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Analytical and antimicrobial study of herbo-mineral formulation *Shwasakasari Rasa* in selected respiratory micro-organism

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ABSTRACT

Shwasakasari Rasa (SKR) is mentioned in *Rasa Yoga Sagar,*[1] (part 2) as a remedy of *Shwasa* & *Kaasa Roga.* This type of *Rasa Aushadhi* are more effective against bacteria. These drugs have a multi dimension effect like Broad-spectrum antibiotics. The *Rasaushadies* mentioned in *Ayurvedic Pharmacopoeia* should be analysed for physical & chemical properties to confirm purity & safety before administration in human beings. Hence it become obligatory to adopt modern analytical methodology for better understanding and interpretation of physio-chemical changes occurred during the process. SKR was analysed on the following parameter Organoleptic parameter, Physiochemical analysis, Advanced physiochemical analysis. SKR sample was tested for antimicrobial activity against standard strains of *Staphylococcus aureus, Klebsiella pneumonia* and *Streptococcus pneumonia.* The prepared *Shwasakasari Rasa* was studied for their MIC (Minimum Inhibitory Concentration & the zone of inhibition. The prepared drug was compared with *Azithromycin* (standard drug) of macrolide group to evaluate its antimicrobial efficacy. These tests was conducted in, S.R Labs & Research Centre, Jaipur.

Key words: *Shwasakasari Rasa, Rasa Aushadhi, Shwasa, Kasa, Azithromicin.*

INTRODUCTION

The *Rasa Shastra* is one of the aspects of *Ayurveda* which deals with the use of mineral, metal, herbal and animal origin product. The *Rasa Shastra* a branch of *Ayurveda* with binary aim, the foremost being the remedial applicability of metal and minerals i.e., *Dehavada* and another been the conversion of lower metals into higher metals i.e. *Dhatuvada.* In *Ayurveda Shwasa Rog*[2] is described as exposure to causative factors leads to vitiation of *Kapha* along with *Vata* which causes obstruction of *Pranavaha Strotas*. This generates movement of *Vayu* in all direction in *Pranavaha Strotas* and body eventually causes *Shwasa Roga*. Aggravated *Vata* due to exposure to causative factors leads to its *Pratiloma Gati* or reverse movement. Vitiated *Vata* runs through channels and reaches head, neck region. It exaggerates the indigenous *Kapha* by increasing epithelial secretion and produce *Peenasa.* These secretions of *Mala Rupi Kapha* obstructs the passage of air and produces *Ghurghur Shabda* or wheezing sound. Numbers of herbo-mineral preparations are available in various Ayurvedic classics out of which *Shwaskasari Rasa* is unique and contains *Parada, Gandhaka, Pippali,*

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ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

Haritaki, Gorakhmundi, Vasa, Vibhitaki and indicated in all type of *Shwasa* and *Kasa Roga*. An Analytical Study encompasses test of any drug or formulations. Analytical examine of any drug is crucial to standardize it. Analytical examine is achieved to test drug quality. For this cause a few analytical assessments are achieved and their results are as compared with standard parameters. The drug gratifying those standards may be taken as standard drug and may be uses for therapeutic purpose Azithromycin[3] is from a group of medicines called macrolide antibiotics. Macrolide antibiotics work by killing the bacteria that cause the infection.

An antimicrobial[4] is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are microbicides, while those that merely inhibit their growth are called bacteriostatic agents. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. The Antimicrobial effect of spices extracts also helps to prevent diseases in many forms. In antimicrobial activity, Minimum inhibition concentrations were determined.

Aim and Objectives

1. To evaluate the analytical study of *Shwasakasari Rasa.*
2. To evaluate the antimicrobial effect of *Shwasakasari Rasa* in respiratory tract infections caused by *Staphylococcus aureus, Klebsiella pneumonia and Streptococcus pneumonia.*[7]
3. To compare and evaluate the antimicrobial properties of *Shwasakasari Rasa* with *Azithromycin (standard drug)* of macrolide group on organisms by Agar disc diffusion method.
4. To evaluate minimum inhibitory concentration of *Shwaskasari Rasa.*

Materials and Methods

A) Drugs

1. *Shwaskasari Rasa*
2. Standard drug -Azithromycin
3. DMSO as a negative control.

B) Microorganism

Table 1: Showing micro-organism with culture

SN	Microorganism	Culture
1.	Streptococcus pneumonia	MTCC 1935
2.	Staphylococcus aureus	MTCC 737
3.	Klebsiella pneumonia	MTCC 39

Methodology

- SKR sample was tested for antimicrobial activity against standard strains of *Staphylococcus aureus, Klebsiella pneumonia and Streptococcus pneumonia.*
- The prepared *Shwasakasari Rasa* was studied for their MIC (Minimum Inhibitory Concentration & the zone of inhibition.
- The prepared drug was compared with *Azithromycin* (standard drug) of macrolide group to evaluate its antimicrobial efficacy.

Procedure

In vitro antibacterial activity of formulations was carried out by using *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 bacterial strain. Azithromycin used as a standard antibiotic, and control DMSO for comparison of the results. Muller Hinton agar plates for bacteria were seeded with liquid culture of bacterial strains and allowed to stay at 37°C for 24

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

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 were determined by measuring the sizes of inhibitory zones (including the diameter of well) on the agar surface with comparison to the standard antibiotic zones.

Diameter of Well - 8 mm, Vol. applied in each well- 100 µl, Control as DMSO

RESULTS

Chart 1: Showing Zone of inhibition (in mm)of standard drug with 50% & 100% *Shwaskasari Rasa* sample.

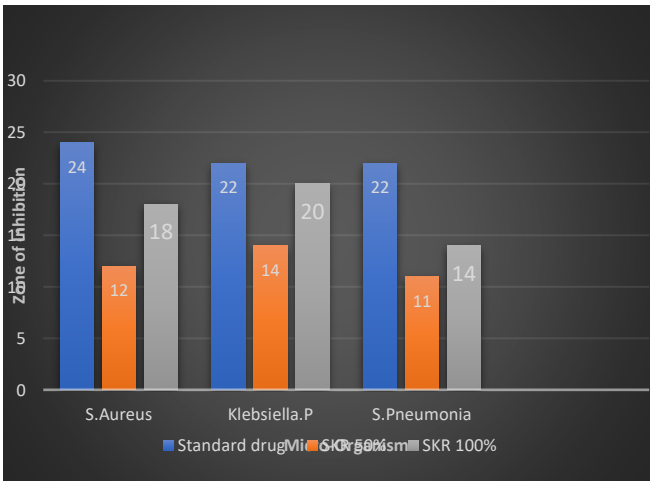

Table 2: Showing result of zone of inhibition of *Shwaskasari Rasa.*

Antimicrobial Activity (Values are mean of triplicate)	As per standard antimicrobial sensitivity protocol of pharmacopoeia	Standard	Test Sample (in DMSO)		
		Positive control	50 %	100 %	DMSO Negative control
Staphylococcus aureus		24	12	18	8
Klebsella pneumonia		22	14	20	8
Streptococcus pneumonia		22	11	14	8

Azithromycin (2.5 µg/mL) as Antibacterial Control

DMSO as Blank for all study

Minimum inhibitory concentration (MIC)[5]

- **MIC** defines in vitro levels of susceptibility or resistance of specific bacterial strains to applied antibiotic.
- **MIC** can be determined by culturing microorganism in liquid media or on plates of solid growth medium.
- A lower MIC value indicates that less drug is required for inhibiting growth of the organism. Therefore, drugs with lower MIC scores are more effective antimicrobial agents.

Chart 2: Showing minimum inhibitory concentration (MIC) in different concentration of *Shwaskasari Rasa* sample.

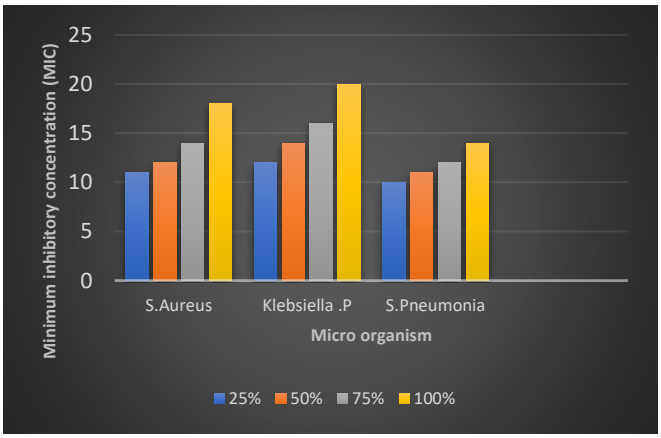

Table 3: Showing result of Minimum inhibitory concentration (MIC) in different concentration of *Shwaskasari Rasa* sample.

SN	MIC Determination (Sample Concentration in %)	DMSO	25%	50%	75%	100%
1.	*Staphylococcus aureus*	8	11	12	14	18
2.	*Klebsiella pneumonia*	8	12	14	16	20
3.	*Streptococcus pneumonia*	8	10	11	12	14

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

Table 4: Showing Results of Physiochemical Analysis[6] of *Shwasakasari Rasa.*

SN	Test Parameters	Test method	Unit	Results
1.	pH (2%w/v Aq. Sol.)	API Part I, Vol.-VI, 2009	-	4.98
2.	Loss on drying	API Part I, Vol.-VI, 2009	%w/w	2.63
3.	Total Ash Value	API Part I, Vol.-VI, 2009	%w/w	10.41
4.	Acid Insoluble Ash	API Part I, Vol.-VI, 2009	%w/w	3.62
5.	Water Soluble extractive	API Part I, Vol.-VI, 2009	%w/w	25.48
6.	Alcohol Soluble extractive	API Part I, Vol.-VI, 2009	%w/w	17.38
7.	Disintegration Time	API Part I, Vol.-VI, 2009	Minute	130.0
8.	Hardness	API Part I, Vol.-VI, 2009	Kg/cm^2	10.0
9.	Average Weight	API Part I, Vol.-VI, 2009	Mg	173.2
10.	High Performance Thin Layer Chromatography	By HPTLC	-	Data Attached
11.	XRD	By XRD	-	Data Attached
12.	EDAX	By EDAX	-	Data Attached
13.	Mercury (as Hg)	By AAS	mg/Kg	139.35
14.	Arsenic (as As)	By AAS	mg/Kg	0.24
15.	Mercury (as Hg)	By ICP-MS	mg/Kg	139.48

DISCUSSION

Discussion on Analytical Study

Classical Parameters: On observation SKR has *Krishna Varna* i.e. black in color. *Katu* in *Rasa* i.e. bitter in taste, *Katu Gandha* & *Shlahshnatva* was felt by simple touch with finger.

Physical Parameters:

1) pH: The pH of SKR is 4.98.

pH of SKR is 4.98 indicating acidic nature of the sample. According to pH partition concept, weak acidic are better absorbed from the stomach and weak bases from the intestine. All the values indicates the partial acidic nature of the drugs which might be due to heat treatment as well as the drugs used in its preparation.

2) Loss on drying:

Loss on drying of SKR is 2.63%w/w. It is the amount of moisture content in the SKR. The least loss on drying at 110°C the better will be the drug stability. SKR have least amount of moisture content & very rare chance of bacterial & fungal growth.

3) Total Ash Value:

The total Ash value of SKR was 10.41%w/w. Ash value is the direct indicator of inorganic content of the material. SKR has low ash value due to *Putpaka* process low temp is given.

4) Acid insoluble Ash:

Acid insoluble Ash of SKR is 3.62%w/w. It indicates the presence of inorganic content which is a criteria to judge the identity & purity of crude drug.

5) Water Soluble Extractive:

Water soluble extractive of SKR was 25.48 %w/w. It indicates the presence of water soluble constituent & amount of active constituents in SKR. It determine solubility & Polarity of Phytochemicals in the formulation SKR.

6) Alcohol Soluble Extractive:

Alcohol Soluble extractive of SKR is 17.38 %w/w. It indicates the amount of active constituents in SKR. It determine solubility & Polarity of Phytochemicals in the formulation SKR.

7) Tablet Disintegration time:

Tablet disintegration time of SKR is 130 min. It indicates the more time required to disintegrate in the body. 2 hr 10 min required for disintegration of SKR. It indicates the quality & purity of drugs. It determines biologically duration of time for breakdown of SKR in body fluids that important for onset of action.

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

8) Tablet Hardness:

Tablet hardness of SKR was 10.0 Kg/cm^2. It is the time in which tablet breakdown inside the stomach that is directly related to therapeutic activity. It indicates good strength of tablet in handling. Hardness was more as no softener material was added in making. More Hardness & Disintegration time suggest softener material would be required for better & Faster therapeutic activity.

Discussion on Advanced Analytical Parameter of *Shwaskasari Rasa*

1) Discussion of HPTLC

HPTLC is high performance thin layer chromatography. At 254 nm highest 9 spots were detected and at 366 nm highest 7 spots were detected .It indicates an accurate number of components present in a SKR based on small variations in Rf values that may be tolerated by that drug ,it indicates the purity of the drug.

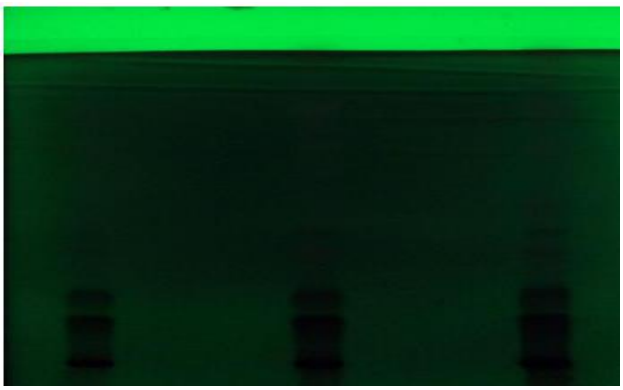

Fig. 1: At 254 nm

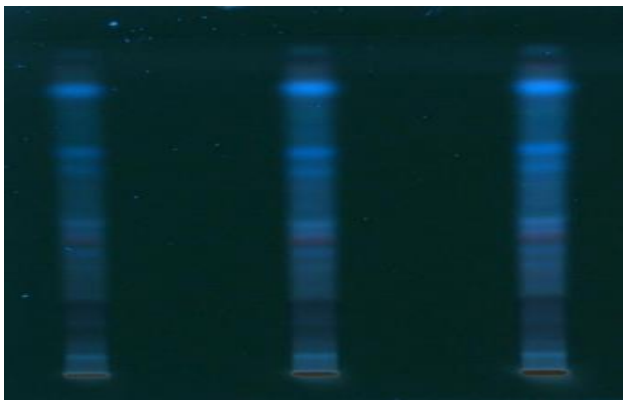

Fig. 2: At 366nm

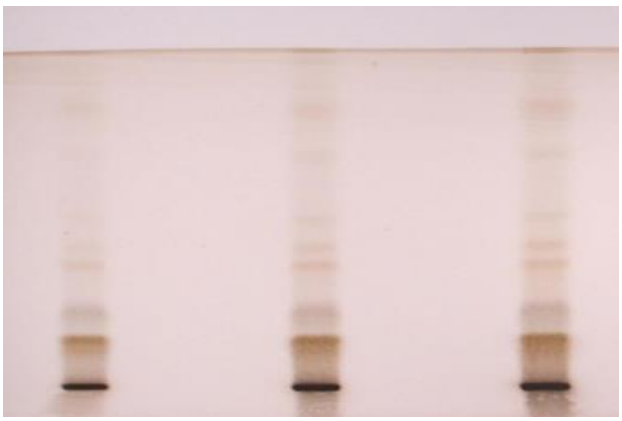

Fig. 3: At 510 nm

2) Discussion of XRD

XRD is X-ray Diffraction. The main compound diffraction peaks corresponding with d spacing are mentioned. SKR sample shows sharp peaks that major compounds of Mercuric sulphide majorly at 100% intensity and 26.7708 at 2-theta values. Spectral data shows SKR sample was perfectly crystallized.

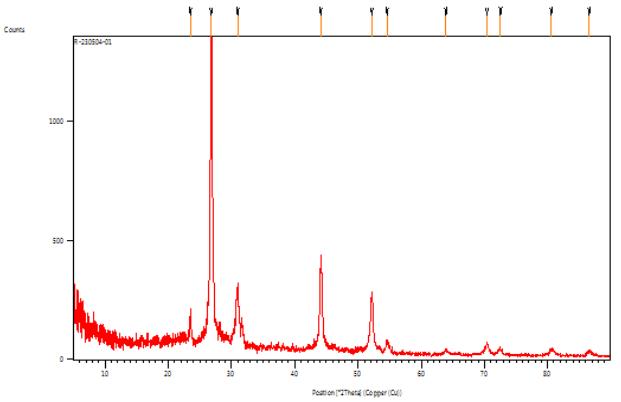

Fig. 4: Showing graph of XRD pattern of SKR

3) Discussion of EDAX

EDAX analysis indicates the presence of only eight elements e.g. highest C (58.06%), S (16.49%), K (6.51%), Mg (1.67%), Al (1.58%) and Si (3.31%). Presence of other elements carbon and oxygen due to natural organic ingredients (*Kasth Aushadi*) used in preparation. Atom balance shows that all Si, K, Mg, Al and S are present more than 20%. It indicates the atomic ratio majorly distributed in Sio2, K compounds, Alumina and S compounds.

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

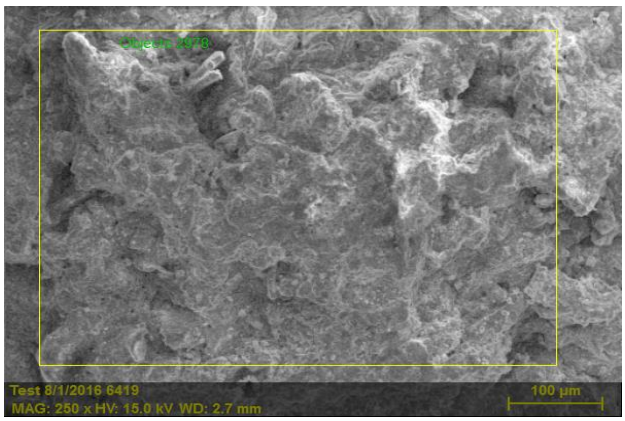

Fig. 5: EDAX Photomicrograph of SKR magnification of 250x

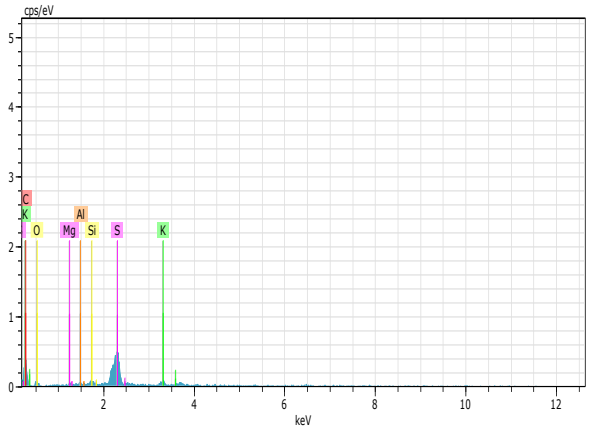

Fig. 6: Presence of 8 elements in EDAX analysis of SKR

4) Discussion of AAS

AAS is Atomic Absorption Spectroscopy. Heavy metal is a group of tests that measures the quantity of specific potentially toxic metals in *Ayurveda*. These heavy metals are lead, cadmium. Mercury & arsenic. Analysis of mercury was done according to API parameters and its result is 139.35 mg/kg (ppm). Analysis of Arsenic was done & its value is 0.24 mg/Kg. Result of mercury and Arsenic were not more than permissible limit. Hence it is concluded that SKR safety profile is good.

5) Discussion of ICP-MS

Analysis of mercury was done by ICP-MS & free mercury was present 139.48 mg/Kg in SKR. Result of mercury were not more than permissible limit. Hence it is concluded that SKR safety profile is good. It shows proper formation of *Kajjali* which was confirmed by *Nishchandratva* and proves safety of the sample SKR.

Discussion on Antimicrobial Study

Discussion of Zone of Inhibition

In Gram positive bacteria Staphylococcus aureus, Standard drug *Azithromycin* solution showed 24 mm of Zone of inhibition. Zone of inhibition of sample drug *Shwaskasari Rasa* in 50% & 100% solution showed 12mm & 18 mm respectively. In Gram positive bacteria Streptococcus Pneumoniae, Standard drug *Azithromycin* solution showed 22mm of Zone of inhibition. Zone of inhibition of sample drug *Shwaskasari Rasa* in 50% & 100% solution showed 11mm & 14 mm respectively. In Gram negative bacteria Klebsiella pneumonia, Standard drug *Azithromycin* solution showed 22 mm of Zone of inhibition. Zone of inhibition of sample drug *Shwaskasari Rasa* in 50% & 100% solution showed 14mm & 20 mm respectively. Staphylococcus aureus, Streptococcus Pneumoniae and Klebsiella pneumoniae have shown good result to 100 mg/ml of *Shwasakasari Rasa* than DMSO. *Shwaskasari Rasa* compared to Standard drug (Azithromycin). *Shwaskasari Rasa* has shown good response to bacterial organisms in all concentrations. *Shwaskasari Rasa* shows good susceptibility against gram negative bacteria Klebsiella pneumoniae i.e. 20 mm of Zone of inhibition. *Shwaskasari Rasa* shows less antimicrobial susceptibility in comparison to standard antibiotics.

Discussion on Minimum Inhibitory Concentration

In vitro studies “Agar well diffusion method” was undertaken to assess the antimicrobial properties of SKR. SKR was tested indifferent concentration on different species of bacteria like *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia.* Lower MIC value indicates that less drug is required for inhibiting growth of the organism, therefore drug with lower MIC scores are more effective antimicrobial agents. SKR showed Minimum Inhibitory Concentration at 25% in bacteria. *Staphylococcus aureus, Klebsiella pneumonia* & *Streptococcus pneumonia* showed MIC 11 mm, 12 mm, 10 mm respectively at 25% concentration.

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

Fig.7,8,9: Showing Zone of inhibition SKR at 50% and 100% concentration, Standard drug (Azithromycin) and DMSO on microorganism by Agar well diffusion method.

Fig. 10,11,12: Showing MIC (Minimum inhibitory concentration) At 25%, 50%, 75%, 100% of SKR and DMSO

ISSN: 2456-3110 ORIGINAL ARTICLE December 2023

Conclusion

In analytical study, SKR were tested on Organoleptic characters, Physico-chemical parameters and Advanced analytical parameters. The sample of SKR on performing organoleptic test like color, odor, taste, appearance. The sample of SKR was then subjected to physiochemical parameter as pH value was 4.98, Loss on drying was 2.63%w/w, Total Ash Value 10.41%w/w. Acid Insoluble Ash was 3.62%w/w, Water Soluble extractive 25.48%w/w, Alcohol Soluble extractive value was 17.38%w/w, Disintegration Time 130 min, hardness 10.0 Kg/cm2, Average weight 173.2 mg. The sample of SKR on performing Advanced analytical test as HPTLC at 254 nm highest 9 spots were detected & at 366 nm highest 7 spots were detected, XRD shows sharp peaks that major compounds of mercuric sulphide majorly at 100 % intensity & 26.7708 at 2-theta values. EDAX analysis indicates the presence of only eight elements e.g. highest C (58.06%), S (16.49%), K (6.51%), Mg (1.67%), Al (1.58%) and Si (3.31%). Heavy metal analysis by AAS for Mercury was 139.35 mg/kg & Arsenic was 0.24 mg/kg, & by ICP -MS for Mercury was 139.48 mg/kg. XRD shows SKR sample was perfectly crystallized.

Antimicrobial study was carried out on *Shwaskasari Rasa* by agar well diffusion method against the Gram-positive bacteria (Staphylococcus aureus and Streptococcus pneumoniae & Gram negative bacteria (Klebsiella pneumoniae). Azithromycin (Standard drug) antibiotic was used as positive control & DMSO was used as negative control. *Shwaskasari Rasa* compared to Standard drug (Azithromycin). *Shwaskasari Rasa* has shown good response to bacterial organisms in all concentrations. *Shwaskasari Rasa* shows good susceptibility against gram negative bacteria Klebsiella pneumoniae i.e., 20 mm of Zone of inhibition. *Shwaskasari Rasa* shows less antimicrobial susceptibility in comparison to standard antibiotics (*Azithromycin).* SKR showed Minimum Inhibitory Concentration at 25% in bacteria. *Staphylococcus aureus, Klebsiella pneumonia* & *Streptococcus pneumonia* showed MIC 11mm, 12mm, 10mm respectively at 25% concentration. It was observed that *Shwaskasari Rasa* shows antimicrobial activity by the study conducted by Agar Well diffusion method. *Shwaskasari Rasa* shows good susceptibility against gram negative bacteria *Klebsiella pneumoniae*. *Shwaskasari Rasa* shows less antimicrobial susceptibility in comparison to Standard antibiotics Azithromycin. This work has resulted in a better understanding of the pharmaceutical preparation of *Shwaskasari Rasa* as per Ayurvedic protocol.

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