Journal of Ayurvedic and Herbal Medicine 2015; 1(2): 35-39

Research Article

J. Ayu. Herb. Med.
2015; 1(2): 35-39
September- October

www. ayurvedjournal.com

Micromatrix and physico-chemical quality parameters of Shwasakuthara Rasa prepared by two different methods

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ABSTRACT

Background: *Shwasakuthara Rasa* (SKR) is a known herbo-mineral formulation indicated in *Shwasa* (Bronchial Asthma). **Aims and Objectives:** To study the Microscopic and Physicochemical profiles of SKR prepared by two different methods. **Materials and Methods:** SKR was prepared by adding Maricha (*Piper nigrum* L) one by one [SKR(A)] and adding Maricha choorna as a whole [SKR(B)] in the mixture of Kajjali of Parada, Gandaka, Vatsanabha, Manahshila and Tankana. Their comparative Pharmacognostical and Physico-chemical studies were carried out. **Observations and Results:** SKR(A) sample took around 12 hours where as SKR(B) took an average of 5 hours of *Mardana* (Trituration) to become a homogenous mixture with desired smoothness respectively. Microscopic studies on SKR(A) showed that individual ingredients of the formulation could not be identified and most of cellular components were damaged and released into the *Kajjali* where as in SKR(B) cellular components of the individual ingredients could be identified. The bottle neck shaped cells of Maricha, Parenchyma cells of Vatsanabha, and Scalariform vessels of Shunti were damaged. Particle size was also less in SKR(A). An increased quantity of Piperine when compared to SKR(B) was also observed. **Conclusion:** SKR prepared by classical method by adding Maricha one by one [SKR (A)] showed better results in microscopic, quantity of Piperine and particle size distribution parameters.

Keywords: Evaluation, Maricha, *Shwasakuthara rasa*, *Shwasa*, Microscopic, Quality parameters.

INTRODUCTION

S*hwasakuthara Rasa* (SKR) is a well known herbo-mineral formulation indicated in *Shwasa* (Bronchial Asthma). Its ingredients are *Shuddha Parada (*Processed Mercury), *Shuddha Gandhaka* (Processed Sulphur), *Shuddha Vatsanabha (*Processed *Aconitum chasmanthum* Stapf.), *Shuddha Tankana* (Processed Borax), *Shuddha Manahshila* (Processed Realgar), Shunthi (*Zingiber officinale* Roscoe.), Maricha (*Piper nigrum* Linn.) and Pippali (*Piper longum* Linn.) [1]. The proportion of ingredients in *Shwaskuthara Rasa* varies according to different texts. There is significant variation in the quantity of Maricha in different texts, whose proportion changes from one part to ten parts. Pharmaceutical preparation remains constant in all references except in *Yogaratnakara* to mix and triturate Maricha one by one [2]. This exclusive process may help in increasing effectiveness of the drug. Maricha is known for *Pramathi* [3] activity i.e the drug which by its own potency eliminates doshas located in channels of circulation and hence its role is important in treatment of diseases such as Bronchial asthma. Moreover, Piperine an alkaloid in Maricha showed to possess bioavailability-enhancing activity with various structurally and therapeutically diverse drugs [4]. Thus in the present work two methods were followed to prepare SKR to develop comparative microscopic and physicochemical profiles.

MATERIALS AND METHODS

Collection, identification and authentification

The raw materials Hingula(Cinnabar), Gandaka, Vatsanabha, Tankana, Manahshila, Shunti, Maricha, Pippali were collected from Pharmacy, GAU, Jamnagar. Herbal drugs were authenticated in the Pharmacognosy laboratory, IPGT & RA, Jamnagar.

Processing of raw materials

Parada was extracted from Hingula by following classical guidelines [5]. *Shodhana* of Gandhaka was carried out by *Ghalana* method using cow's milk and ghee [6], Vatsanabha was purified by keeping in Gomutra(Cow's urine) for 7 days [7], Tankana was purified by *Bharjana*(frying) method [8] and Manahshila

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by *Bhavana* method using Ardraka swarasa(Ginger juice) for 7 days [9]. Later these two were separately powdered and collected. *Kajjali* was prepared from Hingulottha Parada and Shuddha Gandhaka by constant trituration till the appearance of fine black colour powder [10]. Herbal drugs were powdered and passed through sieve no 85.

Preparation of SKR(A):

Kajjali was taken in iron mortar and it was added with fine powders of processed Vatsanabha, Tankana, Manahshila were mixed. It was triturated till homogenous mixture obtained. Then Maricha was added one by one followed by trituration after each addition till uniform mixing. At last fine powders of Pippali and Shunti were added and triturated properly for homogenous mixture and SKR(A) was stored in airtight container. (Table 1) Three batches of SKR(A) were prepared.

Table 1: Showing ingredients of SKR and their proportion

Sr. No.	Drug Name	Quantity	In gms
1	Kajjali	2P	25
2	Shuddha Vatsanabha	1P	12.5
3	Shuddha Tankana	1P	12.5
4	Shuddha Manashila	1P	12.5
5	Pippali	1P	12.5
6	Shunti	1P	12.5
7	Maricha	9P	112.5

Preparation of SKR(B)

The composition was same in this preparation, but all the components were added into mortar at a time and triturated for 5 hours till homogenous mixture was obtained. Three batches of SKR(B) were prepared. (Table 1)

Organoleptic evaluation :

Evaluation of the SRK(A) and SKR(B) by organoleptic characters like, colour, texture, odour, taste [11].

Microscopic evaluation:

Powders of SKR(A) and SKR(B) were dissolved in water and filtered through filter paper, and the filtrates were studied under the Corl zeiss Trinocular microscope attached with camera, without stain. The microphotographs were taken under the microscope [12].

Physico-chemical evaluation:

The routine parameters mentioned in Ayurvedic pharmacopeia of India were considered [13]. Particle size distribution study was evaluated at SICART, Vallabh Vidhya Nagar Gujarat.

High-Performance Thin Layer Chromatography study:

Methanol extract of two samples of SKR were spotted on precoated silica gel GF 60 aluminium plate as 5mm brands apart and 1 cm from the edge of the plates, by means of a Camang Linomate V sample applicator fitted with a 100µ L Hamilton syringe. The linearity in standard was developed by using Piperine (Mark) 90% purity. Equivalent response was observed in chromatographic development of samples using solvent system Toluene: Ethyl acetate: Acetic acid (7:2:1 v/v) as the mobile phase. After development, Densitometric scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 320 nm under control of win CATS software (v 1.2.1, Camag). The slit dimensions were 6 mm x 0.45mm and the scanning speed was 20mm per second. All HPTLC plates were scanned with filter faction Savitsky-goloy 7, minimum spot -5, minimum height 10AU, minimum area 50AU, and maximum height 990AU with absorption unit. The area under the curve obtained for Piperine was calculated in equivalence to Piperine using linear curve [14].

OBSERVATIONS AND RESULTS

Trituration of SKR(A) took 12 hours out of which addition of Maricha alone took 8 hours. Addition of individual took 25-30sec for mixing. SKR(B) took 5 hours of trituration. Average 97.5% of yield was obtained in SKR(A) (Table 2) whereas 99% of yield was obtained in SKR(B). (Table 3)

Table 2: Preparation of SKR(A)

Batch	Initial wt	Total time taken for Mardhana	Total time taken for mardhana of Maricha	Final weight in gm	% of yield
1	200	11hrs30min	8hr	200	100
2	200	12hrs	8hrs30min	191	95.5
3	200	11hrs	8hrs	195	97.5
Average	200	11hr 30min	8hrs	195	97.5

Table 3: Preparation of SKR(B)

Batch	Initial wt	Total time taken for Mardhana	Final weight in gm	% of yield
1	200	5hr 30min	198	99
2	200	5 hrs	200	100
3	200	5hrs	196	98
Average	200	5hrs	198	99

Organoleptic evaluation:

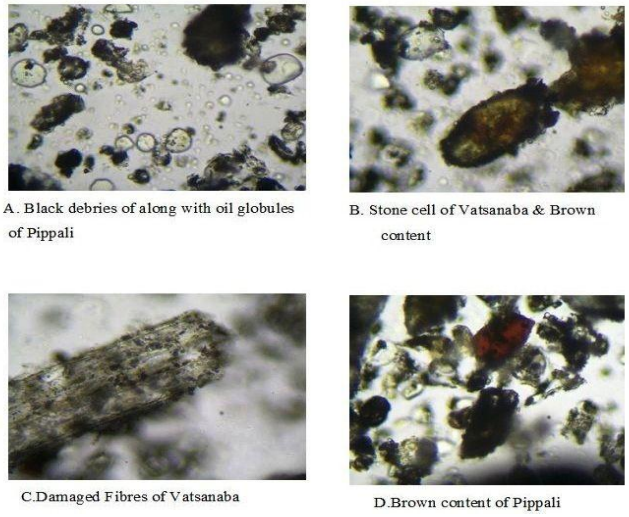
Both samples are having slate black colour, pungent odour and taste, fine in touch having tingling sensation (Table 4).

Table 4: Organoleptic characters of *Shwasakuthara Rasa* powder

Parameters	SKR (A)	SKR(B)
Colour	Black	Black
Odour	Pungent	Pungent
Taste	Pungent	Pungent
Touch	Fine	Fine
Tingling sensation	Present	Present

Microscopic evaluation of SKR(A):

Microscopic study of SKR(A) showed cellular constituents are damaged and completely released into the *Kajjali.* Only black debris along with oil globules and brown content was observed. Stone cells of Vatsanabha damaged and walls are disturbed. Fibers are crashed and most of components are disturbed. (Fig A-D. Plate 1)



A. Black debries of along with oil globules of Pippali

B. Stone cell of Vatsanaba & Brown content

C.Damaged Fibres of Vatsanaba

D.Brown content of Pippali

Plate 1

Microscopic evaluation of SKR(B):

Microscopic study of SKR(B) showed cellular constituents of individual ingredients. Stone cells, black debris of Maricha were identified. The bottle neck shaped cells of Maricha, Parenchyma cells of Vatsanabha, and Scalariform vessels of Shunti were damaged. Cork cells with brown content of Vatsanabha, starch grains of Shunti and stone cells of Pippali were also identified. (Fig A-H Plate 2)

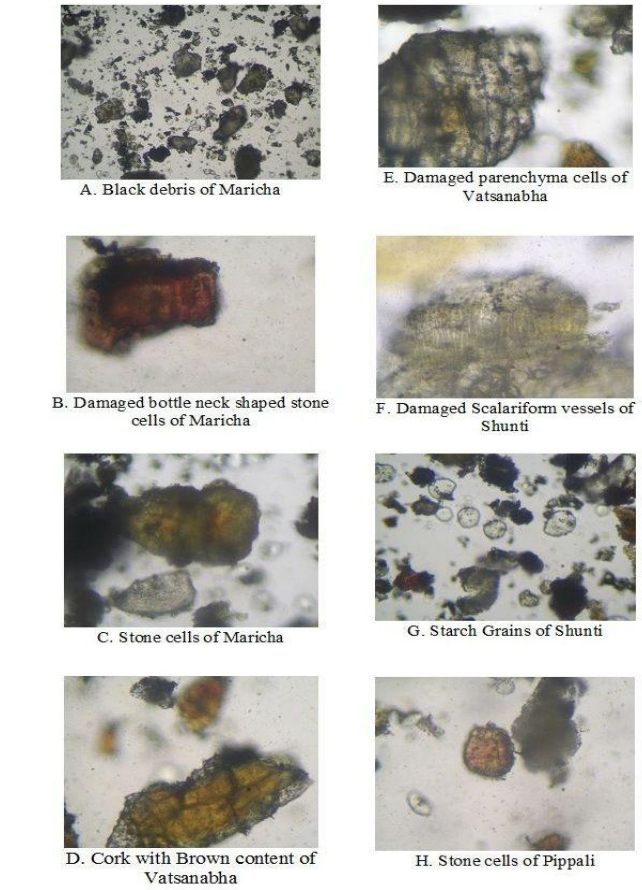


Plate 2

Comparison of microscopic characters of SKR(A) and SKR(B) with normal characters

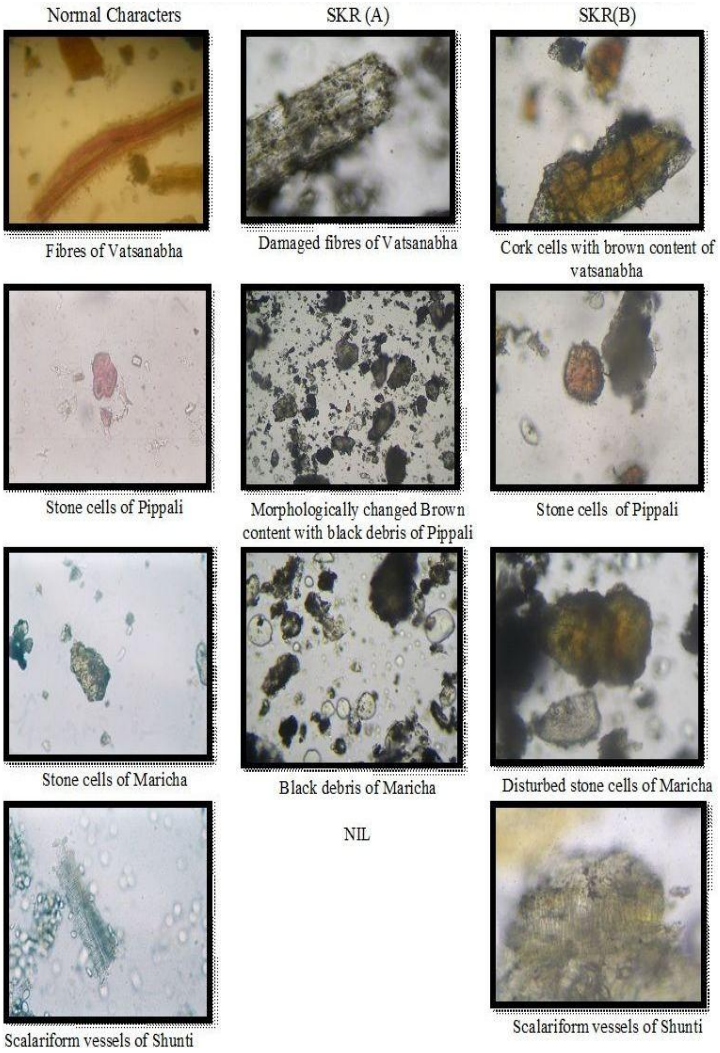


Plate 3

Physicochemical study

Physicochemical parameters are within normal range of API standards depicted in table 5. Both samples showed the presence of alkaloids, tannins, flavanoids, steroids and cardiac glycosoides (Table 6). The particle size distribution of SKR(A) ranges from 4.45 µm to 263.87 µm and SKR(B) ranges from 4.25µm to 329.83µm. (Table 7).

Table 5: Physicochemical parameters of two types of *Shwasakuthara Rasa*

Parameters	SKR(A)	SKR(B)
pH of SKR in 5% water solution	9	9
Loss on drying (%w/w)	8.071	7.502
Ash value (%w/w)	17.32	19.24
Water soluble extract (%w/w)	18.7	18.1
Alcohol soluble extract (%w/w)	16.2	16.5
Presence of free sulphur in %	4.4	5.3

Table 6: Showing the presence of functional groups

	SKR(A)	SKR(B)
Functional groups	Present(+)/Absent (-)	Present(+)/Absent(-)
Proteins	-	-
Alkaloids	+	+
Aminoacids	-	-
Flavanoids	+	+
Tannins and Phenolic compounds	+	+
Steroids	+	+
Cardiac Glycosoides	+	+

Table 7: Particle size distribution of SKR (A) and SKR (B)

Sr. No.		SKR (A)	SKR (B)
1	X10	4.45µm	4.25µm
2	X16	6.97µm	6.67µm
3	X50	28.92µm	32.88µm
4	X84	108.80µm	169.14µm
5	X90	142.66µm	208.32µm
6	X99	263.87µm	329.83µm
7	VMD	54.45µm	74.32µm
8	SMD	12.09µm	12.14µm

X10: 10% of the material is below the mentioned micron value, X16: 16% of the material is below the mentioned micron value, X50: 50 % of the material is below the mentioned micron value, X84: 84% of the material is below the mentioned micron value, X90: 90% of the material is below the mentioned micron value, X99: 99% of the material is below the mentioned micron value, VMD: Volumetric mean diameter, SMD: Standard mean diameter

HPTLC Study

HPTLC study of both the samples showed 5 peaks at 320nm with Rf Value 0.01, 0.57 and 0.64 were present in both samples (Table 8, Plate 4). Quantity of Piperine of SKR(A) was 126.89mg/gm ext and SKR(B) was 114.54mg/gm ext. (Table 9)

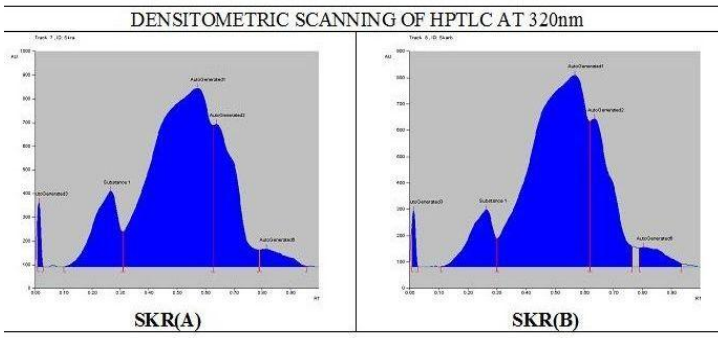


Plate 4

Table 8: Showing the peaks of SKR(A) and SKR(B) at 320 nm when compared with Piperine standard

Drug	No of spots	R_f value
SKR (A)	5	0.01,0.27, 0.57,0.64, 0.81
SKR (B)	5	0.01,0.26,0.57,0.64,0.80

Table 9: Showing quantity of piperine in SKR(A) and SKR(B)

	Quantity of Piperine
SKR (A)	126.89mg/gm ext
SKR (B)	114.54mg/gm ext

DISCUSSION

Khalveeya Rasayanas are most commonly used preparations as they are easily prepared by mixing herbal and mineral drugs in specified proportions and levigating with required liquids. SKR is a commonly prescribed *Khalveeya Rasayana* but there is no any liquid found mentioned in its preparation. Maricha is the chief ingredient in many formulations of SKR that contain Piperine, which acts as an efficient bioavailability enhancer [4]. There is a special method mentioned for the preparation of SKR to add Maricha one by one [2]. This gradual addition of ingredients and trituration may help to release active component directly into the formulation and prevent the loss of volatile components. This even increases the duration of *Mardana* (Trituration), which accentuates the hidden medicinal properties of the drug thus increasing the efficacy of the formulation. Previous reported facts on SKR, maximum Maricha in the formulation showed increase in % of Piperine [15], increasing the bioavailability of other drugs [16] and better bronchodilator activity [17].

In the preparation of SKR(A), the duration of *Mardan*a was increased due to addition of Maricha one by one and there was a need to triturate each and every Maricha individually and continuously to prepare homogenous mixture. But in case of SKR(B), the duration of *Mardana* was less because there was only a mere mixing of powders and no need to go for longer continuous grinding. Yield of SKR(A) is 97.5% where as SKR(B) is 99% because as the duration of *Mardana* increased in SKR(A) there was more loss of product manually.

Microscopic study of SKR(A), very less herbal characters were remained in original state. Most of the cellular components were damaged and released into the *Kajjali* as the duration of *Mardana* was increased. But in SKR(B), most characters retained and individual herbal ingredients could be identified. Only some of the characters were disturbed by *Mardana.* (Plate 3) This result was supported by a study on *Chatushasta prahari Pippali*. After 64 prahara (192 hours) of *Mardana* of Pippali leads a significant changes in epidermal cells, mesocarp cells, fibres and stone cells. These changes suggest that as a result of *Mardana* the intracellular contents are freed that might result in increased bioavailability of the drug [18].

In Physico-chemical study, the pH, LOD, ash value, water soluble extractive, and alcohol soluble extractive did not show significant difference in both samples. Both samples of SKR shows the presence of free sulphur. Previous studies show that some amount of free sulphur is present in Kajjali [19]. Thus free sulphur was present in both samples of SKR. But the presence of percentage of free sulphur was more in SKR(B) when compared to SKR(A). Thus in SKR(A), there was more formation of stable compound due to increased duration of trituration.

Particle size distribution showed that in SKR (A) size of particles range between 4.45µm to 263.87µm and in SKR(B) from 4.25µm to 329.83µm. It also confirms that increase in duration of *Mardana* decreases the particle size. This reduction in particle size helps in increasing the surface of the drug results in quick absorption and assimilation of the formulation.

HPTLC study of two samples of SKR in comparison to Piperine standard showed the quantity of Piperine was more in sample SKR(A) when compared to SKR(B).

Thus SKR prepared by classical method by adding Maricha one by one, followed by triturating after each addition, showed better result in microscopic, quantity of Piperine and particle size distribution parameters. This may help to increase the efficacy of the drug in therapeutics.

CONCLUSION

SKR is a *Khalveeya Rasayana* where duration *Mardana* plays an important role in increasing the efficacy of the drug. SKR(A) took around 12 hours where as SKR(B) took an average of 5 hours of *Mardana* for completion of their preparation respectively. Microscopic studies on SKR(A) showed that individual ingredients of the formulation could not be identified and most of cellular components are damaged and released into the *Kajjali* where as in SKR(B) cellular components of the individual ingredients could be identified. Particle size was also less in SKR(A). An increased quantity of Piperine when compared to SKR(B) was also observed. Thus it could be concluded that SKR prepared by classical method by adding Maricha one by one [SKR (A)] showed better results in studied microscopic and quality control parameters.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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HOW TO CITE THIS ARTICLE

Bhagyalakshmi BR, Galib R, Harisha CR, Shukla VJ, Prajapati PK. Micromatrix and physico-chemical quality parameters of Shwasakuthara Rasa prepared by two different methods. J Ayu Herb Med 2015;1(2): 35-39.