Research Article.

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Screening Of Free Radical Scavenging Activity and Immuno-Modulatory Effect Of *Jwaramurarirasa*

Dr. Omkar K. Dhumale[1], Dr. Mahantesh B. Rudrapuri[2] and Dr. G. Vinay Mohan[3]

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1. PG Scholar, Dept. of Rasa Shastra &Bhaisajya Kalpana, Sri Shivayogeeshwara Rural Ayurvedic Medical College, Inchal, Belagavi, Karnataka.
2. Professor & HOD, Dept. of Rasa Shastra &Bhaisajya Kalpana, Sri Shivayogeeshwara Rural Ayurvedic Medical College, Inchal, Belagavi, Karnataka.
3. Principal, Dept. of Rasa Shastra &Bhaisajya Kalpana, Sri Shivayogeeshwara Rural Ayurvedic Medical College, Inchal, Belagavi, Karnataka

Corresponding Author :- Dr. Omkar K. Dhumale, PG Scholar, Dept. of Rasa Shastra &Bhaisajya Kalpana, Sri Shivayogeeshwara Rural Ayurvedic Medical College, Inchal, Belagavi, Karnataka, Email,Id-

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ABSTRACT: -

Jwaramurari Rasa is one of the herbo mineral formulation having property of *Rasayana* (rejuvenation therapy). Recent study shown the presence of phytochemical like alkaloids, carbohydrates, steroids, flavonoids, proteins, saponins, and tripenoids. Hence antioxidant and immunomodulatory activity are expected with *Jwaramurari Rasa*. In this regard to provide evidence to establish the facts mentioned in classics an attempt was made to assess the free radical scavenging activity of *Jwaramurari Rasa*. *Jwaramurari Rasa* was subjected for screening of free radical scavenging activity by Superoxide dismutase (SOD), Catalase, Glutathione, Malondialdehyde (MDA) test. Immunomodulatory activity by phagocytosis, candidacidal assay, neutrophil locomotion chemotaxis, and Nitrobluete trazolium (NBT) test. 5%, 12.5%, 25%, 50% and 100% concentration of the samples were used. *Jwaramurari Rasa* has shown significant immunomodulatory activity. 100% (95%) solution of *Jwaramurari Rasa* has shown highly significant result with NBT test stimulation of neutrophils than control. In candidacidal activity of *Jwaramurari Rasa* with100% was highly significant. *Jwaramurari Rasa* has shown significant results with Candidacidal, Phagocytosis and Chemotaxis than control.

Keywords: *Jwaramurari Rasa*, Immunomodulatory, free radical scavenging activity,

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INTRODUCTION:

Now a day's prevention of the diseases is achieved by immunization specifically against the several diseases. But the numbers of the diseases are so much that practically it is impossible to immunize a person against all the diseases. On the other hand, the concept of *Rasayana* (rejuvenation therapy) seems to increase the general immunity so that one can live a long with youthful life and free from the diseases. It may provide an umbrella against the diseases and aging by promoting the physical and mental health.

Jwaramurari Rasa is one of the medicines mentioned in *Bhaisajya Ratnavali*[1]. It is effective in the diseases like *Jwara. Jwaramurari Rasa* improves metabolic & other vital functions of body and nourishes the dhatus from *Rasa* (Plasma) *to Shukra* (Semen). So that, it can be postulated that *rasayana* (rejuvenation) drugs may possess free radical scavenging and immuno-modulatory properties.

Hence to provide a scientific data and statistical validation a study on "Screening of free radical scavenging activity & immune modulatory effect of *Jwaramurari Rasa*" was undertaken.

AIMS AND OBJECTIVES OF STUDY:

1. Preparation of *Jwaramurari Rasa.*
2. Screening of free radical scavenging activity *of Jwaramurari Rasa*.
3. Evaluate the immune modulatory effect of *Jwaramurari Rasa*.

MATERIALS & METHODS:

Preparation of *Jwaramurari Rasa* was followed according *to BhaisajyaRatnavali*[2] 5/887

Materials Required:

Table no.1 showing ingredients of *Jwaramurari Rasa*

S.No.	**Materials**	**English/Botanical Name**	**Quantity**
1	*Shodhith Hingula*	Purified *Cinnaber*	12gm
2	*Shodhith Vatsanabha*	Purified Aconite	12gm
3	*Shodhith Tankana*	Purified Borax	12gm
4	*Pippali churna*	*Piper Longum*	12gm
5	*Marich churna*	*Piper Nigrum*	12gm
6	*Haritaki churna*	*Terminalia Chebula*	12gm
7	*Shunthi churna*	*Zingiber officinale*	24gm
8	*Shodhith Jayapala*	*Purified Croton tiglium*	96gm

***Bhawna Dravya* (Levigation drugs) –**

1. *Nimbuka Swaras* (lemon juice)
2. *Ardraka Swarasa* (Ginger Juice)
3. *Gomutra* (Cow urine)
4. *Godugdha* (Cow milk)

Preparation Of *Jwaramurari Rasa*:

Date of commencement :11-02-2020.

Date of completion: 11-02-2020.

Equipment's: *Khalva Yantra* (pestle and mortar), *tulayantra* (Balance), spoon etc.

Procedure:

Shodhith Vatsanabha (Purified Aconite) , *Shodhith Tankana(* Purified Borax) , *Pippali churna (Piper Longum), Marich churna (Piper Nigrum), Haritaki churna (Terminalia Chebula)* each12 gms ,*Shunthi churna* (*Zingiber officinale*) 24 gms and *Shudha jayapala* (Purified *Croton tiglium)* 96 gms are taken in *khalwa yantra* (pestle and mortar) and mixed till it becomes uniform mixture.[3]

·Homogenous mixture prepared well.

Total weight of *Jwaramurari Rasa*: 192 gms

Free Radical Scavenging Activity :

Laboratory Name : Maratha Mandal's Central Research Laboratory, R.S.No.47A/2, Bauxite Road, Belgaum -590010

Parameters Taken for Assessment:

1. Lipid peroxidation
2. Superoxide dismutase
3. Catalase
4. Reduced Glutathione (GSH)

Methods

A. **Lipid peroxidation (by Nadiger[4])**

Procedure: for Sample

a. 0.5ml of Sample is taken in test tube

b. To this 3.6ml of TCA (Trichloroacetic acid) is added

c. 1.5ml of TBA (Thiobarbituric acid) is added

d. Kept in water bath, boiled for 10-15 mins and cooled

e. Centrifuge for 10-15 mins at 4000 rpm

f. OD of supernatant at 530 nm is recorded

B. **Superoxide dismutase[5]:**

Tris buffer: 0.6 grams of Tris and 0.037 gms dissolved in 100 ml. 50 ml of Tris buffer (containing 50mm of Tris buffer and 1 mm of EDTA) was prepared. To this 50 mm of HCl was added to adjust the pH at 8.5 and volume was made upto 100ml.

Tris --Mol. Wt. 121.14

1M = 1000mm therefore 50mm = 60 mg in 100ml distilled water

EDTA: 372 gm in 1000ml = 1M

0.0372 GM = 1MM

3.7 mg in 100 ml distilled water

20 mM pyrogallol: 25.2 mg dissolved in 10 ml gives 20 mM solution of pyrogallol. This was prepared freshly and used. *Jwaramurari Rasa* Sample is diluted in distilled water at a ratio of 1:10

1.1 ml of *Jwaramurari Rasa* is taken in test tube.

The contents of the above tubes are mixed and absorbance at 420 nm were measured.

Table No.2 showing reaction mixture content in SOD test

Sl.No.	Reaction mixture contents	Quantity	Group
1	Sample A + Tris buffer + Pyrogallol	0.1 ml + 2.85 ml + 0.1 ml	Test sample 1
2	Tris buffer + Pyrogallol	2.85 ml + 0.1 ml	Control

The activity of SOD was assessed OD at 420 nm after 1 min 30 sec & 3min 30 sec. Each test tube added with 2.8 ml of Tris buffer solution. Just before checking its optical density reaction mixture was added with freshly prepared 0.1 ml of 20 NM pyrogallol.

Immediately after addition of pyrogallol OD was seen using UV spectrophotometer at 420 nm at interval of 1.5 min and 3 min. ΔA obtained for control and test.

SOD was calculated by formula.

$$A/min = \frac{\text{Absorbance at 3 min 30 sec - Absorbance at 1 min 30 sec}}{2}$$

Calculation –

$$\frac{\Delta A/min \text{ of control - } \Delta A/min \text{ of test x 100 x conc of standard}}{\Delta A/min \text{ of control x 50 vol of sample}}$$

$$\frac{C\text{-}T \times 100 \times 1}{CX50\ 0.1}$$

$$C\text{-}T \times 1000$$

$$CX50 \text{ --------/units/ml}$$

C. Estimation of GSH(Reduced Glutathione)[6]

GSH Assay mixture

a) 0.2ml of compound
b) 0.7ml of 0.3m Na2HPO4
c) 0.1ml DTNB – citrate solution

Prepared only one blank. All cellular GSH is oxidized by adding 0.005ml of10mm diamide prior to addition of DTNB. Allow the diamide to react with the supernate for approximately one minute before adding the DTNB. It should becolorless .

Read the absorbance of the solution at 412nm. Reading was taken within 15 to 20min after adding DTNB.

Calculation – By graph

D. ESTIMATION OF CATALASE[7]

Optimal condition for the assay was as follows: 0.1ml of sample was incubated in 1.0ml substrate (65umol/ml hydrogen peroxide in 60mmol/l sodium potassium phosphate buffer, pH7.4 at 370c for 60secs).

The enzymatic reaction was stopped by addition of 1.0ml of 32.4 mmol/l ammonium molybdate (NH4)6 Mo7O24 4H2O) and yellow complex of molybdate and hydrogen
peroxide was measured at 405nm against blank 3.

Serum catalase activity(KU/L) = A(sample) – A(blank 1) X 271 A(blank 2) – A(blank 3)

Blank 1 contained 1.0ml substrate, 1.0ml molybdate ad 0.2ml sample
Blank 2 contained 1.0ml substrate, 1.0ml molybdate ad 0.2ml buffer
Blank 3 contained 1.0ml buffer, 1.0ml molybdate ad 0.2ml sample

Table No.3 showing reaction mixture content

	Substrate	Molybdate	Sample	Buffer
Blank 1	1.0ml	1.0ml	0.2ml	-
Blank 2	1.0ml	1.0ml	-	0.2ml
Blank 3	-	1.0ml	-	0.1ml/0.2ml

Immuno-Modulatory Activity

Jwaramurari Rasa was subjected for screening of immuno-moduloatory activity with four parameters

A. NitrobluetetrazoliumTest.(NBT)

B. Phagocytosis and candidacidal assay.(Two parameters in sametest)

C. Neutrophil locomotion &Chemotaxistest.

OBSERVATIONS & RESULT

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Free Radical Scavenging Activity:

Table No.4: Showing concentration of MDA in estimation of LPO

Test	Sample
First Test	9.0
Second Test	8.3
Third Test	7.8
Mean	8.35

Maximum decrease in concentration of MDA was seen in sample.

Table No.5: showing concentration of SOD in estimation of SOD (in units/mgprotein)

Test	Sample
First Test	4.0
Second Test	3.58
Third Test	3.0
Mean	3.52

Maximum utilization of SOD was noted with Sample.

Table No. 6: showing concentration of Glutathione (units/mg protein)

Test	Sample
First Test	15.5
Second Test	15.0
Third Test	15.3
Mean	15.2

Maximum utilization of GSH was noted with sample.

Table No.7: showing umol of H2O2 consumed/min/mg protein estimation of catalase

Test	Sample
First Test	1939
Second Test	2341
Third Test	2923
Mean	2401

Maximum consumption of H2O2 seen with sample.

Immunomodulatory Effect:

Table no: 8 Shows results of NBT- test (Stimulated cells in %)

Concentration of Drug	Sample	Positive Control	Negative Control
5% JMR	56%	90%	20%
12.5% JMR	62%		
25% JMR	92%		
50% JMR	92%		
100% JMR	95%		

Table No: 9 Shows results of Phagocytosis Assay (In Meanparticle number).

LeucocyteSuspension	Leucocyte Suspension					PC	NC
Concentrationof drug%	5	12.5	25	50	100	4	2
No of candidaengulfed	2	2	4	4	5		

With 100% *Jwaramurari Rasa* maximum no of candida were engulfed by Neutrophils.

Table no: 10 Shows results of Candidacidal Assay (Dead candida cells in %).

LeucocyteSuspension	Leucocyte Suspension					Negative Control
Concentrationof drug%	5	12.5	25	50	100	18
Dead candidacells in %	18	18	30	30	39	

Maximum candidacidal activity was seen with 100% .

Table no: 11 Shows results of Neutrophil Locomotion & Chemotaxis test (Inmm.).

NeutrophilSuspension	Neutrophil Suspension				
Concentrationof drug %	5	12.5	25	50	100
Movement ofneutrophils inmm	0.8mm	1.0mm	2.0mm	2.0mm	2.3mm
Control	FMLP- 2.2mm Media – 0.5mm				

Maximum movement was seen with 100% *Jwaramurari Rasa*.

DISCUSSION:

Jwaramurari Rasa was subjected for screening of Immuno-modulatory activity on Human Polymorph neutrophils with four parameters.

1. NBT test
2. Phagocytosis assay
3. Candidacidal assay
4. Neutrophil locomotion and chemotaxis test.

Mechanism of action of *Jwaramurari Rasa* on immune system:

1. *Jwaramurari Rasa* stimulates the neutrophils for phagocytosis
2. *Jwaramurari Rasa* potentiates neutrophils to kill foreign organisms.
3. *Jwaramurari Rasa* facilitates neutrophils locomotion towards the stimulus or infected site.

From the above results of immuno-modulatory assay with four parameters it is noted that *Jwaramurari Rasa* demonstrated significant immuno- modulatory activity. *Jwaramurari Rasa* contains phytochemical constituents like alkaloids, carbohydrates, steroids, flavonoids, proteins, saponins, and tripenoids, and even in individual ingredients too hence these might have contributed in immuno-modulatory activity of sample.

CONCLUSION:

Free Radical Scavenging Activity:

Jwaramurari Rasa was subjected for screening of the free radical scavenging activity with the following four parameters:

1)SOD

·SOD Activity in sample was 3.5units/ml and control was 9.2 units/ml.

· Sample shown significant free radical scavenging activity than control.

2) CATALASE

· The mean result of Sample was 2401 KU/L and control was 58.5KU/L.

· Sample shown significant effect compared to control.

3)GSH

· The mean reading of serum has shown 15.8umol/L and Control – serum sample 0.88umol/L.

·Sample shown significant antioxidant activity than Control.

4)MDA

· The mean reading of sample was 8.35mmol/L and control serum sample 20.4mmol/L was noted.

·Sample has shown significant than to control.

Among these sample with SOD, MDA, GSH and Catalase parameters has shown significant free radical scavenging activity.

Individual ingredients of *Jwaramurari Rasa* contains phytochemical constituents like alkaloids, carbohydrates, steroids, flavonoids, proteins, saponins, and tripenoids might have contributed in free radical scavenging activity.

Immunomodulatory Effect:

1. NBT test:

i)All the concentrations of *Jwaramurari Rasa* have shown highly significant stimulation of neutrophils for phagocytic activity, with 5% (56%),12.5% (62%), 25% (92%),50% (92%), and 100% (95%).

ii) 100% (95%) solution of *Jwaramurari Rasa* has shown highly significant result with NBT test stimulation of neutrophils than 5% (56%),12.5% (62%), 25% (92%), and50% (92%).

2. Phagocytosis:

i) 5%, 12.5%, 25%, 50% and 100% *Jwaramurari Rasa* significantly stimulated

neutrophils in blood samples to ingest candida albicans, thus promoted the phagocytosis.
ii) Mean particle number per neutrophils engulfed by neutrophils in blood samples with 5%(2MPN), 12.5%(2MPN), 25%(4MPN), 50%(4MPN) and100%(5MPN) was noted. This was equal to that of standard 5.
iii) Highly Significance was seen in 100% of the comparisons.

3. Candidacidal assay:
i) 5%, 12.5%, 25%, 50% and 100% of *Jwaramurari Rasa* significantly stimulated neutrophils in samples separately for candidacidal activity.
ii) % of candida cells killed with *Jwaramurari Rasa* 5%(18%), 12.5%(18%), 25%(30%),50%(30%) and 100%(39%).
iii) In candidacidal activity of *Jwaramurari Rasa* with 100% was highly significant, 25%and 50% was significant and 5% and 12.5% was non-significant compared to controlwithout drug.

4. Neutrophil locomotion and chemotaxis test:
i) 5%, 12.5%, 25%, 50% and 100% of *Jwaramurari Rasa* significantly stimulated neutrophils in blood samples to migrate for neutrophil locomotion and chemotaxis test.
ii) Distance of migration by neutrophils towards *Jwaramurari ras* 5%(0.8mm), 12.5%(1.0mm), 25%(2.0mm), 50%(2.0mm) and 100%(2.3mm)
iii) Highly significance was seen in 100% with control, non significance was seen in all other comparisons

From results significant Immuno-modulatory activity of *Jwaramurari Rasa* was observed. In this study from the different methods results obtained clearly shown immuno-modulatory activity of *Jwaramurari Rasa. Jwaramurari Rasa* as the name indicates towards promotion and protection of health which is witnessed with this scientific study and given further scope for clinical studies.

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Conflict of interest- None
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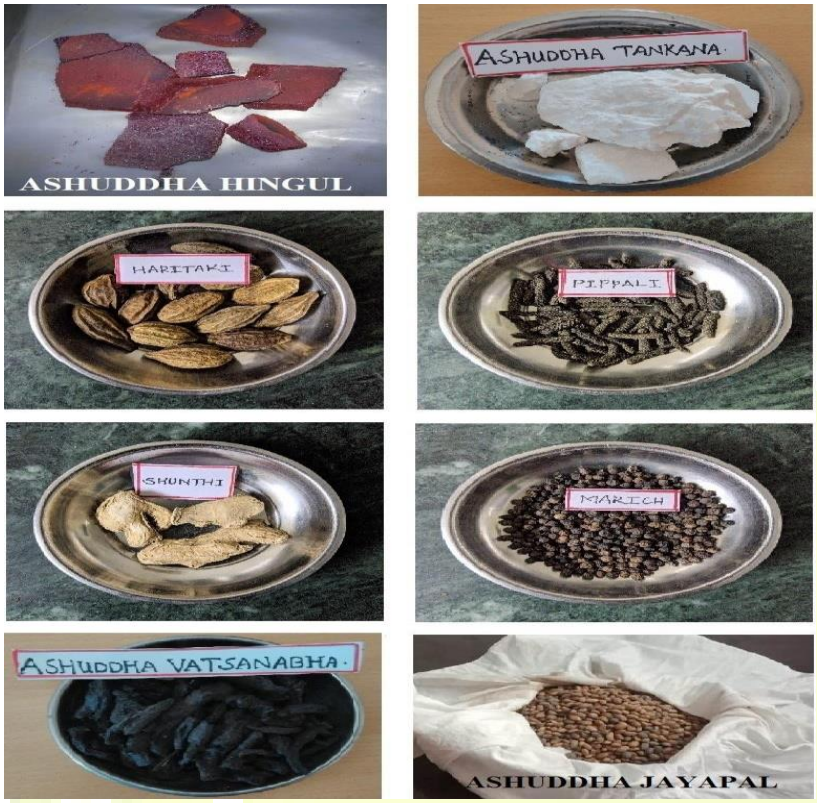
RAW INGREDIENTS OF JWARAMURARI RASA



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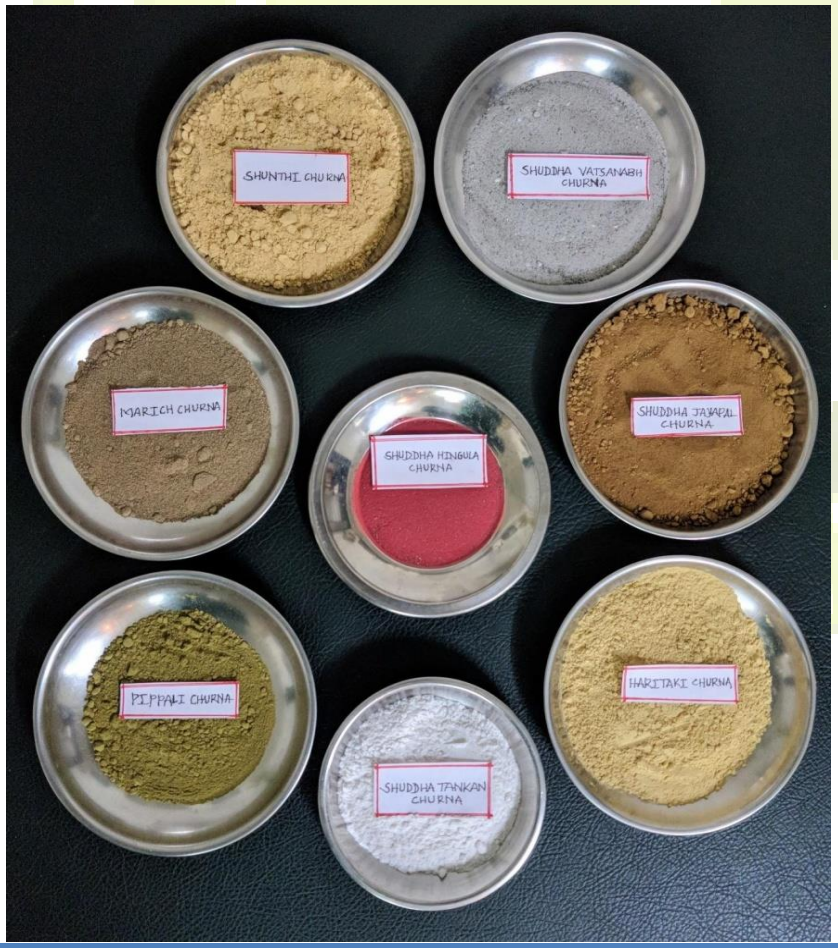
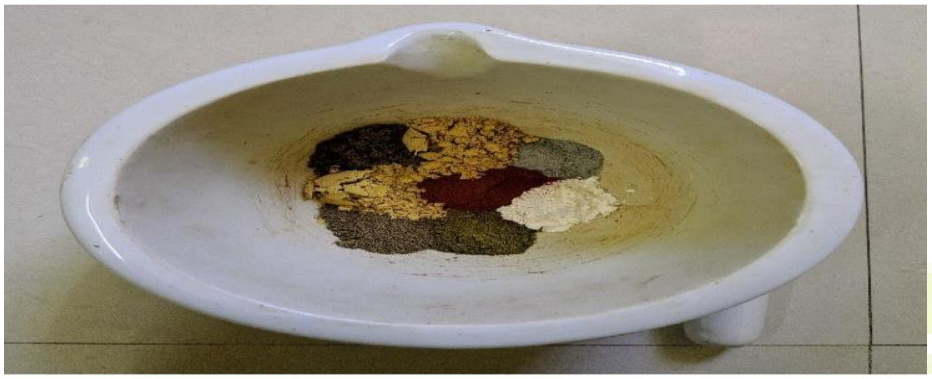
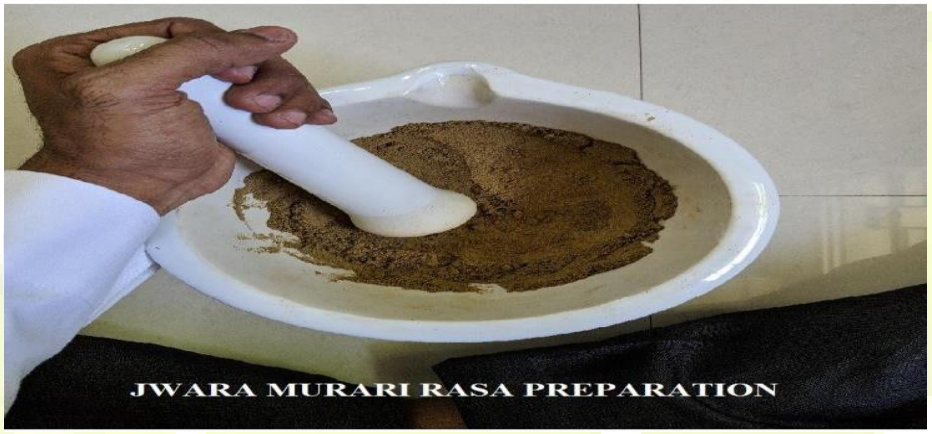


PREPARATION OF JWARAMURARI RASA





SCREENING OF FREE RADICAL SCAVENGING ACTIVITY ESTIMATION OF SOD, MDA, GSH, AND CATALASE