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CHARACTERIZATION OF RASASINDURA: A METALLIC PREPARATION USED IN AYURVEDIC MEDICINE PREPARED BY TWO DIFFERENT METHODS

R. S. Sawant [1]*, S. S. Savrikar [2]

[1] PhD Scholar, Deptartment of Rasashastra & Bhaishajya Kalpana, R. A. Podar Medical (Ayu.) College, Mumbai, Maharashtra India

[2] Ex-Professor, Deptartment of Rasashastra & Bhaishajya Kalpana, R. A. Podar Medical (Ayu.) College, Mumbai, Maharashtra India

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***Corresponding author**
E-mail: drranjeet.sawant@gmail.com

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ABSTRACT

Rasasindura [RS] is a unique mercurial formulation prepared by the Kupi-pakwa Rasayana method, widely used by the physicians of Ayurveda in various diseases. Parada (Mercury) and Gandhaka (Sulphur) are prime ingredients of Rasasindura; however, their proportion varies from formulation to formulation, ranging from 1:1/6 to 1:6. Characterization of the drug helps develop its pharmacopeial standards. An attempt was made to characterize the prepared samples of Samaguna Rasasindura [RSM] & Shadguna Rasasindura [RSD] by X-ray diffraction (XRD) and Field Emission scanning electron microscopy (FESEM), Thermogravimetric Analysis (TGA), Particle size distribution (PSD) with zeta potential & Fourier-transform infrared spectroscopy (FTIR). A significant difference was observed between the pharmacopeial characteristics of Samaguna and Shadaguna balijarita Rasasindura. Variation in the pharmacopeial characteristics is believed to cause the difference in therapeutic actions of two formulations of Rasasindura, which may result from variation in the proportion of ingredients.

Keywords: Ayurvedic Drug, Rasasindura, Kupipakwa Rasayana, Characterization

INTRODUCTION

Rasasindura [RS] is a unique mercurial formulation prepared by the Kupi-pakwa Rasayana method in which the material under process, kept in a narrow-mouthed short-necked glass bottle covered with multiple layers of clay-coated muslin cloth; is digested by subjecting it to gradually increasing intense heat to obtain the final product of expected quality.

Rasasindura [RS] is widely used by the physicians of Ayurveda in various diseases such as Prameha (Polyuria), Shoola (colicky pain), Bhagandara (fistula in ano), Jwara (fever). It is and Rasayana (healthy tissue generator) and Vajeekarana (virility enhancer) in action.[1] Parada (Mercury) and Gandhaka (Sulphur) are prime ingredients of Rasasindura; however, their proportion varies from formulation to formulation, ranging from 1:1/6 to 1:6. [2] Apart from variation in the proportion of mercury and sulfur, some other ingredients are also found added in the elemental composition of Rasasindura. [3] Such addition of ingredients appears to serve the object of value addition, retaining the basic therapeutic efficacy of Rasasindura. Recent advances in the study of herbo-mineral-metallic formulation include characterization studies to analyze the structural changes in raw materials, in-process materials, and end products. [4-7]

Characterization of the drug helps develop its pharmacopeial standards. Singh *et al*., in their studies on the characterization of Rasasindura, have observed the presence of several trace elements along with HgS, which may be interfering or adding value to the therapeutic action of Rasasindura. [8]

In the present study, an attempt was made to characterize prepared samples of Samaguna & Shadguna Rasasindura by X-ray diffraction (XRD) and Field Emission scanning electron microscopy (FESEM), Thermogravimetric Analysis (TGA), Particle size distribution (PSD) with zeta potential & Fourier-transform infrared spectroscopy (FTIR). The morphology, crystallite size, and optical properties of both Rasasindura nanostructures were investigated and compared.

MATERIALS AND METHODS

The essential raw materials of required quality were collected from the local market. They were authenticated and subjected to Shodhana (purification) and Pachana (digestion) by the Kupipakwa method.

Purification of raw mercury

An equal amount of lime clay was mixed with mercury in the mortar, and the mixture was ground for three days [36 hours]. It was then strained through twofold cloth and again taken into the mortar. This time it was mixed with a fine paste of peeled garlic cloves and Saindhava (rock salt). It was then triturated till the mixture turned blackish. The mixture was then washed with hot water to obtain purified mercury. This procedure is performed once only. [1]

Purification of raw sulfur

Raw Sulphur was melted completely in steel wok smeared with cow ghee, which was then poured into a vessel containing lukewarm cow milk through a cotton cloth and was allowed to settle down at the bottom and then collected. After that, it was washed several times with hot water and dried thoroughly in the shade. [9]

Preparation of Sama Guna Kajjali [K1]

Purified Mercury and Purified Sulphur were weighed and taken in a mortar and triturated till the total mass was converted into a fine black, smooth, lusterless powder. This prepared Kajjali was taken into the mortar pestle, and Juice of the aerial roots of the Banyan tree was added to it till the mixture became muddy. Then this mixture was triturated for 18 hours [6 hours daily] until it became dry and fine. [1]

Preparation of Shadguna Kajjali [K2]

Purified Mercury (1part) and Purified Sulphur (6parts) were weighed & taken in the mortar and triturated till the total mass was converted into a fine black, smooth, lusterless powder. Obtained Kajjali was also taken into the mortar pestle, and Juice of the aerial roots of the Banyan tree was added till the mixture became muddy. Then this mixture was triturated for 24 hours [6 hours daily] until it became dry and fine. [1]

Preparation of Samaguna Bali jarit Rasasindur. [RSM] [1]

Kajjali was prepared as described earlier. It was filled in 3 bottles (300 gms each) carefully. The Kajjali containing bottles were placed in a Valuka yantra (sand bath). The assembly, along with bottles, was kept on a wooden furnace. Gradually increasing heat was provided in the furnace by burning the wooden logs. The internal temperature was measured by immersing a galvanometric pyrometer inside the bottle every 30 minutes. External temperature was measured by immersing the pyrometer in the sand at the level of the 2/3rd part of the bottle. White fumes start oozing from the mouth of the bottle, followed by yellow and blue fumes one after another. When the blue flame disappeared, and the bottom of the bottle became red hot, the bottle mouth was sealed using plaster of Paris coated muslin cloth. Before sealing the bottle, a red hot iron stick was introduced through the bottle's mouth to burn the sulphur accumulated at the bottle's neck. After continuous heating for 6 hours, it was left for self-cooling. The bottle was then taken out from the Valuka yantra (sand bath), and the layers of clay-coated cloth were removed using a sharp knife. Then the bottle was broken at midline, and the material deposited at the neck of a bottle was collected as Rasasindura.

Preparation of Shadguna Bali jarit Rasasindur [1] **[RSD]**

Shadguna Bali jarit Rasasindur was prepared using the same method for Samaguna balijarita Rasasindur except for Mercury and Sulphur was 1:6 for Shadguna balijarita Rasasindur.

The samples collected from both methods [RSM1 RSM2, RSM3 & RSD1, RSD2, RSD3] were analyzed using analytical techniques.

Characterization of Rasasindura using Sophisticated Analytical Techniques

Analysis of study samples was performed at the 1. Dept of Chemical Engg; University Institute of Chemical Technology, Mumbai; 2. Diya Lab, Airoli, New Mumbai & 3. Late Prin. B. V. Bhide Foundation, Pune, India.

Kajjali (K1 & K2) and Rasasindura Samples (RSM & RSD) were analysed to obtain physicochemical characteristics. Rasasindura samples were also analyzed for X-ray diffraction (XRD) on D8 Advance, Bruker, Germany. The Pattern was recorded for the angle (2θ) ranging from 10-90o at a scanning rate of 6 degrees/second at 25ºC. For the characterization of the nanostructure and the defined phases in the sample, a Scanning electron microscope (SEM) was used. The 400-4,000 cm^{-1} region spectra were recorded using Cary 630 FTIR Spectrometer. Thermograms TGA was recorded in a Nitrogen atmosphere on a Pyris Diamond thermal analyzer EXSTAR 6000, Perkin Elmer. Particle size Distribution with zeta potential was carried out by Malvern Zetasizer Ultra Serial no. MAL1036126 (Zetasizer Ver. 7.11) FESEM study carried out using Field emission scanning electron microscope/EDS (FE-SEM/EDS).

Table 1: Characteristics of final product of RSM & RSD

Parameter	RSM	RSD
Weight of Kajjali taken in each batch	300 g	400g
Weight Product obtained (average)	162 g	84 g
% Loss of weight	138 g (46%)	316 g (79%)
Total duration required to obtain the final product	26 hours	42 hours

Table 2: Comparison of characteristics of raw (Kajjali) & final product Rasasindura (RSM & RSD)

Parameter	K1	K2	RSM	RSD
Colour	Black	Black	Brown	Brown
Odour	Odourless	Odourless	Odourless	Odourless
Appearance	Fine powder	Fine powder	Fine powder	Fine powder
Taste	Tasteless	Tasteless	Tasteless	Tasteless
Touch	Soft	Soft	Soft	Soft
pH	5.7	4.2	5.4	6.9
Loss on Drying (%)	2.3	6.5	3.6	2.1
Loss on ignition (%)	99	98	99.8	99.8
Total Ash Value (%)	0.9	2.3	0.2	0.3
Acid insoluble Ash (%)	0.4	0.9	0.8	0.5
Water Soluble Ash (%)	0.6	0.3	0.8	0.4
Total Mercury (%)	45.5	39.3	83.4	81.1
Total Sulphur (%)	7.3	1.5	8.5	9.8
Free Sulphur (%)	45.5	91.6	2.4	3.6

*K1- Kajjali (1:1), K2 – Kajjali (1:6), RSM – Samaguna Rasasindura prepared using K1, RSD - Shadguna Rasasindura prepared using K2,

Table 3: Comparision of XRD findings in RSM & RSD Samples

RSM			RSD		
Angle 2θ	Intensity count / sec	Intensity	Angle 2θ	Intensity count / sec	Intensity
24.7818	155.51	8.76	24.8233	115.84	7.42
26.5333	8500.06	100	26.5745	4250.12	100
28.2052	362.63	20.43	28.2383	636.49	40.77
31.2248	1518.09	85.53	31.254	1520.08	97.37
31.313	1005.76	56.66	31.3418	1058.18	67.78
37.8896	85.12	4.8	37.9232	71.33	4.57

Table 4: Comparision of PSD findings in RSM & RSD Samples

Sample	Z-Average (d.nm)	Zeta Potential (mV)	Stability behaviour of sample
RSM	428	-21.13mV ± 6.79	Moderate
RSD	332	-25.93mV ± 11.26	emergent

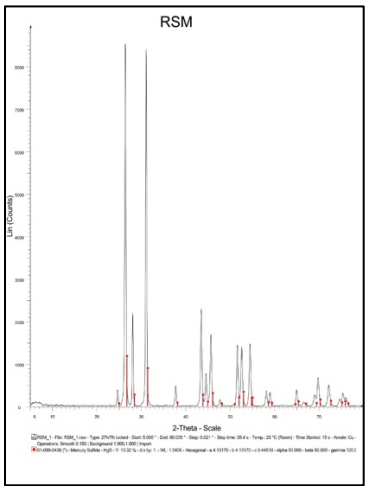

Figure 1: XRD Graph of Samaguna balijarita Rasasindura [RSM]

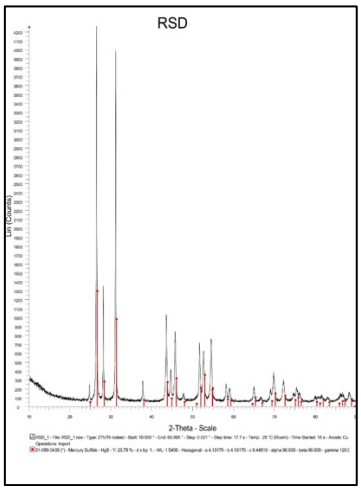

Figure 2: XRD Graph of Shadguna balijarita Rasasindura [RSD]

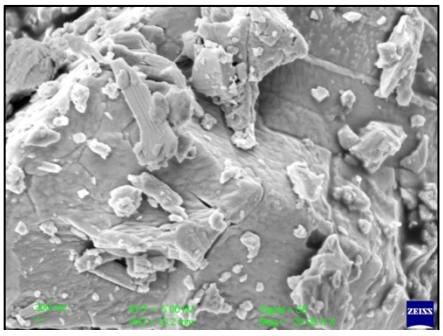

Figure 3: SEM Image of Samaguna balijarita Rasasindura [RSM] [200nm]

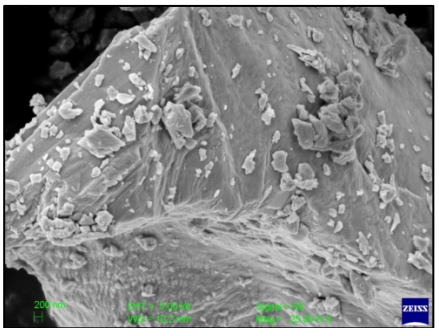

Figure 4: SEM Image of Shadguna balijarita Rasasindura [RSD] [200nm]

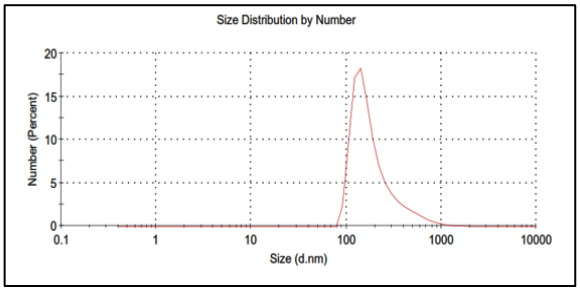

Figure 5: PSD Image of Samaguna balijarita Rasasindura [RSM]

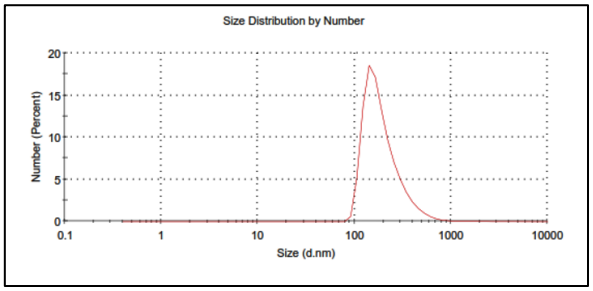

Figure 6: PSD Image of Shadguna balijarita Rasasindura [RSD]

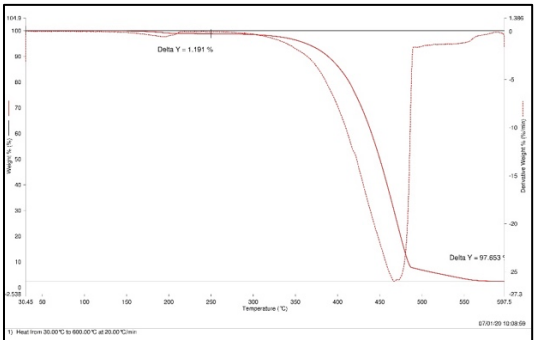

Figure 7: TGA Graph of Samaguna balijarita Rasasindura [RSM]

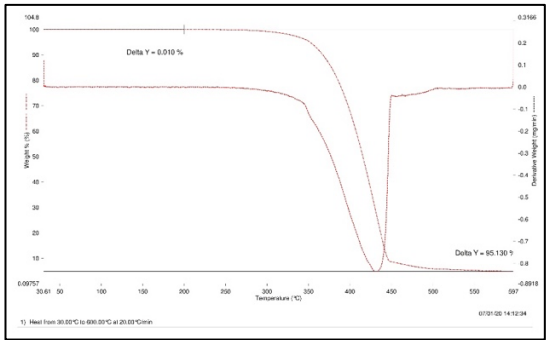

Figure 8: TGA Graph of Shadguna balijarita Rasasindura [RSD]

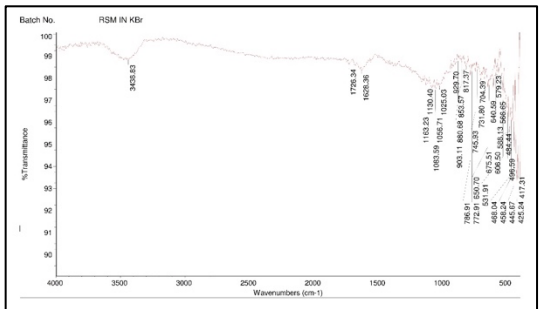

Figure 9: FTIR Graph of Samaguna balijarita Rasasindura [RSM]

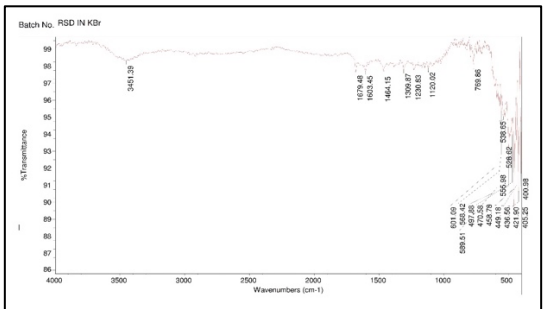

Figure 10: FTIR Graph of Shadguna balijarita Rasasindura [RSD]

RESULT AND DISCUSSION

Developing a bond between mercury and sulfur is a vital stage in preparing metallic dosage forms in Rasashastra. During the preparation of Kajjali, it was observed that after 30 minutes color of mass changed to blackish yellow, which was converted to yellowish black. Mercury particles were visible in the mass after 60 minutes. After six hours, the whole mass converts into a black mixture. After seven hours, almost all Sulphur gets mixed with mercury. The compound turns into fine, black, smooth, lusterless material named Kajjali. It appears like a collyrium by the end of 21 hours.

In the present study, the time required for RSM & RSD was 26 hours & 42 hours respectively. Khedekar S *et al*., in their research, stated that in the Jarana process, the duration of heat plays an important role. As the duration of heat increases, the quality of the product also intensifies. If the Rasasindura is prepared in a short duration (~7 hours), the final product resembles synthetic Cinnabar, and when it is prepared with maximum time (~7days), it resembles natural Cinnabar. [10, 11] The physical characters of both RSM & RSD are compiled in Table 1. The final products were obtained to comply with the Ayurvedic criteria mentioned in the pharmacopeial standard of Indian medicine. Other physicochemical values observed in both drugs are summarized in Table 2.

Free Sulphur present in all samples may be responsible for deciding the drug's pH. The pH of both drugs was investigated. There was a difference observed in the pH of RSM and RSD, which may be due to the availability of more free sulfur in RSD. [12] Ash values of K1 and K2 were found to be 0.9% and 2.3%, respectively, while ash values of RSM and RSD were observed as 0.2% and 0.3%. The Ash values of Rasasindura samples were slightly higher due to silica particles and repetitive ash insertion of a hot and cold iron rod. The acid-insoluble ash value of both K1 and K2 was 0.4% and 0.9%, respectively. Acid insoluble ash value of RSM & RSD was 0.8% and 0.5%. There was a proportionately decrease in the acid-insoluble ash value of RSD, but the Ash value of the RSM sample increased. It might be due to the impurity of silica particles during the process. Loss on drying value of K1, K2, RSM and RSD were observed as 2.3%, 6.5%, 3.6%, and 2.1%, respectively. This test was performed to detect the moisture content in the sample. This value was comparatively higher in all samples; it might be due to the storage of powdered samples for a long time & humid conditions in the Mumbai region.

Mercury is present in various compounds as either mercurous (Hg+) or mercuric (Hg++). However, mercuric salts are more stable than mercuric salts. Mercurous salts are capable of transforming into mercuric form. Sulfides can be reduced to smaller operational groups. They possess ionic bonding. Some of them may have metallic bonding. Sulfates are firmly bound groups and do not share oxygen. These contain covalent bonds. The four possibilities of forming mercurial compounds in the final product are mercuric sulfate, mercuric sulfide, mercurous sulfate, and mercurous sulfide. Sulfides of mercury are easily formed, whereas procedures like mere trituration do not quickly form mercury sulfates. No free mercury was present in all samples, which verifies the Nischandratva test [Absence of lustre] of Kajjali. It also shows that all procedures were properly carried out. Total mercury percentage in K1 and K2, RSM, and RSD was found to be 45.5%, 39.3%, 83.3%, and 81.1%, respectively. During the procedure, excessive sulfur was burned; hence Hg% is more in both the Rasasindura; more mercury concentration in Rasasindura indicates that corking was done at the proper time. Free sulfur content in K1 and K2 was observed as 39.76% and 41.07%, respectively. As mercury forms a stoichiometric compound with sulfur, it was evident that free sulfur will be more in K2. Free sulfur was present in RSM and RSD as 2.4% & 3.6%, which indicates that the corking was done a little earlier before the complete jarana of the sulfur.

On Analysis by XRD, all Rasasindura samples contained HgS with a Hexagonal crystal system. [13] They all were identified chemically as Mercury sulfide (red). [14] The diffraction peaks of mercury sulfide are sharper and more intense. This finding

supports the hypothesis that heat treatment of Kajjali aids in the production of mercury sulfide and boosts the sample's crystallinity. The highest mean peak count in RSM was 8500, whereas it was 4250 in RSD, clearly showing that RSM is more crystalline than RSD. Although both Rasasindura [RSM & RSD] were identified as HgS, a significant difference in intensity indicates substantial variation in the degree of structural order in both crystals. The material crystallinity is bound to rise when the more considerable particle size and the high molecular aggression. RSM has higher crystallinity than RSD, implying that the crystal size of RSD is significantly finer than RSM, and hence RSD is more potent therapeutically. (Table 3; Figure 1, 2)

FESEM study of both samples showed the surface of RSD is exceptionally smooth and porous. On the surface, several nanometer-sized particles were observed. The morphology of RSM was consistent. The particles ranged in size from 180 nanometers to several micrometres. [15] Because the particle size was in the nanometres range, and the medication would absorb more quickly. (Figure 3, 4).

In the present study, the average particle size recorded of RSM and RSD was 428d.nm & 332d.nm respectively. Also, zeta potential noted were -21.13mV $\pm$ 6.79 and -25.93mV $\pm$ 11.26 respectively. (Table 4; Figure 5, 6). RSM appears to stay in a colloidal condition for longer than RSD. At first glance, it appears that RSM is more easily and extensively reaching all human tissues than RSD since it is less likely to be disseminated in colloidal form than previously. According to traditional resources, RSD is more potent than RSM. Several shreds of evidence suggest that RSD absorbs considerably better than RSM. The intestinal mucosa is negatively charged due to the presence of glycocalyx. Zeta potential analysis predicts medication absorption from the mucosa because it influences particle uptake.

The intestinal mucosa is generally attracted to particles having a high positive surface charge, such as chitosan, which aids in the intestinal absorption of the encapsulated medication. There is solid electrostatic contact between the positively charged particles and the negatively charged glycocalyx. It may impede the advancement and penetration of these particles towards the epithelial cell surface, limiting their absorption. Non-ionized particles also have a higher affinity for M cells than ionized and positively charged particles. RSM and RSD particles with low negative zeta potential and nano-size have been suggested to be uptaken similarly. [16]

The TGA analysis (Figure 7, 8) showed that for sample RSM and RSD, loss in mass was negligible up to 600oC (<0.5%), whereas, for sample RSM and RSD, the weight loss was 1%

and 1.5% respectively. The mass loss was highest for samplers, which lost approximately 8% mass up to 250^{o}C and 11% up to 600^{o}C. The mass loss of SB-A indicated the presence of other composite materials, having low degradation temperature, and mercury.

FTIR spectrum of Rasasindura was studied in the region (50-400 cm^{-1}). The absorption peaks of crystalline mercury sulfide (HgS) were at 83, 92, and 100 $cm^{-1,}$ respectively and their existence in the FTIR spectra indicates that Rasasindura is Mercury sulfide. [17] FTIR spectrum of sample RSM and RSD has many peaks denoting different functional groups, respectively. These are most likely derived from the aerial root of *Ficus benghalensis* Linn. [8] The existence of considerable concentrations of functional groups of C, N, and O also indicates the drug's organic nature. (Figure 9, 10) FTIR analysis revealed that when the percentage of sulfur to mercury in Rasasindura formulation grew, the number of functional groups reduced. The presence of an active group is critical for medication absorption since it speeds up the process. On the other side, multiple functional groups may obstruct dosage form absorption by interfering with the actions of different functional groups. As a result, RSM may have superior absorption than RSD. In RSM, FTIR confirmed the existence of more unsaturated carbon and exchangeable proton than RSD. RSD may be more stable as a result of this than RSM. Although FTIR detected the functional group, the quantity and duration of heat used during preparation caused it to dissolve; thus, their relevance is uncertain.

CONCLUSION

A significant difference in pharmacopeial characteristics was found between Samaguna and Shadaguna balijarita Rasasindura. The difference in the proportion of ingredients leads to variation in pharmacopeial characterization, which might explain the difference between the therapeutic action of these two Rasasindura [RSM & RSD].

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