WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 588-597. Review Article ISSN 2277– 7105

A COMPARATIVE PHARMACEUTICAL STUDY OF RASAPUSHPA PREPARED BY TWO DIFFERENT FORMULATIONS.

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Article Received on
29 Jan 2016,
Revised on 22 Feb 2016,
Accepted on 15 Mar 2016
DOI: 10.20959/wjpr20164-5942

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INTRODUCTION

Rasapushpa is a sagni, *nirgandha moorchana* (without sulphur) of Mercury prepared by adopting *bahirdhuma* procedure. For the preparation of *Rasapushpa*, pure mercury along with equal quantities of pure *Kasisa* (ferrous sulphate) & *Saindhava lavana* (Rock Salt) in *kupipakwa* method are used & other formulation which contains same ingredients mentioned above, along with pure *Kankshi* (double potash of allum) by *Damaruyantra* method are mentioned. Based on use of *Gandhaka* (sulphur) in the preparation of Kupipakwa Rasayana, they are called as sagandha & nirgandha kalpanas. The two important nirgandha preparations mentioned in Rasa literature are *Rasapushpa* & *Rasakarpoora*. Not much research work has done in this direction. Hence for present research work, *Rasapushpa* was selected as trial drug on virtue of its importance in therapeutics as well as skills involved in its pharmaceutical preparations.

AIM AND OBJECTIVES

- To compare the physico-chemical properties of two different formulations of *Rasapushpa.*
- Preparation of *Rasapushpa* Formulation 1 & 2 by *Kupipakwa vidhi* according to *Rasatarangini.*
- Study of Physico-chemical properties of both different formulations of *Rasapushpa*.

MATERIALS AND METHODS

All the materials were procured from market & were authenticated from experts.

Materials-

1. *Parad* (Mercury, Hg)
2. *Kasis* (Ferrous sulphate $FeSO_4$)
3. *Kankshi* (Double potash of allum, K_2SO_4, Al_2O_3, 24 H_2O)
4. *Saindhav* (Rock Salt, NaCl)
5. *Bhringaraj swaras* (Expressed juice of eclipta alba)
6. *Rasona* (allium sativum)
7. *Sudha* (calcium oxide; $Ca(OH)_2$)

Method

Study was divided into following parts.

a. Purification of raw material
b. Preparation of kajjali
c. Preparation of *Rasapushpa* by formulation 1 & 2
d. Physico – chemical properties study of both *Rasapushpa* formulation

a. Purification of raw materials:

1. Parad shodhan: Reference : R.T 5/27-30

Total time required : 50 hrs **Total days required :** 18 days

1 Kg of *Parad* & equal quantity of *sudharaja* (lime powder) was taken in *khalwa yantra* & *mardan* (trituration) done for 36 hrs. After 36 hrs the whole mixture filtered through two layered cloths. Then, filtered *Parad* was weighed & again taken in *khalwayantra* with equal quanity of *Rason kalka* & half quantity of *parad, saindhav* was added. It was triturated till the mixture became black paste like consistency. Then the mixture carefully washed with the warm water in plastic tray. Then the *shuddha Parad* collected carefully & kept in glass container.

- Initial weight of mercury : 1000 gms
- Weight of mercury after filtration : 730 gms

2. **Kasis Shodhan:** Referenc : R.T 21/ 230

Total time required : 1 hr 12 mins **Total days required :** 2 days

Impure *kasis* was taken & powdered well. It was then wrapped in a cloth & *Pottali* was prepared in mud pot. *Kasisa Pottali* was hanged in *Dolayantra* containing sufficient amount of *Bhringaraja Swarasa. Swedana* was done for 1 hour & 12 min (1 *Ghatika* = 24 min & 3 ghatika = 72 mins i.e. 1 hour & 12 mins.). After 1 hour & 12 mins. *Pottali* was taken out. When *kasis* was totally dried, it was powdered & weighed. Colour of *kasis* changed from yellowish green to dark green. Impure *kasis* was 500 gms while pure *kasis* was 415 gms after *swedan*.

Ashuddha kasis- 500 gms Shuddha kasis- 415 gms

3. Kankshi Shodhan: Reference: Ayurved Prakash 1/258.

Total time required: 2hrs 20 mins. **Total days required:** 1 day

Kankshi was crushed into small size & was spread over vessel. Heat was given *Tivragni* was given. When water present in *Kankshi* was completely evaporated & *Kankshi* was blown up fully, it was then subjected for self-cooling. After self-cooling *kankshi* was taken out, powdered & weighed. Pure *Kankshi* was white shiny crystalline while pure *kankshi* was white opaque powder.

Ashuddha kankshi- 1000 gms shuddha kankshi- 400 gms

4. Preparation of kajjali of formulation 1: Reference R.T. 6/29

Shuddha Parada, shuddha Kasisa & Saindhava lavana were taken in equal quantity & *mardana* was done in *Khalva yantra* for 22 hours till mixture became lustreless.

5. Preparation of kajjali of formulation 2: Reference R.T. 6/ 33-34

Shuddha Parada, shuddha Kasisa, shuddha kankshi & Saindhava lavana were taken in equal quantity & mardana was done in Khalva yantra for 26 hours to become lustreless mixture.

	Ingredients	Initial wt	Loss	Final wt	Time required
Formulation 1	Shuddha Parada Shuddha Kasis Saindhav	380 gms	25 gms	365 gms	22hrs
Formulation 2	Shuddha Parada Shuddha Kasis Saindhav Shuddha kankshi	400gms	20gms	380gms	26hrs

6. Preparation of Rasapushpa formulation 1: Reference R.T. 6/29-31

120 gms of Kajjali was taken & cautiously filled into kachakupi (7 times clay smeared beer bottle) for each batch. The kupi in valuka yantra was heated for 6 hours in three stages of

graded heating i.e. mrudu agni, madhyamagni & tikshna agni in valuka yantra; The temperature was maintained at 3 stages ranging from mrudu agni 100°C to 150°C for 2hours, madhyamagni 150°C to 250°C for 2 hours, tikshnagni 250°C to 400°C for 2 hours. Corking done after cessation of vapours.

Table no. 1 Temperature pattern of Rasapushpa formulation 1

In Hrs	Batch-1		Batch-2		Batch -3	
	Int.	Out.	Int.	Out.	Int.	Out.
0	32	35	25	27	30	32
1	35	65	38	70	32	75
2	65	140	76	116	69	150
3	74	186	84	180	80	210
4	89	255	90	225	89	160
5	94	325	99	310	95	350
6	94	379	105	400	102	400

Table no. 2- showing observation observed during formation of Rasapushpa formulation 1

In Hrs	Batch - 1	Batch - 2	Batch - 3
0 hr	Kajjali is in powder form	Kajjali is in powder form	Kajjali is in powder form
1 hr	Kajjali was moistened Water vapours present Mild fumes present	Kajjali was moistened. Water vapours present Mild fumes present	Kajjali was moistened Water vapours present Mild fumes present
2 hr	Water vapours and fumes increased	Water vapours and fumes increased	Water vapours and fumes increased
3 hr	Fumes decreased and crystals of RP seen at the neck of kupi	Fumes decreased and crystals of RP seen at the neck of kupi	Fumes decreased and crystals of RP seen at the neck of kupi
4 hr	Water vapours decreased and RP deposition increased	Water vapours decreased and RP deposition increased	Water vapours decreased and RP deposition increased
5 hr	Water vapours disappeared	Water vapours disappeared	Water vapours disappeared
6 hr	Water vapours disappeared completely.	Water vapours disappeared completely.	Water vapours disappeared completely.

7. Preparation of Rasapushpa formulation 2: Reference R.T. 6/33-38

Above same procedure was used for preparation of Rasapushpa formulation 2.

Table no. 3- temperature pattern of Rasapushpa formulation 2

In Hrs	Batch -1		Batch -2		Batch -3	
	Int.	Out.	Int.	Out.	Int.	Out.
0	25	25	20	26	25	30
1	26	62	21	70	40	70
2	80	135	35	160	66	140
3	88	170	62	255	79	190
4	95	220	86	365	95	250
5	96	300	95	400	100	310
6	98	380	100	420	102	360

Table no. 4- showing observation observed during formation of Rasapushpa formulation 1

In Hrs	Batch -1	Batch -2	Batch -3
0 hr	Kajjali is in powder form	Kajjali is in powder form	Kajjali is in powder form
1 hr	Kajjali was moistened Water vapours present, Mild fumes present	Kajjali was moistened Water vapours present, Mild fumes present	Kajjali was moistened Water vapours present, Mild fumes present
2 hr	Water vapours & fumes increased	Water vapours & fumes increased	Water vapours & fumes increased
3 hr	Fumes decreased	Fumes decreased	Fumes decreased & crystals of RP seen at the neck of kupi
4 hr	RP crystals deposition started at the neck of kupi & Water vapours decreased	RP crystals deposition started at the neck of kupi Water vapours decreased	Water vapours decreased & RP deposition increased
5 hr	RP crystals deposition increased, Water vapours decreased & fumes disappeared	Water vapours decreased & fumes disappeared, RP crystals deposition increased.	Water vapours disappeared., RP crystals deposition increased.
6 hr	Water vapours disappeared & Copper coin test positive, corking done	Water vapours disappeared & Copper coin test positive.	Water vapours disappeared completely, Corking done, Coin test positive

Table No 5 Showing yeild of Rasapushpa

Samples	Kajjali	RP	Yield in %	Residue
Rasapushpa formulation 1	120 gms	14 - 36 gms	11%-30%	75gm (average)
Rasapushpa formulation 2	120 gms	10 - 16 gms	8%-13%	78gm (average)

Table No 6 Showing organoleptic characteristics of Rasapushpa

Samples	Color	Touch	Appearance
Rasapushpa formulation 1	Yellowish white	Soft	Parpati (layered)
Rasapushpa formulation 2	Crystal white	Soft	Crystalline

Table No 7 Showing XRPD (X-ray powder diffraction) analysis of Rasapushpa

Sr. No.	Name of drug	Hg_2Cl_2	NaCl	Fe_2O_3
1	Kajjali of formulation 1	√	√	–
2	Kajjali of formulation 2	√	√	–
3	Rasapushpa formulation 2	√	–	–
4	Rasapushpa formulation 1	√	–	–
5	Residue of formulation 2	–	√	–
6	Residue of formulation 1	–	√	√

Table No 8. Showing SEM (Scanning electron microscope) analysis of Rasapushpa

Samples	Magnification	Particle size
Formulation 1	×4000	5.76 µm - 7.29 µm
Formulation 2	×2000	4.41 µm– 21.3 µm

Table No. 9. Showing the % of Mercury & % Chlorine in both formulations of Rasapuhpa by Atomic Absorption Spectrometry (AAS):

Sr. No.	Sample	% Of Mercury	% Of Chlorine
1.	Formulation 1	65.1%	34.9%
2.	Formulation 2	75.7%	24.3%

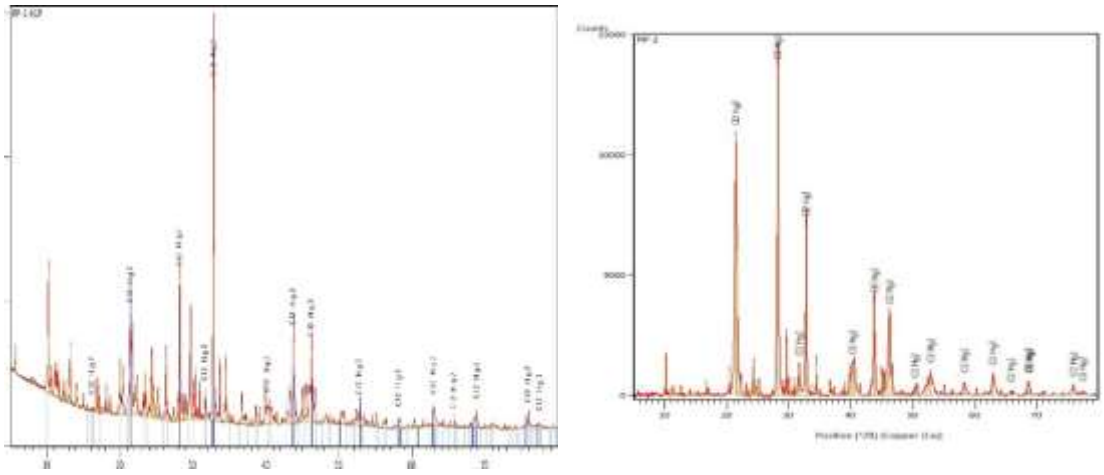
Inductive Coupled Plasma-Atomic Emission Spectroscopy.

0ICP-AES showed following elements present (in ppt level) in the Formulation 1& 2.

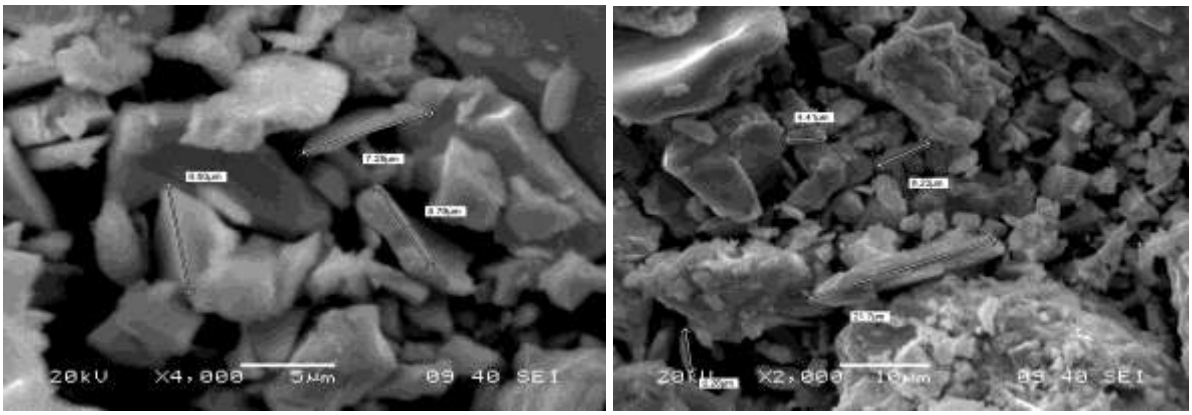
Table no. 10. Showing elements present in ppt level in the samples

Sr. No.	Sample Name	Elements Present
1	Formulation 1	Hg, Ca, Cr, Hg, Sr, Zn
2	Formulation2	Al,Ba,Ca,Co,Cr,Cu,Fe,Hg,K,Mg,Mn,Mo,Ni,Na, P,S,Si,Sr,Ti,Zn,Yb

Table No. 11. XRD Graph of Rasapushpa Formulation 1& 2 respectively



SCANNING ELECTRON MICROSCOPE (SEM)



Formulation 1 **Formulation 2**

DISCUSSION

Rasapushpa Nirman

As per the topic of dissertation two Rasapushpa with two different formulations were compared pharmaceutically as well as analytically.

1. Duration & temperature pattern was same for the both formulations i.e. 6 hrs & observation like melting of kajjali, starting of water vapours, fumes & corking time were observed at relatively same temperature.
2. Mrudu agni was given for 2 hrs from room temperature 22˚C-30 ˚C to 100 ˚C to 150 ˚C
3. After this Madhyamagni 150˚C-250 ˚C for next 2 hrs given for expulsion of watery vapours & crystals of Rasapushpa started to deposit at the neck of the kupi.
4. Tivragni 250˚C-400˚C given. At this stage the watery vapour disappears & copper coin test was positive. Corking done. Then, the valukayantra was left for cooling on its own for 12 hrs.then the kupi broken & Rasapushpa was collected.

5. Kajjali was taken 120 gm as the standard for every batch. Lowest yield was found in formulation 2 i.e. 10 gm & highest was found in formulation 1 i.e. 36 gms.
6. Decreased in the yield of formulation 2 was because the proportion of mercury & saindhav was less as compared to formulation 1.
7. The residue left on the breaking of kupi of all batches of formulation 1 & formulation 2 was ranges from 65 gm to 80 gm
8. Colour of Rasapushpa of formulation 1 is white shiny crystalline whereas Rasapushpa of formulation 2 was dull white & was found in *parpati* (layered) form. Rasapushpa obtained by scrapping the sides of neck of the bottle as it was adherent to the bottle being less in quantity & was in the form of thick layer in formulation 2 & rather than crystalline in formulation 1.
9. The colour of residue was also different in both formulations. It was observed Greyish in residue of formulation 2 & Brick Red in formulation 1. As the Rasapushpa is not water or alcohol soluble, therefore the solubility in the both media & pH can't be determined.

SEM Analysis

it can be concluded that there is variation in the particle size from 4.41µm to 21.3 µm. As our product was not uniform so exact size of particle cannot be determined. The particle size is lesser in formulation 1 than formulation 2.

XRD Analysis

XRD analysis of Rasapushpa and residues of formulation 1 and 2 showed Hg_2Cl_2 as the major compound. Interestingly RPR & RPSR also showed Hg_2Cl_2. Also, NaCl was found in both residues. But the residue of formulation 1 showed Fe_2O_3 as a major compound.

ICP-AES Analysis

The interest was to conduct ICP (Inductive Coupled Plasma) Full scan to know the presence of other elements other than Hg and Cl. In formulation 1 Ca, Cr, Hg, Sr, Zn detected but other than these elements many more elements were found in the formulation 2 like Al, Ba, K, Mg, Mo, Na, Ni, P, Yb, Co, Cu. On the other hand, in residue of formulation 1 , Ba, Ca, Cr, Fe, Hg, Mg, S, Sr, Zn found and in residue of formulation 2 other than this Al, K, Na, Ni, Ti were found. These elements might have come from mines and bhringaraj swaras which was used for purification method and also from the mortar during the preparation of kajjali.

CONCLUSION

Following conclusion drawn are considering the results obtained from above procedures & tests.

1. Kajjali was taken 120 gm as a standard for every batch. Yield found less in formulation 2 than formulation 1. Lowest was 8% in formulation 2 & highest in 30% in formulation 1.
2. The quantity of residue left is not much varied. It was seen the residue in the both formulation was ranges from 65gm-80gm.
3. The formulation 1 was crystalline, white, shiny in nature whereas formulation 2 was dull, white, & lustreless.
4. XRD analysis showed Hg_2Cl_2 in all samples i.e. both of formulation 1 and 2
5. In SEM analysis particle size varied from 4.21µm to 21.3µm.
6. Rasapushpa i.e. Hg_2Cl_2, which was obtained is not only calomel containing Hg & Cl but ICP- AES showed that there are other elements which may help to reduce the toxicity of Rasapushpa.
7. Hence it is concluded that both Rasapushpa i.e. formulation 1 & formulation 2 have same chemical formula but differ in following aspect such as chemical composition as shown in ICP-AES, The % of Mercury & Chlorine seen in AAS & particle size as seen in SEM.

RASAPUSHPA PREPARED BY FORMULATION 1

RASAPUSHPA PREPARED BY FORMULATION 2

Preparation of Rasapushpa

Kajjali (moistened)

kajjali (dry)

filling of kupi

Bhashtri

Rasapushpa crystal deposition At neck

Breaking of kupi