



Research Article

PHARMACEUTICAL ANALYTICAL STUDY OF *PARIBHDRA RASA* AND ITS ANTIFUNGAL STUDY ON *ASPERGILLUS FUMIGATUS* AND *CANDIDA ALBICANS*

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ABSTRACT

Rasashastra deals with the use of metals, minerals, gemstones, certain poisonous herbs and their processing. In *Rasashastra*, mercury is used as chief material and *Rasaushadhis* considered as more effective and beneficial due to lesser therapeutic doses, enhancement of action of other ingredients of formulation, more shelf life, quicker action and palatability as compared to herbal preparation. *Rasaushadhis* are being the backbone of Ayurveda due to its *Rasayana* and therapeutic properties. As the *Paribhdra Rasa* is mentioned in the *Dadru Kushtha*. In Ayurveda, all skin diseases are categorized under the broad term *Kushtha*. Skin diseases in Ayurveda are further classified into *Maha Kushtha* and *Kshudra Kushtha*. *Dadru* is one type of *Kushtha* and known for its fast-spreading and invasiveness in nature. *Dadru* can be associated with fungal infections in modern science. Therefore "Pharmaceutical Analytical Study of *Paribhdra Rasa* and its Antifungal Study on *Aspergillus Fumigatus* and *Candida Albicans*" is chosen for present study.

INTRODUCTION

Rasa Shastra, meaning "Science of Mercury," is a specialized branch of Ayurveda focused on *Rasa dravyaas* (substances with mercury). These substances (*Rasa Drayas*) have three key attributes: instant effectiveness, require very small doses, and have broad therapeutic uses, regardless of individual constitution.

अल्पमात्रोपयोगित्वादरूवेरप्रसङ्गतः ।

क्षिप्रमारोग्यदायित्वात्तौषधे भ्योऽधिको रसः ।

साध्येषु भेषजं सर्वमीरितं तत्त्ववेदिना ।

असाध्यैष्वपि दातव्यो स्सोऽतः श्रेष्ठ उच्यते । (R.Sa.San. 1/4-5)

Ayurveda's history is divided into three periods: the *Vaidika* period, the *Samhita* period, and the Post-samhita period, based on the evolving approaches to health and disease. The post-*Samhita* period is marked by the influence of the renowned alchemist Siddha Naagaarjuna, considered the "Father of Rasa Shastra."

Rasa Shastra, a specialized branch of Ayurveda, is believed to have been formally established around the 8th century. Naagaarjuna famously proclaimed, "सिद्धे रसे करिष्यामि निर्दारिद्र्यमयं जगत्" meaning "I am experimenting with mercury to eliminate poverty from this world." His work emphasized that the goal of mercury science was not only alchemy (*Dhaatuvaada*) but also to maintain health, strengthen the body, and ultimately achieve *Mukti* (salvation).

न च रसशास्त्रं धातुवादार्थं इति मन्तव्यम्वेदवेधाद्वारा मुक्तिरेव परमप्रयोजनात्: ।।

Paribhdra Rasa is mentioned in the *Rasender Sara Sanghrah* for treating *Dadru Kushtha*, a type of skin disease classified under the broad category of *Kushtha* in Ayurveda. *Kushtha* is divided into two main categories: *Maha Kushtha* (severe) and *Kshudra Kushtha* (less severe). *Dadru Kushtha* is known for its rapid spread and invasive nature.^[1] *Kushtha* refers to skin conditions that cause both skin damage and discoloration. *Dadru* is categorized differently by various *Acharyas*: *Acharya Charaka* in *Kshudra Kushtha*^[3], while *Acharya Vagbhatta* and *Acharya Sushruta* place it in *Maha Kushtha*.^[4,5] It involves the clinical features like *Kandu* (itching), *Utsanna* (elevated circular lesions), *Mandala* (circular patches), *Raaga* (erythema), and *Pidikas* (papule).^[6] *Vagbhatta*

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specifically labels *Dadru* as *Anushangika*.^[7,8] *Kushtha* types are generally considered *Tridoshaja*, but their *Dosha* identity depends on the dominant *Dosha* in the disease progression. *Dadru* is primarily associated with *Kapha*. Its *Samprapti* involves the vitiation of *Pitta* and *Kapha Doshas*, along with the disturbance of *Rasa* and *Raktavaha Strotas*. Due to its similar symptoms, *Dadru* is often associated with fungal infections in modern science. The fungi (*L. fungus mushroom*) have traditionally been regarded as "plant-like". Most species grow by continuous extension and branching of twig-like structures. Fungi grow either as single cells, the yeasts, or as multicellular filamentous colonies, the molds and mushrooms. *Candida albicans* is dimorphic and at the surface of rich agar medium it grows as oval budding yeast cell but deeper in the medium hyphae are found and both forms are characteristically seen in infected tissue and in most cultures. *Candida albicans* and other species of *Candida* are frequently present on the normal mucous membranes of mouth, vagina, and intestinal tract and causes mucocutaneous candidiasis, cutaneous candidiasis, esophageal candidiasis, urinary tract candidiasis. Many species of *Aspergillus* have been recognized in nature, but only seven have so far been associated with human disease (aspergillosis). Of these, *Aspergillus fumigatus* accounts for over 90% of all infections. Farmers and individuals working with decaying vegetation are frequently exposed to spores of *Aspergillus*, a type of mold. Exposure to these spores can lead to allergic reactions, such as asthma and rhinitis, due to sensitivity to the antigens in the spores. Additionally, under certain predisposing conditions, *Aspergillus* can cause progressive infections, especially in individuals with weakened immune systems or pre-existing respiratory issues.

AIMS AND OBJECTIVES

- To prepare *Paribhdra Rasa* as per classical text *Rasender Sar Sangrah*.
- To evaluate analytical study and antifungal study of *Paribhdra Rasa*.

MATERIALS AND METHODS

Preparation of *Paribhdra Rasa*

Firstly, all raw drugs were identified and authenticated by PG Department of Dravya Guna of Institute for Ayurved Studies and Research, Shri Krishna AYUSH University, Kurukshetra, according to macroscopic characteristics given in API.

The physicochemical analysis done for raw drugs from Govt. Ayurvedic State Drug Testing Laboratory, Department of AYUSH, Kurukshetra and

Satiare Research & Anatech Pvt. Ltd. Panchkula (Haryana), S.R LABS AND Research Centre, Jaipur.

Five steps are involved in the preparation of *Paribhdra Rasa*

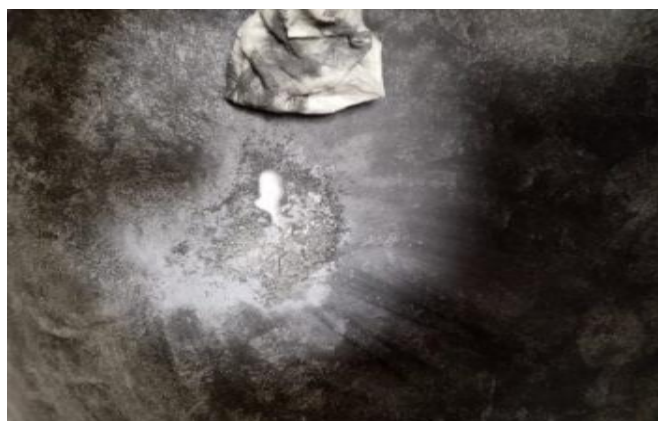
1. *Parad* extraction from *Hingul* in two batches^[9]
2. *Gandhaka Shodhana*^[10]
3. *Samaguna Kajjali* preparation^[11]
4. *Samaguna Rasa Sindura* preparation through traditional method.^[12]
5. Powder of all ingredients then followed by *Bhawana* of *Khadir Kwath*^[13].

Parad extraction from *Hingul*

The extraction of *Parad* from *Hingul* was carried out by three methods i.e., *Adhah Patana*, *Urdhva Patana*, *Tiryanka Patana*. The different *Yantra* were used for extraction of *Parad* like *Damaruyantra*, *Vidhayadharayantra* and *Patana yantra* etc. In present study *Nadh yantra* is used because it is assumed that *Parad* extraction from *Hingul* by using *Nadh yantra* was more convenient as compared to others *Yantra* and also average yield of *Parad* is also more in *Nadhyantra* as compared to others. *Parad* extraction from *Hingul* by using *Nadh yantra* is carried out in two batches.



Nadh Yantra for *Parad* extraction



Parad extracted from *Hingul*

Table 1: Amount of Parad extracted from Hingul

S ₂ No	Weight of Sudh Hingul	Weight of Parad obtained from Hingul	Loss of Parad
1	150 gm	80 gm	70 gm
2	150 gm	90 gm	60 gm

Gandhaka Shodhana

For purification of the *Gandhak*, the traditional method using cow's milk and cow's ghee was used. In this method, sulfur in powder form mixed with ghee was heated up to its melting temperature and the resulting liquid is poured through a filter into a vessel containing cow's milk. After pouring into cow's milk vessels *Gandhak* was settled on the bottom of vessel in granules form. This process was repeated three times and the final deposited product was taken out, washed with hot water and dried.

Table 2: Observations during Gandhaka Shodhana

S ₂ No	Weight of initial Gandhak	Shodhan Type	Wt. after Shodana of Gandhak	Weight after Dry
1.	150 gm	1 st	146gm	146 gm
2.	146 gm	2 nd	144 gm	144 gm
3.	144 gm	3 rd	141 gm	140 gm

Samaguna Kajjali Preparation

In the process, *Shodita Parad* (purified mercury) and *Shodita Gandhaka* (purified sulfur) were taken in equal quantities (100 grams each) and triturated together in a *Khalva Yantra* (a traditional stone mortar). During the trituration process, the white color of mercury and the yellow color of sulfur gradually disappear, and a black fine powder forms. The trituration continued until the powder became very fine, dark black, and similar in texture to *Kajala*. The powder was then confirmed to meet the criteria of *Kajjali* (a finely processed, black-colored compound) and was characterized as *Nishchandrika*. Afterward, *Kajjali* was treated with *Vatankur Swarasa* (the juice of the *Vatankur* plant) for three cycles of *Bhavana* (a process of moistening and grinding). Once the *Bhavana* was complete, the final product was stored in a 7 layered *Kachkupi*.

**Sudha Gandhak****Sudha Parad****Kajjali Nirman (Sudh Parad, Sudha Gandhak)****Kajjali**

Samaguna Rasa Sindhur preparation through traditional method: *Rasa Sindhur* preparation involves following steps

- *Purva Karma*
- *Pradhana Karma*
- *Paschata Karma*

Purva Karma: *Purna Karma* includes preparing of *Samguna Kajjali*, seven layered *Kachkupi*.

Pradhana Karma: *Pradhana Karma* seven layered *Kajjali* filled *Kachkupi* was placed in *Valukayantra* and *Valukayantra* was placed in the centre of traditional *Bhatii* and then followed by the ignition of *Agni in Mridu, Madhyam* and *Triva Agni* pattern as mentioned in *Rasa* classical texts.

Paschata Karma: *Paschata Karma* involved removal of *Kachkupi* from *Valukayantra* after complete self cooling of Traditional *Bhati* and breaking of it (*Kachkupi*) and after braking collect *Kanthsth Rasa Sindhur*.

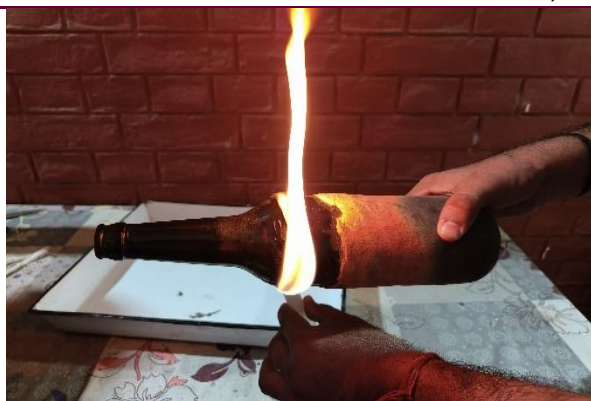
Table 3: Observation with Recorded Temperature

Time in hours	Temperature Reading	Specific Observation
11.30 am	30°C	<i>Kupi-sthapana</i>
12.05 pm	100°C	<i>Kajjali</i> was dried
12.25 pm	150°C	Sand start heating
12.40 pm	200°C	<i>Kajjali</i> was dried
12.47 pm	220°C	<i>Kajjali</i> start melting
12.55 pm	240°C	White fumes appear
01.10 pm	240°C	Dense white fumes start
01.30 pm	280°C	Dark yellow color fumes
04.25 pm	360°C	Yellow fumes disappeared blue flame start appear
04.40 pm	420°C	Flame height increased about 2-3inches
05.54 pm	440°C	Flame disappeared
05.56 pm	440°C	Bottom of <i>Kupi</i> become red hot <i>Shita shalaka</i> test and copper coin test indicates presence of <i>Parad</i> particles
05.58 pm	420°C	Immediately cork was applied on <i>Kupi</i> .
6.10 -6.45 pm	420-550°C	Temperature was increased from 440-550°C.
06.50pm-8.45pm	550°C	Heat was given for 2 hours.

Rasa Sindhur Observations



Blue flames apperas during *Rasa Sindhur Nirman* Copper coin Test- Shows *Parad* Particle

Broking of the *Kachpuki**Kanthstha Rasa Sindhur**Rasa Sindhur Powder*

Results: Total time taken for *Rasa Sindhur* preparation: 9 hours 30 minutes.

Weight of *Kajjali* was taken: 110 gm

Weight of *Samaguna Rasa Sindhur* obtained: 48 gm

5. Preparation of *Paribhdra Rasa*

Fine powder of *Amla*, *Nimb* and *Rasa Sindhur* was kept in *Kharal* and *Khadir kwath* was prepared for *Bhawana*. After mixing of all fine powder, *Khadir kwath* (10-15 ml) was used for trituration and after completing trituration. The final powder (*Paribhdra Rasa*) was put in air tight container and sent to S.R Labs and Research Centre, Jaipur for its Physio-chemical Analysis as well as its Antifungal Study on *Candida Albicans* and *Aspergillus Fumigatus*.

Physio-Chemical Analysis of *Paribhdra Rasa*

Table 4: Analytical Analysis of *Paribhdra Rasa*

S.No.	Parameters	Test method	Unit	Result
1	pH	API Part 1, Vol.-VI, 2009	-	3.28
2	Loss on drying	API Part 1, Vol.-VI, 2009	% w/w	6.43
3	Total ash	API Part 1, Vol.-VI, 2009	% w/w	4.23
4	Acid insoluble ash	API Part 1, Vol.-VI, 2009	% w/w	1.15
5	Water soluble extractive	API Part 1, Vol.-VI, 2009	% w/w	29.53
6	Alcohol soluble extractive	API Part 1, Vol.-VI, 2009	% w/w	26.83
7	Particle size -Zeta potential	By particle size Analyzer	Nm	930.8
8	XRD	By XRD		Data Attached
9	Touch	Qualitative analysis		Smooth
10	Lusterless	Qualitative analysis		Positive
11	<i>Rekhapurnatav</i>	Qualitative analysis		Positive
12	<i>Varitaratav</i>	Qualitative analysis		Positive
13	Tasteless	Qualitative analysis		Positive

14.	<i>Apurnabhava</i>	Qualitative analysis		Positive
15.	Colour	Sense organ		Brick red
16.	Odour	Sense organ		Characteristics
17.	Appearance	Sense organ		Fine powder
Heavy Metal Analysis				
18.	Lead	SRL/CHEM/SOP-ICP-MS/13	Mg/kg	9.01
19.	Cadmium	SRL/CHEM/SOP-ICP-MS/13	Mg/kg	BLQ (LOQ 0.1)
20.	Mercury	SRL/CHEM/SOP-ICP-MS/13	Mg/kg	41962.39
21.	Arsenic	SRL/CHEM/SOP-ICP-MS/13	Mg/kg	BLQ (LOQ 0.1)
Microbiological Analysis				
22.	Total bacterial count	API Part 1, Vol.-VI, 2009	Cfu/g	20
23.	Total fungal count	API Part 1, Vol.-VI, 2009	Cfu/g	Less than 10

Ayurvedic Classical Parameters



Varitar Pariksha of Paribhdra Rasa



Rekhapurnatav Pariksha of Paribhdra Rasa



Apurnabhava Pariksha of Paribhdra Rasa

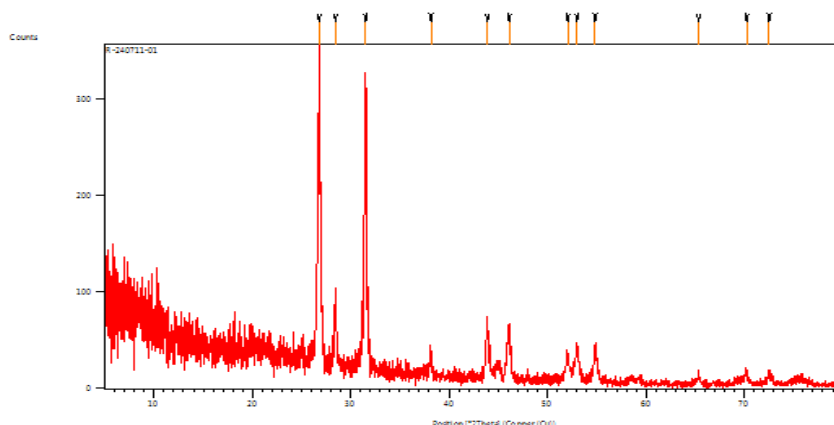
X-Ray Diffraction of *Paribhdra Rasa*

Powder XRD analysis of *Paribhdra Rasa* was carried out using Philips 1710 X-ray diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$) operating at 30 kV and 30 mA. Pattern was recorded for angle (2θ) ranging from 10 to 100° at a scanning rate of 0.03 degree and a dwell time of 0.5 second. Higher the value of counts represents higher the crystallinity of the phase. For identification of each phase, minimum 3 strong peaks were chosen and compared with standard X ray Powder Diffraction file i.e. JCPDS File number: ICDD- 04-004. Sharp peaks observed that major compounds as Mercuric sulphate of majorly at 100% intensity on 26.8082, 2 theta value with crystalline shape and structure.

Table 5: X-Ray Diffraction of Paribhdra Rasa

Sample Name	Obs. Max	d (Obs. Max)	Net Height	Net Area	Intensity
	2-Theta °	Angstrom	Cps	Cps x 2-Theta °	%
HgS	26.8082	3.32561	307.05	0.2362	100.00
HgS	28.4526	3.13705	71.89	0.2362	23.41
HgS	31.5454	2.83619	256.97	0.1968	83.69

Graphic



Particle size by Laser Diffraction: Laser diffraction has become one of the most used particle size techniques, particularly for particles in the 0.5 to 1000 μ range. It is based on the idea that when a laser beam is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size (i.e., the smaller the particle size, the larger the angle of light scattering).

Graphic (Particle Size Paribhdra Rasa)

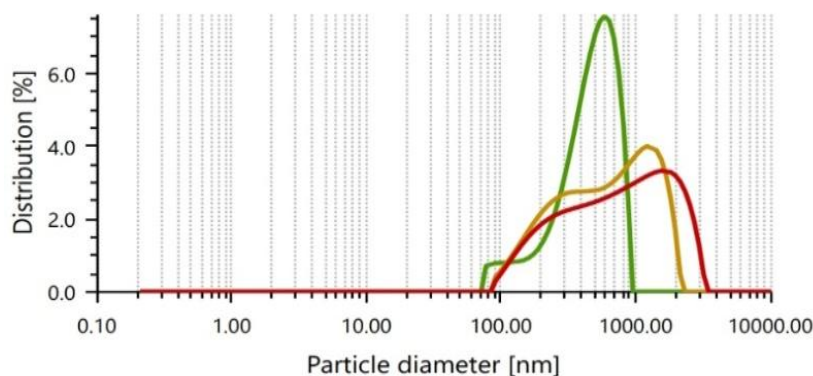


Table 6: Measurements (intensity) Particle Size Paribhdra Rasa

Name	Colour	Hydrodynamic diameter	Polydispersity index (%)	Peak 1	Peak 2	Peak 3	Transmittance	Diff. Coef.
R.S 1	-----	985.6 nm	30.6	476.9 nm	(Intensity)	(Intensity)	1.6 [%]	0.5 [μm ² /s]
R.S 2	-----	901.9 nm	34.3	752.2 nm	(Intensity)	(Intensity)	2.0 [%]	0.5 [μm ² /s]
R.S 3	-----	904.9 nm	33.1	986.0 nm	(Intensity)	(Intensity)	1.4 [%]	0.5 [%]

Antifungal Study of Paribhdra Rasa

Materials/Methods for Antifungal Study

Selection of Micro-organisms: Micro-organisms like Candida Albicans & Aspergillus Fumigatus were selected for the antifungal study of Paribhdra Rasa.

Table 7: Micro-organisms

Sr.no	Species	ATCC No.
1.	Aspergills Fumigatus	MTCC 227
2.	Candida Albicans	MTCC 870

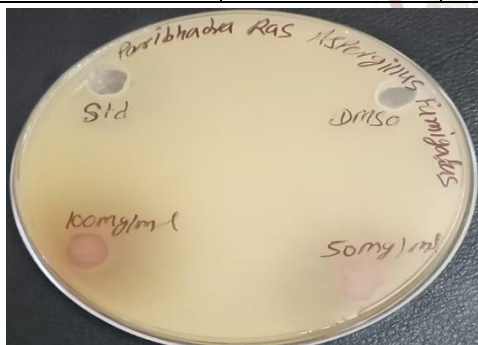
The viable micro-organisms used in the test must not be more than five passage removed from the original ATCC (American type culture collection) culture or any other equivalent cultures.

Test Procedure

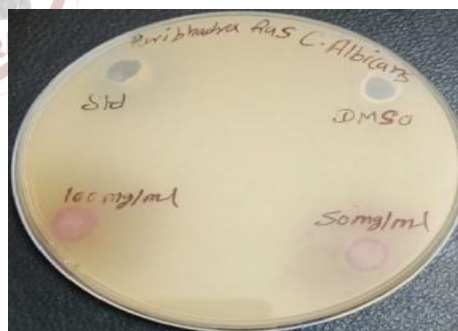
In vitro antibacterial activity of formulations was carried out through usings Kirby- Bauer Agar Well diffusion method. This classic method yields a zone of inhibition in mm result for the amount of antibacterial that is needed to inhibit growth of specific microorganisms. Each formulation was used as such and in diluted in DMSO at 5mg/ml, 10mg/ml or 50 and 100%. Sample dissolved in to DMSO and pour in to wells. For the determination of zone of inhibition (ZOI), liquid suspension culture of each bacterial and fungal strain. Gentamycin used as a standard antibiotic, fluconazole as an antifungal agent and control DMSO for comparison of the results. Muller Hinton agar plates for bacteria and fungus were seeded with liquid culture of bacterial strains and allowed to stay at 37°C for 24 hours. The zones of growth inhibition around the wells were measured after 18 to 24 hours of incubation at 37°C for bacterial and 48 to 72 hours for fungal at 25°C. The sensitivity of the microorganism species to formulation was obtained by measuring the sizes of inhibitory zones (including the diameter of well) on the agar surface with comparison to the standard antibiotic zones. Control as DMSO and positive control or standard control Gentamycin 2.5 ug/ml as antibacterial and Fluconazole 5 ug/ml as antifungal.

Table 8: Anti-fungal Result

A	Antimicrobial activity					
	Antimicrobial activity (values are mean of triplicate)	As per standard antimicrobial sensitivity protocol of pharmacopoeia	Zone of inhibition			
			Standard	Test samples (in DMSO)		
			Positive	50mg/ml	100 mg/ml	DMSO Negative Control
1.	Aspergillus fumigatus		24	14	18	8
2.	Candida Albicans		24	16	20	8



Zone of Inhibition of Aspergillus Fumigatus



Zone of Inhibition of Candida Albicans

DISCUSSION

Color of *Paribhdra Rasa* is brick red, odor-characteristic, pH of 10% aqueous solution of *Paribhdra Rasa* was 5.8 shows *Paribhdra Rasa* acidic in nature, moisture content of *Paribhdra Rasa* is 6.43%. The water and alcohol soluble extractive value of *Paribhdra Rasa* is 29.53% & 26.83% shows that *Paribhdra Rasa* had good water and alcohol solubility. Particle size distribution of *Paribhdra Rasa* mean average size of particles found 930.8nm and distribution of particles are in between 901.9 to 985.6nm. XRD of *Paribhdra Rasa* shows sharp peaks observed that major compounds as mercuric sulphate

of majorly at 100% intensity on 26.8082, 2 theta value with crystalline shape and structure. Concentration of heavy metals in *Paribhdra Rasa* i.e., Lead is 9.1mg/kg, mercury 41962.39 mg/kg, arsenic and cadmium were below permissible limits in *Paribhdar Rasa*. The estimation of microbial contamination shows there was absence of the specific pathogens like *E. coli*, *Pseudomonas aeruginosa*, *Salmonella sp.* etc. Microbial analysis of *Paribhdra Rasa* show total bacterial count 20 cfu/g and total fungal count less than 10 cfu/g. *Paribhdra Rasa* passes all Ayurvedic classical parameters i.e., *Varitar*, *Rekhapurnatav*, *Nisawadu* etc.

Discussion on Antifungal Study of Paribhdra Rasa

To evaluate the comparative anti-fungal activity of the test drug, with positive control (fluconazole). DMSO solutions of *Paribhdra Rasa* were prepared and this study was performed. Since, no documented study is found mentioned in Ayurveda regarding antifungal activity, basic microbiological techniques mentioned for evaluating antifungal activity in the modern medicine were followed. This study was done at S.R. LABS. Pratap Nagar, Jaipur, against two common pathogenic strains of fungus already mentioned in anti-fungal study earlier. I have taken following microbes as *Aspergillus Fumigatus* and *Candida Albicans* in present study. For antifungal study in the sample of *Paribhdra Rasa*, Zone of inhibition of *Aspergillus Fumigatus* is 14 in 50mg/ml and 18 in 100mg/dl (in DMSO) and in case of *Candida albicans* zone of inhibition is 16 in 50mg/ml and 20 in 100mg/dl (in DMSO).

CONCLUSION

In present study, preparation of *Rasa Sindhur* through (*Kupipakav Rasayan*) traditional method, however was complex but can be prepared without any difficulty. After preparing, final drug (*Paribhdra Rasa*) was subjected on anti-fungal study, by means of antifungal study it is came to know that this drug (*Paribhdra Rasa*). At the given concentration, *Paribhdra Rasa* was found bioactive against *Aspergillus Fumigatus* and *Candida Albicans* and DMSO (negative control) did not show any activity against test organisms. *Paribhdra Rasa* is less effective than standard drug fluconazole, so there is need to do more research in this field like the dose of the drug can be increased i.e., more than 50mg/100 mg, to check the effectiveness of the *Paribhdra Rasa* for anti-fungal study.

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