can be categorized as:-

- Kharaliya Rasayanas
- Kupipakwa Rasayanas
- Pottali Rasayanas
- Parpati Rasayana

The first reference of Kupipakwa Rasayana is found in Rasaprakash Sudhakar of Acharya Yashodhara in 12th century where the formulation is named as Udayabhaskara Rasa in the context of Rasa bhasma. Kupipakwa Rasayanas are the unique Murchana yogas of Parada. The formulation are prepared in glass bottle with the aid of Valukayantra.

कुप्याम् अग्निनां पक्वं यद्ररसायनं तत् कूपीपक्वरसायनम् ।।

Many processing types have been mentioned in Indian alchemy, most of the processes mentioned are for making Parada develops lohavada and dehavada property.

There are also procedures and samskaras mentioned to potentiate the formulations or drugs. One such samskara (process) is mardana samskara that is used as a purification procedure of Parada as well as a bhavana for preparing Khalviya yogas (preparation made in mortar and pestle).

The term **Mardana** is used in the following areas of rasashastra:

- 1. As a procedure of general method of **shodhana**(purification)of Parada(mercury)
- 2. A samskara(process) of Parada-Mardana samskara(grinding process).
- 3. As a sub procedure for **Patana samskara**(distillation process), **Utthapana samskara**(processes of mercury)
- 4. Mardana done as a sub process for **satvapatana**(extraction)procedure . ex. Hingula Shodhana.
- 5. Mardana as **a bhavana**(trituration)procedure to triturate the drugs with liquid medial like swarasa(juice)Kashaya(decoction), etc

2. Material and methods :

Mardan is special pharmaceutical procedure subjecting the drug for preparation of Kajjali. Parada and Gandhak was the materials for preparation of Kajjali.

2.1 Pharmaceutical Study

3 batches of Samaguna Gandhak Kajjali prepared with 2 days of mardan3 batches of Samaguna Gandhak Kajjali prepared with 7 days of mardan

The following practical studies have been carried out -

- Gandhak Shodhan
- Parada Shodhan
- Kajjali Nirmana Two types

- a) Triturated for two days.
- b) Triturated for seven days.

Material : Shudhha Parada and Shudhha Gandhak

Apparatus : Mortar and Pestle

Procedure:

Sample 1-

- Kajjali was prepared following SOP acc to textbook Rasatarangini.
- 800gms kajjali(2 days of trituration) was prepared in to two batches.
- Equal quantity of Shudhha Parada(200gms) and Shuddha Gandhak(200gms) was taken in khalva yantra.
- After trituration of 24hrs (2days) ,colour of Parada and Gandhak disappear and black powder was formed.
- During trituration ,minimum 30 rotation per minute was done in eight shape for Kajjali formation.
- Trituration was done till the powder become black in colour and fulfilled all the criteria of Kajjali.

Sample -2

- Kajjali was prepared following SOP acc to textbook Rasahridaya Tantra.
- 800gms kajjali(7 days) was prepared in to one batches due to increased trituration hours (84hrs).
- Equal quantity of Shudhha Parada(400gms) and Shuddha Gandhak(400gms) was taken in khalva yantra.
- After trituration of 84hrs (7days) ,colour of Parada and Gandhak disappear and black and lusterless powder was formed.
- During trituration , minimum 30 rotation per minute was done in eight shape for Kajjali formation.
- Trituration was done till the powder become black in colour and fulfilled all the criteria of Kajjali.
- Precautions : Wear a mask to avoid inhalation of kajjali.
- Trituration was done carefully to avoid spillage of kajjali.
- Mortar and pestle was cleaned and dried before starting the process.

Observations:

sample 1 (mardan for 2 days that is 24hrs)

Hours	Colours	Odour	Consistency	Other observations
After 10mins	yellow	Sulphur and goghrit	Thick powder due to goghrit in Gandhak	
After 20mins	Light grey	Sulphur and goghrit	Thick powder dur to goghrit in Gandhak	
After 1.5hrs	Light grey	Sulphur and goghrit	Thick podwer due to goghrit in Gandhak	Light greyish yellow colour with shiny particles of Parada.
After 3hrs	Grey	Sulphur and goghrit	Powder	Yellow streaks of Gandhak seen
After 6hrs	Grey	Sulphur and goghrit	Powder	Parada was not seen separately in churna.
After 9hrs	Grey	Sulphur	Powder	Lots of shiny particles of parada as its got scattered in powder.
After 12hrs	Dark grey	Sulphur	Fine Powder	It turned in to fine powder.shiny particles were reduced.
After 16hrs	Black	Sulphur	Fine Powder	Attained Varitaratva and Rekhapurnatva
After 18hrs	Black	Sulphur	Fine powder	Fine powder started spilling out of the mortar.
After 22hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny particles present.
After 24hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny particles present.

Sample 2 : (mardan for 7 days that is 84 hours)

Hours	Colours	Odour	Consistency	Other observations
After 10mins	yellow	Sulphur and	Thick powder due	Shudhha Gandhak and Shudhha
		goghrit	to goghrit in	Parada starts mixing.
			Gandhak	
After 40mins	Light grey	Sulphur and	Thick powder dur	Parada was completely
		goghrit	to goghrit in Gandhak	disintegrated with Gandhak.
After 2hrs	Light grey	Sulphur and	Thick podwer due	Light greyish yellow colour with
Alter 2013	Light givy	goghrit	to goghrit in	shiny particles of Parada.
		202mi	Gandhak	sinity particles of Farada.
After 5hrs	Grey	Sulphur and	Powder	Many Yellow streaks of Gandhak
		goghrit		seen
After 12hrs	Grey	Sulphur and	Powder	Parada was not seen separately in
		goghrit		churna.
After 14hrs	Grey	Sulphur	Powder	Lots of shiny particles of parada as
		- 		it <mark>s got scattere</mark> d in powder.
After 18hrs	Dark grey	Sulphur	Fine Powder	It turned in to fine powder.shiny
<u> </u>				particles were reduced.
After 24hrs	Black	Sulphur	Fine Powder	Attained Varitaratva and
4.6. 201		0.11		Rekhapurnatva
After 30hrs	Black	Sulphur	Fine powder	Fine powder started spilling out of the mortar.
After 36hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny
		1	1	particles present.
After 42hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny
				particles present.
After 48hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny
				particles present.
After 54hrs	Jet black	Sulphur	Fine powder	Jet black, smooth powder, shiny
				particles still present.
After 60hrs	Jet black	Sulphur	Fineness increased	Jet black, smooth powder, few
				shiny particles present.
After 66hrs	Jet black	Sulphur	Fineness increased	Jet black, smooth powder, shiny
A & 701	T , 1 1 1		F ' '	particles reduced
After 72hrs	Jet black	Sulphur	Fineness increased	Jet black, smooth powder, shiny

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				particles reduced.
After 78hrs	Jet black	Sulphur	Fineness increased	Jet black, smooth powder, shiny
				particles absent
After 84hrs	Jet black	Sulphur	Very Fine powder	Jet black,smooth powder,shiny
				particles absent.

Results :

Sample 1 (mardan for 24hrs)

Quantity taken	Obtained	Weight	Weight	No. of	No. of days
	Quantity of Kajjali	loss(gms)	loss(per)	hours	
400gms(batch 1)	397gms	3gms	0.75per	24hrs	8days
400gms(batch2)	390gms	10gms	0.5per	24hrs	8days
800gms(total)	787gms	13gms	1.625per	48hrs	16days

Results of organoleptic characterstics:

Sr. no.	Parameters	Kajjali
1.	Colour	Jet black
2.	Odour	Characterstics
3.	Touch	Smooth and fine
4.	Consistency	Very fine
5.	Taste	Tasteless

L	lts of Class	sical Ayurvedic characterstics(Co	nfirmative test)	JCR
Sr.	no.	Test	Observation	Results
1.		Varitara	Floats on water	+ve
2.	1	Rekhapurnatava	Enters the furrows of the window	+ve
3.	<u> </u>	Slakshnatvam	Smoothness	+ve
4.	1	Nishchandratavam	Lusterless	-ve

Sample 2: (mardan for 84 hrs)

Quantity taken	Obtained	Weight	Weight	No. of	No. of days
	Quantity of Kajjali	loss(gms)	loss(per)	hours	
800gms(total)	790gms	10gms	1.25per	84hrs	60days

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Results of organoleptic characteristics:

Sr. no.	Parameters	Kajjali	
1.	Colour	Jet black	
2.	Odour	Characterstics	
3.	Touch	Smooth and fine	
4.	Consistency	Very fine	
5.	Taste	Tasteless	

Results of Classical Ayurvedic characterstics(Confirmative test):

Sr. no.	Test	Observation	Results
1.	Varitara	Floats on water	+ve
2.	Rekhapurnatava	Enters the furrows of	+ve
		the window	
3.	Slakshnatvam	Smoothness	+ve
4.	nishchandratavam	Lusterless	+ve

2.2 Analytical Study :

Showing different physicochemical and analytical parameters

A) PHYSICO -CHEMICAL EVALUATION : Kajjali

(2 days of Mardan)

Sr. No. Parameters Result Limit as per API

PHYSICO-CHEMICAL ANALYSIS

1	Total Ash	0.01 %	NA
2	Acid Insoluble Ash	0.0 %	NA
3	Water Soluble Extractive	0.49 %	NA

QUALITATIVE ANALYSIS

1	Assay of sulphur	47.83 %	NA
2	Assay of Mercury	46.01 %	NA

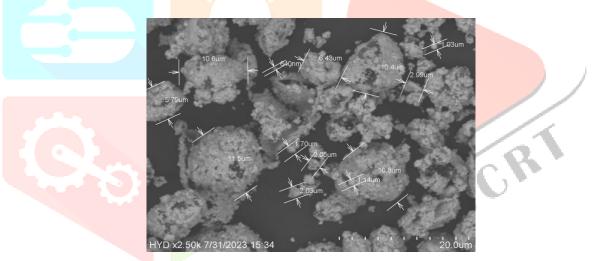
Kajjali

(7 days of Mardan)

Sr. No.	Parameters	Result Limit as per Al	PI				
PHYSIC	PHYSICO-CHEMICAL ANALYSIS						
1	Total Ash	0.35 %	NA				
2	Acid insoluble ash cont	ent 0.0 %	NA				
3	Water soluble extractive	value 0.49 %	NA				
QUALIT	FATIVE ANALYSIS						
1	Assay of sulphur	45.89 %	NA				
2	Assay of Mercury	49.45 %	NA				

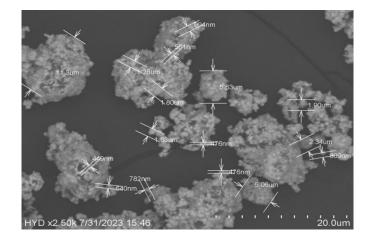
B. Scanning Electron microscopy:

SEM of kajjali(sample 1) prepared with 2 days of trituration



Particle Size Distribution: The SEM images likely reveal a diverse range of particle sizes, spanning from approximately 1 micron to 10 microns. This wide size distribution suggests variations in the preparation process or conditions, which can impact the final particle size and shape.

SEM of Kajjali(prepared with 7 days of trituration).



Particle Size Distribution: The SEM images reveal a wide range of particle sizes, spanning from micron-sized to nano-sized particles. This diverse size distribution suggests variations in the preparation process or conditions over the course of 7 days, leading to a mixture of different-sized particles.

Discussion :

- 1. Varitaratvam, rekhapurnatvam and slakshanatwa test signifies fineness of particles. (more in kajjali prepared in 7 days). REKHAPURNATVAM: rekhapurnatvam shows fineness of particles which increase the free movement of particles which further increase absorption and assiliation to the body. VARITARATVAM: have less specific gravity than water and also shows intermolecular force between particles which shows crystallinity of particles.
- 2. Nishchandra test of classic method is validated complete absence of free mercury in Kajjali prepared in 7 days.
- 3. 1. Interpretation of SEM Data for Kajjali (Prepared with 2 days):

Scanning Electron Microscopy (SEM) analysis offers valuable insights into the morphology and characteristics of materials at the microscale. The SEM data for Kajjali particles, prepared over a span of two days, provides significant information about the structure, size distribution, and surface features of these particles. Based on the description provided, the following interpretation can be made: Particle Size Distribution: The SEM images likely reveal a diverse range of particle sizes, spanning from approximately 1 micron to 10 microns. This wide size distribution suggests variations in the preparation process or conditions, which can impact the final particle size and shape. Particle Morphology: The particles exhibit irregular shapes, which could indicate agglomeration, coalescence, or irregular growth during the preparation process. The presence of irregular shapes suggests potential interactions or transformations occurring during the particle formation. Spherical Structures: The presence of spherical structures is notable within the particle population. These spheres could be individual entities or agglomerates of smaller particles. The OSEM analysis reveals that the surfaces of the spheres are not smooth. The presence of surface irregularities, such as roughness, pits, or uneven textures, might be

attributed to the nature of the materials involved or the processing conditions. Partial Opening of Spheres: The observation that spheres are open at certain areas implies that they are not completely packed or have irregularities on their surfaces. This could be indicative of the dynamic and evolving nature of the preparation process, leading to partially formed or interconnected structures.

4. 2. Interpretation of SEM Data for Kajjali (Prepared with 7 days):

Scanning Electron Microscopy (SEM) analysis provides crucial insights into the morphology and characteristics of materials at the micro- and nanoscale. The SEM data for Kajjali particles, prepared over a period of 7 days, offers significant information about the structure, size distribution, and surface features of these particles. Based on the provided description, the following interpretation can be derived: Particle Size Distribution: The SEM images reveal a wide range of particle sizes, spanning from micron-sized to nano-sized particles. This diverse size distribution suggests variations in the preparation process or conditions over the course of 7 days, leading to a mixture of different-sized particles. Particle Morphology: The particles exhibit a combination of both micron-sized and nano-sized particles. The presence of both size ranges implies complex interactions and transformations occurring during the extended preparation period. Spherical Structures: The observation of spherical structures suggests the presence of well-defined crystalline arrangements or growth patterns. The formation of spheres could be attributed to specific crystallization processes or intermolecular interactions. Surface Texture: The SEM analysis reveals that the surfaces of the spheres are rough. The presence of rough surfaces indicates the possibility of surface irregularities, protrusions, or features that can impact the particle's properties and interactions with the environment. Agglomeration: The presence of agglomerates within the particle population indicates that particles have aggregated or clustered together. Agglomeration could be influenced by factors such as particle-particle interactions, drying, or solvent evaporation.

Conclusion :

SEM study suggests more fine nature of Kajjali prepared in 7 days than prepared in 2 days as size ranges from nano to micron and 1 to 10 micron respectively.

For the effective role in therapeutic bio-availability of metallic and mineral preparation, particle size is one of the important factor as it decides the permeability of drug through cells, tissues and blood capillaries. (Goldberg, M., Langer, R. and Xinqiao, J., Nanostructured materials for applications in drug delivery and tissue engineering. J. Biomater. Sci. Polym. Ed., 2007, 18(3), 241–268).

8) It has been reported that nanoparticles exhibited a size dependent uptake from the intestine and its passage via the mesentery lymph supply and lymph nodes to the liver, with significant absorption of particles <100nm.9) Nishchandra test of classic method is validated complete absence of free mercury in Kajjali prepared in 7 days.

Picture of kajjali formation with two days of mardan



Picture of kajjali formation with seven days of mardan



Acknowledgement:

I acknowledge Deptt. Of Rasa Shastra and Bhaishajya Kalpana, Govt. P.G. Ayurvedic College and Hospital, Varanasi for allowing me to work and perform this study. I would also like to express my sincere thanks to Vasu pharmaceuticals and Nishka lab for carrying out the analytical studies on time.

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