AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

ANTIMICROBIAL ACTIVITY OF *MANJISTHADI AGADA* AND *SHIRISHADI AGADA*: AN IN VITRO STUDY

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Article info

Article History:
Received: 15-04-2026
Accepted: 14-05-2026
Published: 08-07-2026

KEYWORDS:
Manjisthadi Agada, Shirishadi Agada, Ayurvedic Formulation, Antibacterial Activity, Antifungal Activity, Zone of Inhibition.

ABSTRACT

Antimicrobial resistance is a growing global health concern resulting from the irrational and excessive use of antibiotics, thereby reducing the effectiveness of many antimicrobial agents. *Ayurveda* offers several herbal formulations with potential antimicrobial properties. Among them, *Manjisthadi Agada* and *Shirishadi Agada* are traditionally indicated for their *Vishaghana* (antitoxic), *Krimighna* (antimicrobial), and *Shothahara* (anti-inflammatory) actions. **Objective:** To evaluate the antimicrobial activity of *Manjisthadi Agada* and *Shirishadi Agada*. **Materials and Methods:** An in vitro study was conducted using three microbial strains. The antimicrobial activity of both formulations was assessed by measuring the diameter of the zone of inhibition produced against the test microorganisms. **Results:** *Shirishadi Agada* demonstrated superior antimicrobial activity compared to *Manjisthadi Agada*. It exhibited a 12.5 mm zone of inhibition against the Gram-positive bacterial strain, whereas *Manjisthadi Agada* showed only a 2.5mm zone. Against Escherichia coli, *Shirishadi Agada* produced a 16.25mm inhibition zone compared to 11.5mm for *Manjisthadi Agada*. In antifungal testing against Candida albicans, *Shirishadi Agada* showed a larger inhibition zone (26.50mm) than *Manjisthadi Agada* (24.50mm). **Conclusion:** *Shirishadi Agada* exhibited broader and stronger antibacterial and antifungal activity, while *Manjisthadi Agada* showed comparatively weaker and selective antimicrobial effects.

INTRODUCTION

Agadas are herbal compounds used in the treatment of *Sthavar* (plant origin poison) and *Jangama visha* (animal origin poison). Plants are used as medicine since time immemorial. The antimicrobial property of various plant part like leaves, seeds, fruits have been well documented for some of the medicinal plants for the recent decade. In Bharat Bhaisajya Ratnakar one of *Agada* named "*Manjisthadi Agada*" is described in *Makaradimishraprakrana* which act on a *Sthavar* and *Jangama visha* (poison) [1].

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	https://doi.org/10.47070/ayushdhara.v13i3.2790

Shirishadi Agada is mentioned in Ashtanga Hridaya - *Uttar sthana* for the *Sthavar* and *Jangama visha* [2]. In the present era, the emergence of antimicrobial resistance has become a major global health concern. The irrational and excessive use of antibiotics has led to the development of resistant strains of microorganisms, reducing the efficacy of available drugs [3]. Therefore, there is an urgent need to explore safe, effective, and affordable alternatives from natural sources, particularly from Ayurveda, which offers a rich heritage of herbal formulations with proven antimicrobial potential. Among the classical Ayurvedic formulations, *Manjisthadi Agada* and *Shirishadi Agada* are described for their *Vishaghana* (antitoxic), *Krimighna* (antimicrobial), and *Shothahara* (anti-inflammatory) properties. Both formulations contain herbs with documented pharmacological activities- such as *Manjistha* (Rubia cordifolia), *Shirisha* (Albizia

lebbeck), *Haridra* (Curcuma longa) etc, which have shown promising results against microbial infections in various studies.

MATERIAL AND METHOD

Collection and authentication of test drug

All contents of *Manjisthadi Agada* and *Shirishadi Agada* were collected from local market and its surroundings. Authentication was done at well-known authorised institute. All ingredients were cleaned and cut into small fragments. Fragmented plant material was spread on the paper and kept under sunlight for drying procedure. After drying, all ingredients are pulverised separately with the help of grinder. Later, powdered form of plant material is filtered with the help of siew no. 60 for fine powder (*Churna*) and better result. All fine powdered ingredients were taken in the equal quantity as prescribed in the text. Then, with the help of *Khalva* and *Peshani* all ingredients were mixed properly. Final formulation of drug was sent to well-known authorised institute for standardisation process. After the standardisation, it was used for the antimicrobial activities.

Table 1: Ingredients of *Manjisthadi agada*

S.No.	Name of drug	Latin name [4]	Family	Part used	Quantity
1.	*Manjistha*	Rubia cordifolia	Rubiaceae	Roots	1 part
2.	*Ela*	Elettaria cardamomum	Scitaminae	Seeds	1 part
3.	*Draksha (Mrudvika)*	Vitis vinifera	Vitaceae	Fruits	1 part
4.	*Nisha*	Curcuma longa	Scitaminae	Rhizome	1 part
5.	*Jatamansi*	Nordostachys jatamanshi	Valerianaceae	Rhizome	1 part
6.	*Yashtimadhu*	Glycerrhiza glabra	Leguminosae	Roots	1 part
7.	*Harenuka*	Vitex Agnus Castus	Verbenaceae		1 part
8.	*Kshaudra (Madhu)*	-	-	As it is	

Table 2: Ingredients of *Shirishadi agada*

S. No.	Name of drug	Latin name [5]	Family	Part used	Quantity
1.	*Arka ksheer*	Calotropis procera	Asclepiadaceae	Latex	1 part
2.	*Shirisha*	Albizzia lebbeck	Mimosoideae	seeds	1 part
3.	*Pippali*	Piper longum	*Piperaceae*	Fruits	1 part

Experimental antimicrobial strains

Strains of microbes were used for the antimicrobial evaluation. They were as follows:

1. Gram positive bacteria: *Staphylococcus aureus* [6]
2. Gram negative bacteria: *Escherichia coli* [7]
3. Fungus: *Candida albicans* [8]

All above strains were collected from authorised centre. The parts of the drugs which was used for formulation identified by literature comparison and then confirmed by well-known institute.

Experimental study design

An in-Vitro study was conducted followed by 3 strains of microbes were taken for evaluation of antimicrobial activity. *Manjisthadi Agada* and *Shirishadi Agada* were taken to carried out study. The assessment of antimicrobial activity was based on the measurement of diameter of inhibition zone formed.

Study Design

Pre–clinical study / Antimicrobial study: In-Vitro experiment

- Nutrient Broth was prepared using manufactures instructions. After autoclaving at 121°C for 15 minutes, 0.1 ml of each pure culture was pipetted into the media.
- *Staphylococcus aureus, Escherichia coli and C albicans* were incubated in respective media and incubated for 24 hours at 28 ^{0}C for the growth.
- Desired turbidity of each bacterial and fungal suspensions was adjusted to 1.5 x10^8 CFU/mL using 0.5 McFarland standard.
- Gentamicin 100mcg and Norfloxacin were used as standard
- Amphotericin 10 mcg was used as standard for antifungal activity.
- Mueller Hinton Agar plates were prepared with reference to the manufacturer's instructions [9,10].
- Media was poured aseptically (20-25) ml into sterile Petri plates and allowed it

- A sterile swab was dipped into the suspension. The entire surface of the agar plate was then swabbed evenly to ensure a uniform growth of organisms.
- The process was repeated once more to ensure an even distribution of the inoculum. After the plates dried, they were divided into four equal quadrants. A hole was then bored at the centre of each quadrant on the agar surface using a sterile cork borer.
- The test compounds were serially diluted and 0.1ml of each test solution is the added to the respective well. Without inverting, the petri plates were kept for incubation at 37ºC for 24 hours.
- After the observation period of 24 hours of the plates for zones of inhibition, the diameter of the clear zone using a scale was measured.

Shirishadi Agada- exhibited better greater zone of inhibition of 12mm than *Manjisthadi Agada* wherein minimum inhibition of 2.5 was observed. Thus, *Shirishadi Agada* thereby showed better antibacterial activity. *Shirishadi Agada*- showed highest zone of inhibition of 16.25mm against E coli bacteria, whereas *Manjisthadi agada* elicited of 12mm zone of inhibition indicating lesser antibacterial activity than *Shirishadi Agada*. Thus, *Shirishadi Agada* thereby demonstrated to showed good antibacterial activity against E coli. *Shirishadi Agada*- For antifungal activity evaluation, showed highest zone of inhibition of 27mm against *Candida albicans*, whereas *Manjisthadi agada* showed lower zone of inhibition 25 than *Shirishadi Agada*. Thus, antifungal activity of *Shirishadi Agada* was better than *Manjisthadi agada* against *C albicans*.

OBSERVATION AND RESULTS

Table 3: Antibacterial activity against Staphylococcus aureus

Name of *Agada*	Experiment 1 Zone of inhibition (mm)	Experiment 2 Zone of inhibition (mm)	Mean Zone of inhibition (mm)
Manjisthadi agada- Test Sample A	2	3	2.5
Shirishadi Agada- Test Sample B	12	13	12.5

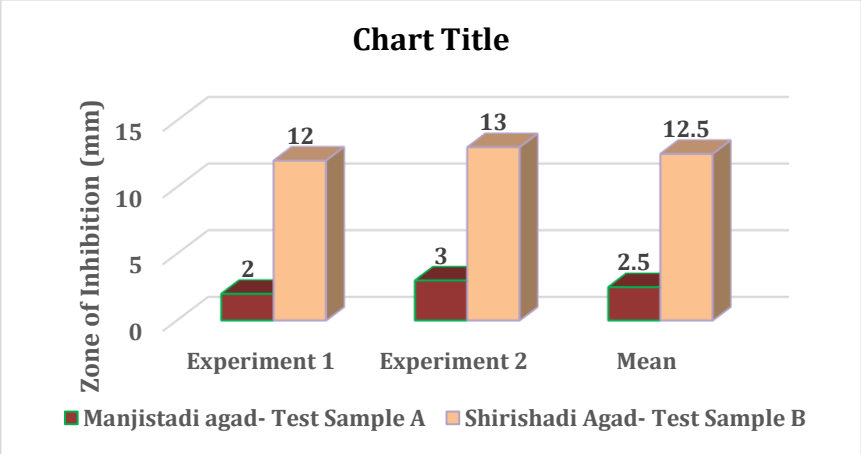


Table 4: Antibacterial activity against E. coli

Name of *Agada*	Experiment 1 Zone of inhibition (mm)	Experiment 2 Zone of inhibition (mm)	Mean Zone of inhibition (mm)
Manjisthadi agada- Test Sample A	11	12	11.5
Shirishadi Agada- Test Sample B	16	16.5	16.25

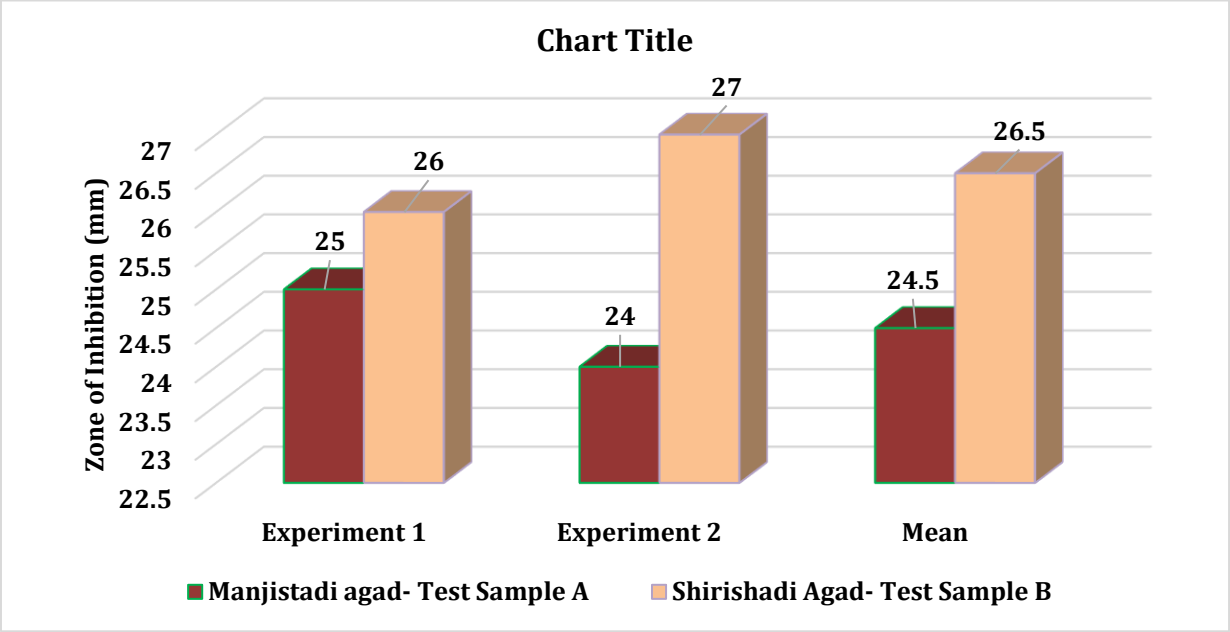
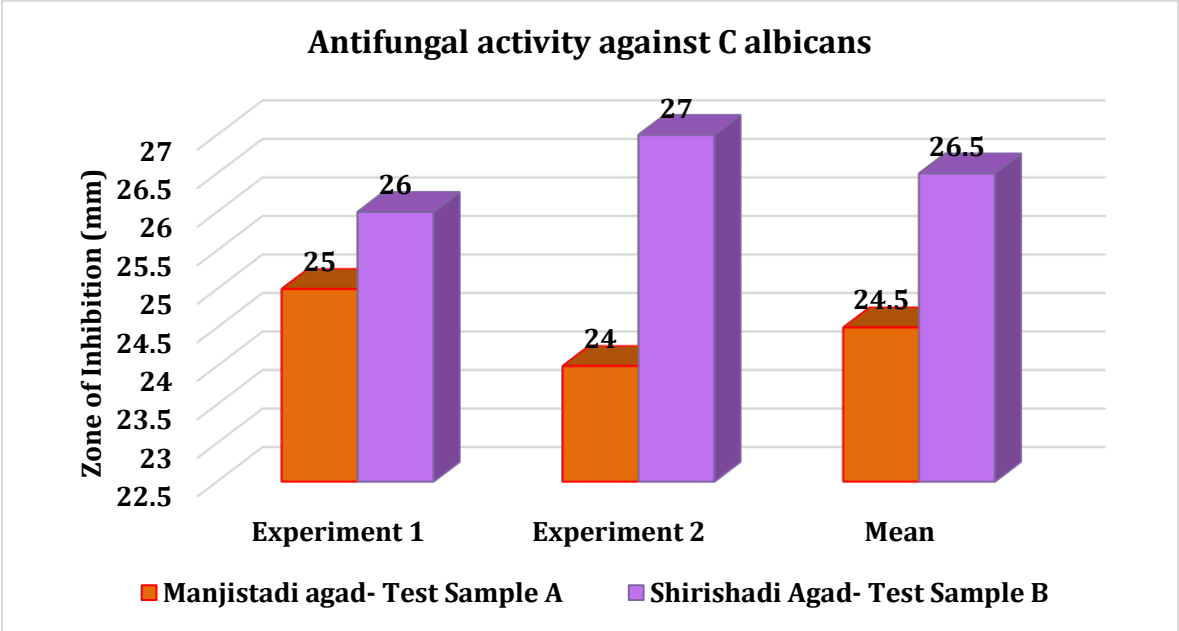


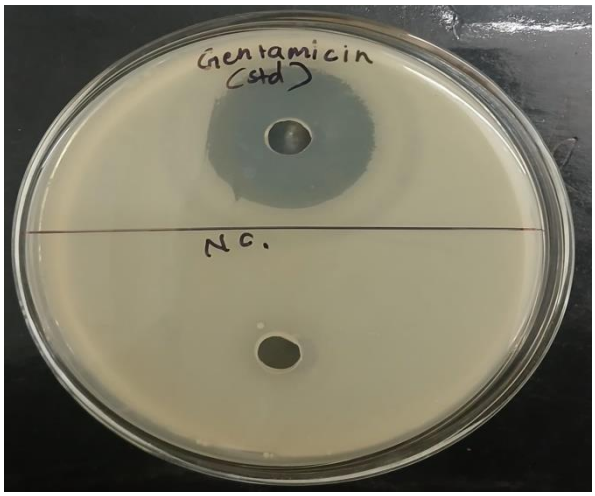
Table 5: Antifungal activity against C albicans

Name of *Agada*	Experiment 1 Zone of inhibition (mm)	Experiment 2 Zone of inhibition (mm)	Mean Zone of inhibition (mm)
Manjisthadi agada- Test Sample A	25	24	24.50
Shirishadi Agada- Test Sample B	26	27	26.50

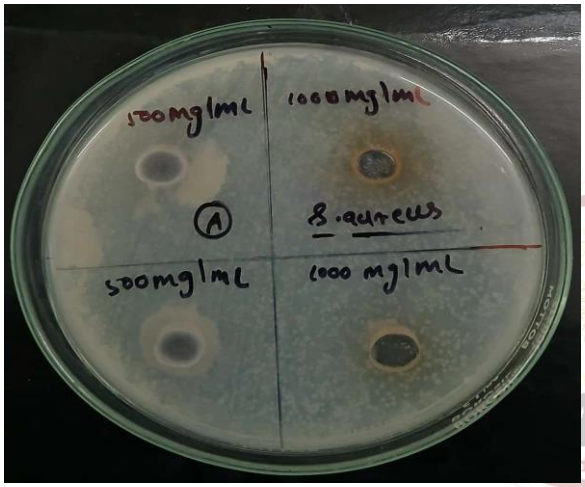


OBSERVATION

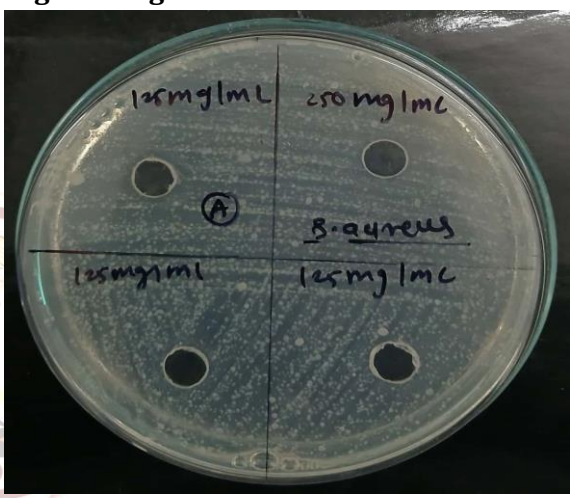
Standard



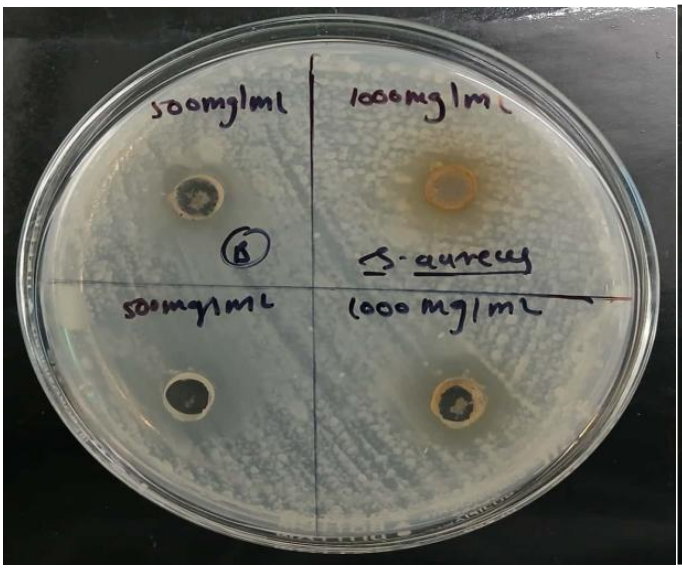
Standard – Gentamicin 100mcg and Negative control



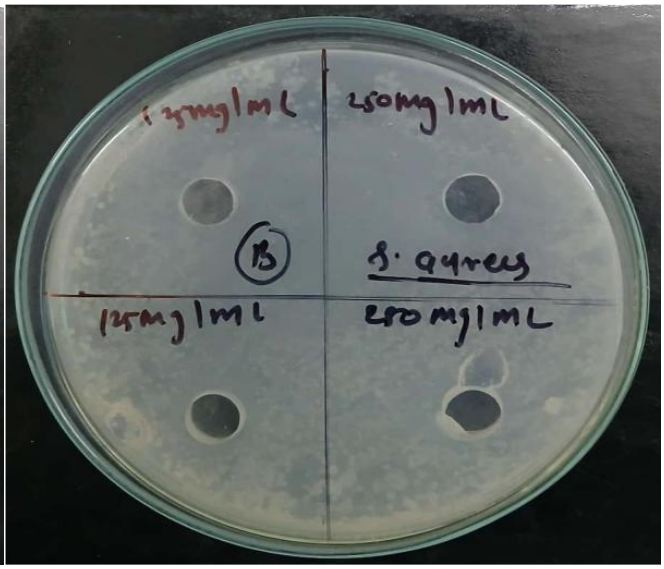
Against ***Staphylococcus aureus*** at 200mg/ml, 100mg/ml, 50mg/ml, 25mg of *Manjisthadi agada*



Against Staphylococcus aureus at 12.5mg/ml, 6.25mg/ml, 3.12mg/ml and 1.56mg/ml of *Manjisthadi agada*



Against Staphylococcus aureus at 1000mg/ml and 500mg/ml of *Shirishadi agada*



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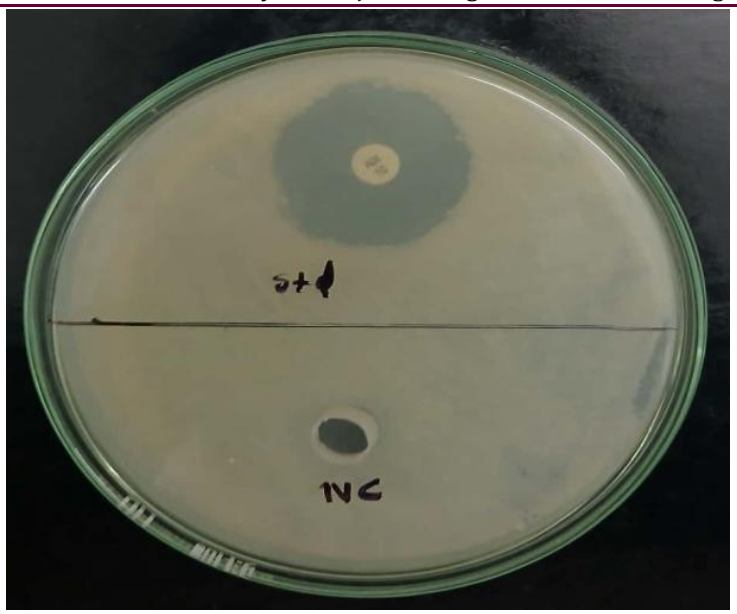
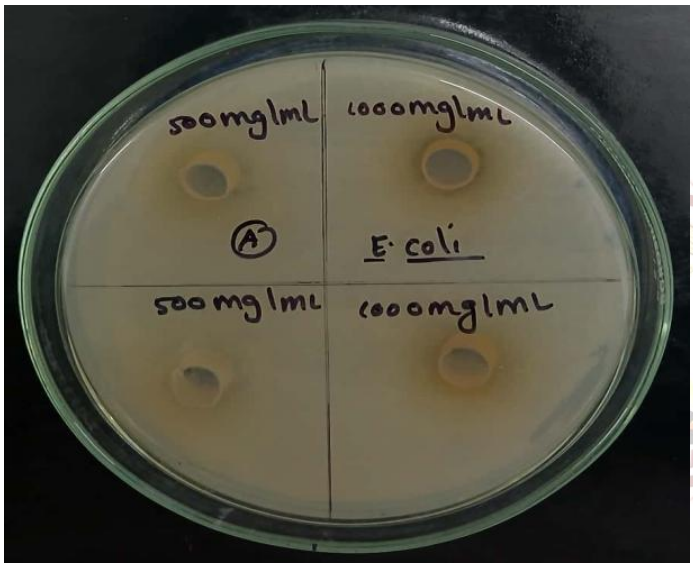
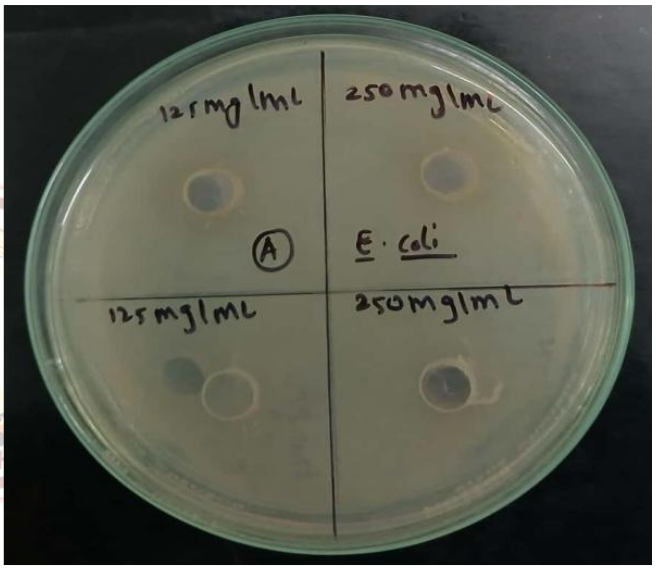


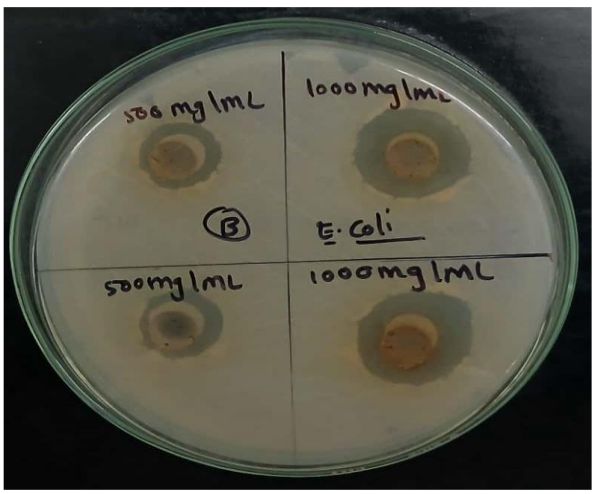
Standard – Norfloxacin 10mcg and Negative control



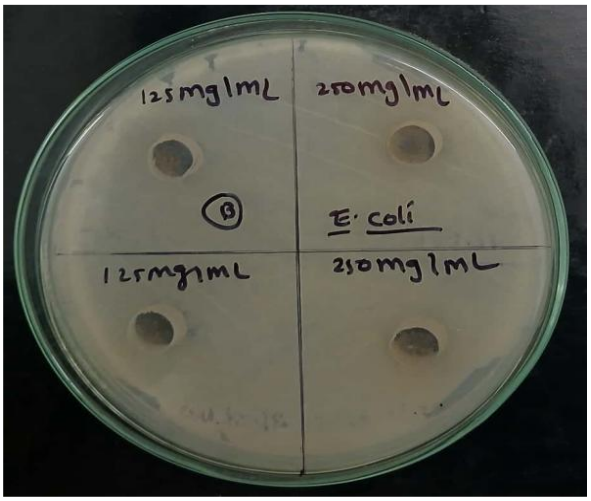
Against Escherichia coli at 1000mg/mL and 500mg/mL of Manjisthadi agada



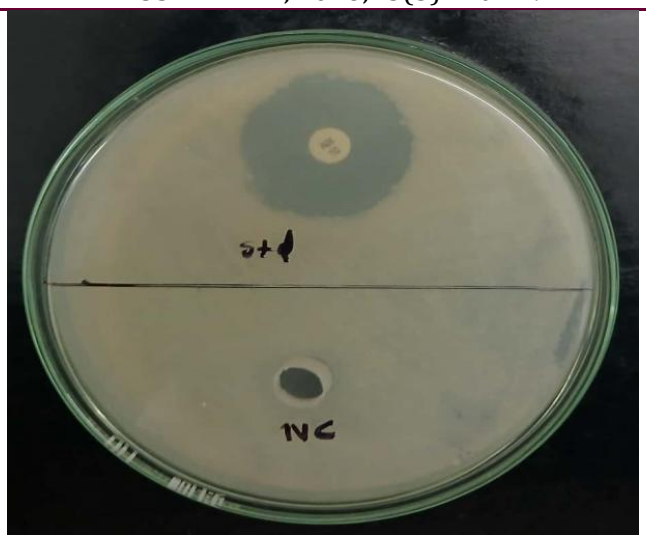
Against Escherichia coli at 250mg/mL and 125mg/mL of Manjisthadi agada



Against Escherichia coli at 500 mg/mL, 1000mg/mL of Shirishadi agada

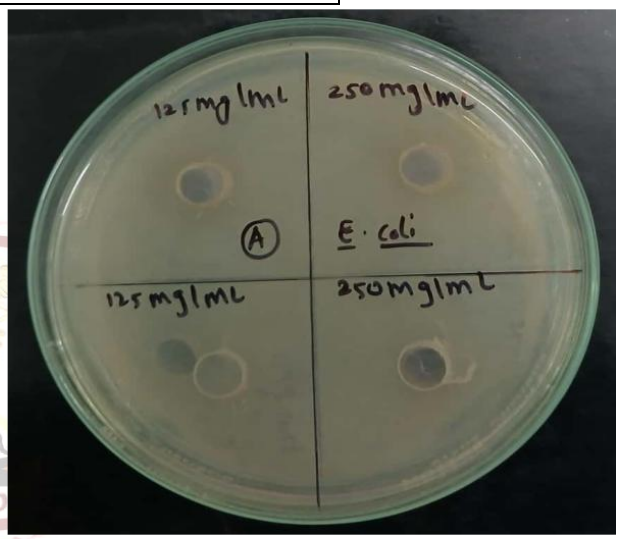


Against Escherichia coli at 250mg/mL and 125mg/mL of Shirishadi agada

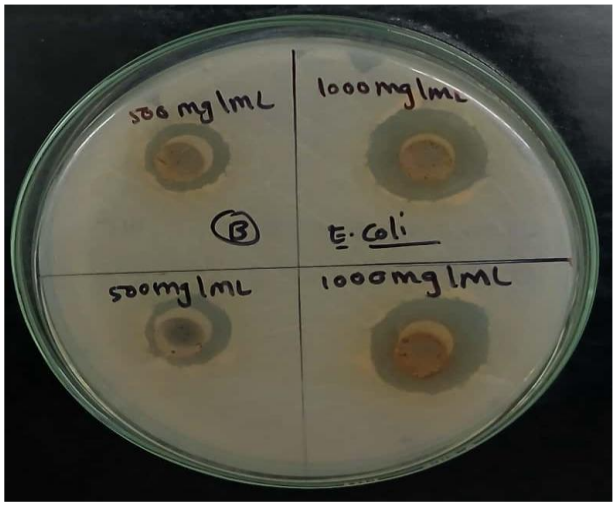


Standard – Norfloxacin 10mcg and Negative control

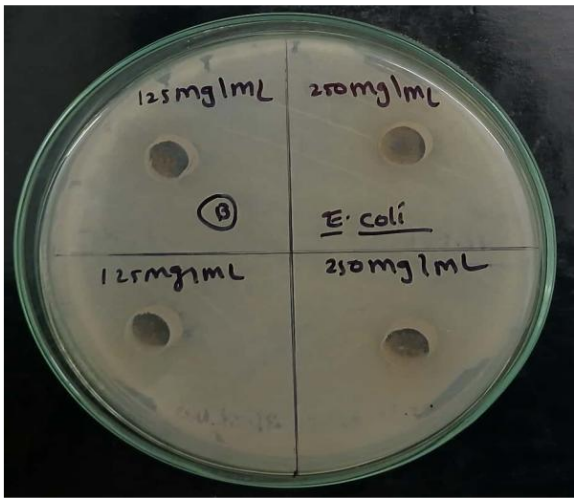
Against ***Escherichia coli*** at 1000mg/mL and 500mg/mL of *Manjisthadi agada*



Against ***Escherichia coli*** at 250mg/mL and 125mg/mL of *Manjisthadi agada*

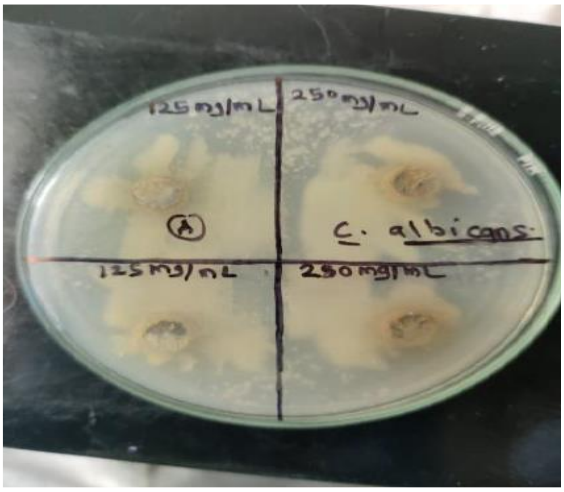


Against Escherichia coli at 500 mg/mL, 1000mg/mL of Shirishadi agada

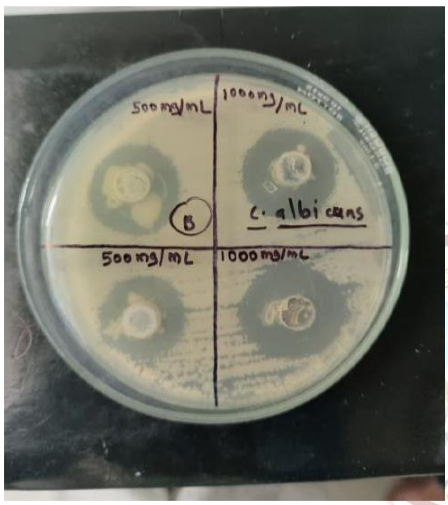


Against Escherichia coli at 250mg/mL and 125mg/mL of Shirishadi agada

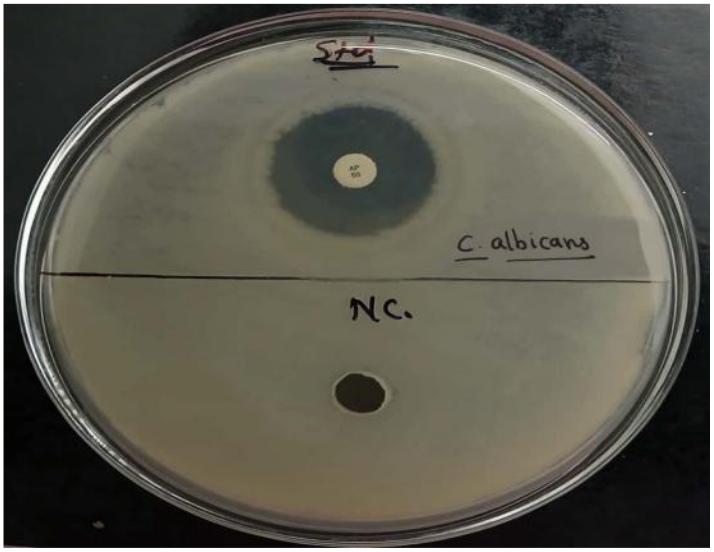
Antifungal activity against C albicans



Against ***C albicans*** at 500 mg/mL, 1000mg/mL of *Manjisthadi agada*	Against ***C albicans*** at 125 mg/mL, 250 mg/mL of *Manjisthadi agada*



Against ***C albicans*** at 500 mg/mL, 1000mg/mL of *Shirishadi agada*	Against ***C albicans*** at 125 mg/mL, 250 mg/mL of *Shirishadi agada*



Against C albicans at 10 mcg, of Amphotericin

DISCUSSION

Infectious diseases are one of the growing problems in recent era. Morbidity and mortality rate increase day by day. Infectious diseases are caused due to pathogenic organisms like bacteria, viruses, fungi or other parasites which may spread directly or indirectly from one person to another or from infected animals to humans. Among the entire population of world more than 20 to 25% children less than 5 years are dead due to diarrhoea which is caused by gram-negative bacteria *E. coli*.

Antibiotics are used against bacterial infection and fungicidal medicines are used against fungal infection. While, since last few decades, the use of antibiotics is less beneficial against certain illness may be due to their adverse reaction. Also, due to frequent usage of this medication resulting into drug resistance to bacteria. So, more antibiotics are used, the more likely they are to become non-infective for managing critical infectious conditions. Another major cause of reduction in the uses of higher antibiotics is harmful effect on human health like gastric ulcer, diarrhoea, vomiting, kidney or liver damage etc. sometimes antibiotics are interacting with other medicines which may leads to toxic effect on health.

It is essential to study newer drugs along with lesser resistance. Nowadays, herbal drugs play an important role in the prevention and management of various diseases. In various developing countries, traditional or Ayurvedic medicines are used since antient time. Herbal drugs and their uses in traditional medicines as well as curative potentials are well documented in literature. Other major problem related to human health is chronic and degenerative diseases of vital systems like neurodegenerative diseases, DM, cardiac diseases, aging, etc. which is caused due to oxidative stress. So, in recent era, there is need of study of antimicrobial activities of herbal medicine for better results.

Many Ayurvedic formulations are used to treat acute as well as chronic illness. In Ayurvedic literature, *Agadas* (antidotes) are used to treat animal as well as plant origin poisoning. As infectious diseases caused by toxins of various bacteria, Previous work shows that antimicrobial activities of individual herbal drugs as well as compound formulations from Ayurvedic literature. The previous researches also show that some of the individual ingredient of *Manjisthadi agada* and *Shirishadi agada* have potential antimicrobial activities. So, we can use various *Agadas* for the management of such diseases, as it acts as a counter poison. *Manjisthadi agada* and *Shirishadi agada* are used in the management of *Sthavara* and *Jangam visha*. So, for evaluation of antimicrobial property of both the *agadas* present laboratory study had been selected. The synergistic action of multiple ingredients supports the Ayurvedic principle of *Samsarga* (synergistic enhancement), where the combination of herbs produces a more potent therapeutic effect than individual drugs.

Shirishadi agada is active against both Gram-positive and Gram-negative bacteria and C albicans in a dose-dependent and reproducible manner and thereby *Shirishadi agada* demonstrated broader and stronger antibacterial and antifungal activity. *Manjisthadi agada* displayed weak and selective antibacterial activity, only effective against E. coli at high concentration. These variations may be due to the phytochemical composition and polarity of active constituents.

CONCLUSION

Present laboratory study following conclusion drawn from the literature, previous work and observations. These were as follows:

Due to frequent use of higher antibiotics, drug resistance to bacteria is occurred to certain illness. So, there is need of newer drugs with higher efficacy and low resistance.

Bacterial infections occurred due to toxins produced by bacteria, so can be corelated with poisoning. In the management of poisoning, various *Agadas* are used. As poisoning as well as bacterial infection are formed due to toxins, *Agadas* are also useful in the management of bacterial infection.

Shirishadi Agada is active against both Gram-positive and Gram-negative bacteria and C albicans in a dose-dependent and reproducible manner and thereby *Shirishadi Agada* demonstrated broader and stronger antibacterial and antifungal activity. *Manjisthadi agada* displayed weak and selective antibacterial activity, only effective against E. coli at high concentration. This study has experimentally validated the antimicrobial activity of *Manjisthadi Agada* and *Shirishadi Agada*.

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Cite this article as:
Hardik V. Solanki, Savita C. Samleti, S. M. Lahankar, Shreyali Sharma. Antimicrobial Activity of Manjisthadi Agada and Shirishadi Agada: An In Vitro Study. AYUSHDHARA, 2026;13(3):140-149.
https://doi.org/10.47070/ayushdhara.v13i3.2790
Source of support: Nil, Conflict of interest: None Declared

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