

IN-VITRO ANTIMICROBIAL ACTIVITY OF ARDRAKA AVALEHA

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ABSTRACT

Antimicrobial activity is a process by which response of an organism to a drug or crude extract can be evaluated. There may be wide variations in the susceptibility of different strains of the same bacterial species to antibiotics or to a drug. Several tests are now available for determination of bacterial sensitivity to antimicrobial agents. *Avaleha* being one of the common dosage forms, is extensively used in *Pranavaha Srotodushthi Vikaras*. *Ardraka Avaleha*, explained in *Yoga Ratnakar- Kasa Chikitsa Adhyaya* is indicated in the treatment of *Pranavaha Srotodushthi Vikaras*. The ingredients of *Ardraka avaleha* are having the properties like *Balya*, *Rasayana*, *Krimighna* and *Kaphahara*. *Loha Bhasma* is *Ayuprada*, *Bala veeryakarta* and is a *shrestha Rasayana*. Hence an attempt was made to evaluate the antimicrobial effect of *Ardraka Avaleha*.

KEYWORDS: *Ardraka Avaleha*, Disc diffusion method, MIC method, Anti-microbial activity

INTRODUCTION

Diseases related to the respiratory system are considerably increasing in developed and developing countries and has become one of the reason of morbidity in this era. Air pollution, constant climatic variations, exposure to smoke and dust are the major causes for respiratory diseases¹. Now a days, indiscriminate, irrational use of antimicrobial drugs has lead to multiple drug resistance and has posed a great challenge to the medical and pharmaceutical fraternity for inventing newer molecules/ medicines. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitive, immuno suppression and allergic reactions². Ayurveda presents with lots of medicines for such kind of problems where these medicaments not only cure diseases but also

modulate immunity to combat various infectious diseases. This has lead to upsurge in usage of Ayurvedic medicines. *Avaleha* being one of the common dosage forms is extensively used in *Pranavaha Srotodushthi Vikaras*³. *Ardraka Avaleha*, a formulation explained in “*Yoga Ratnakar*”⁴ in the context of *Kasa Chikitsa Adhyaya* is used in the treatment of *Pranavaha Srotodushthi Vikaras*. The ingredients of *Ardraka avaleha* are having properties like *vata kaphahara*, *Ushna veerya*, *Bhedhana karma*, *Shrotomukha shodhaka*, *Krimighna*, *Balya*, *Rasayana* thus facilitates in clearing *margavarana* caused by *kapha* during an episode by providing easy movement of *Vayu*. *Loha Bhasma* is *Ayuprada*, *Bala veeryakarta* and is a *shrestha Rasayana*⁵.

In present study, Ardraka Avaleha, was evaluated for its antimicrobial properties against Gram-positive, Gram-negative and fungi which are the commonest pathogens causing upper respiratory tract infection by in-vitro method.

Aims & Objectives of the Study:

1. To Prepare Ardraka Avaleha according to classics⁶.
2. In-vitro Antimicrobial activity of Ardraka Avaleha

Materials and Methods:

-The raw materials required for the preparation of Ardraka Avaleha were

procured from reliable sources and authenticated from CRL, KLE's Ayush approved drug testing laboratory Belagavi, Karnataka.

-Preparation of Ardraka Avaleha was prepared at the BVVS Ayurvedic Medical College and Hospital, Bagalkot-Karnataka.

-Antimicrobial study was carried out at Maratha Mandal's Dental College and Research Institute, Belagavi. Karnataka.

Ingredients of Ardraka Avaleha:

S.N	Drug	Latin Name and Family	Part used	Qt
1	Ardraka	Zingiberofficinale Roscoe /Zingiberaceae	Rhizome	625 gm
2	Dhanyaka	Coriandrumsativum Linn / Apiaceae	Fruit	25 gm
3	Jeeraka	Cuminumcyminum Linn / Apiaceae	Seed	25 gm
4	Tejapatra	CinnamomumtamalaNees / Lauraceae	Leaves	25 gm
5	Ela	Elettariacardmomum Linn / Zingiberaceae	Seed	25 gm
6	Tvak	CinnamomumzeylanicumBl / Lauraceae	Bark	25 gm
7	Ajamoda	Apiumgraveolens Linn/ Apiaceae	Fruit	25 gm
8	Musta	Cyperusrotundus Linn / Cyperaceae	Rhizome	25 gm
9	Guda	Jaggery	Whole	625 gm
10	Loha Bhasma	Iron Oxide (Fe ₂ O ₂)	-	25 gm

Pharmaceutical Study:

Ardraka Avaleha was prepared according to general method of preparation of Avaleha.

-Firstly the freshly collected swarasa from Ardraka was taken into clean stainless steel vessel.

-To this, pounded jaggery was added and heated over mandagni till it gets dissolved completely. It is then filtered to remove physical impurities. The whole procedure was carried out on mandhagni.

-Heating was continued till appearance of proper paka laxanas i.e. Tantumatvam, Apsumajjati and Gandha varna rasodhbhava⁶.

-After ensuring proper paka laxanas, churna and prakshepaka dravyas are mixed and stirred well to attain homogenous mixture.

-Thus prepared Avaleha was tested for pakanantar pariksha i.e. Avaleha siddhi pariksha. If a small portion of the avaleha is poured in a cup filled with water, it should remain stable in water without getting dissolved in water immediately.

-Thus prepared avaleha was allowed for swangasheeta. After swangasheeta, madhu was added and mixed thoroughly.

-Later Ardraka Avaleha was filled in wide mouthed, air tight glass container and stored.

Antimicrobial Study:

Antimicrobial activity is a process by which response of an organism to a drug or crude

extract can be evaluated. There may be wide variations in the susceptibility of different strains of the same bacterial species to antibiotics or to a drug. Ardraka Avaleha, was evaluated for its antimicrobial properties against Gram-positive, Gram-negative and fungi which are the commonest pathogens causing upper respiratory tract infection by in-vitro method. Two tests that are most commonly used are disk diffusion and agar or broth dilution tests.

The disk diffusion test⁷:

This technique is simple to perform and relatively inexpensive. It provides only qualitative or semi-quantitative information on the susceptibility of a given microorganism to a given antibiotic. The test is performed by applying commercially available filter paper disks impregnated with specific quantities of the drug on the surface of the agar plates over which a culture of the microorganism has been streaked. After 18 to 24 hrs of incubation, the size of a clear zone of inhibition around the disk is determined; this is related to the sensitivity of the drug against the test strain. Standards for sensitivity vary for each microorganism and they are based on concentration of the drug that can be achieved safely in plasma without producing toxicity.

Serial Dilution tests⁸:

In the serial dilution technique, the graded doses of test substances are incorporated in to broth and the tubes inoculated with test organism are incubated.

Employ antibiotics in serially diluted concentrations in solid agar or broth media containing a culture of the test microorganism. The lowest concentration of the

agent that prevents visible growth after 18 to 24 hrs of incubation is known as the Minimal Inhibitory Concentration (MIC) and the lowest concentration that result in 99.9% decline in bacterial members is known as the Minimal Bactericidal Concentration (MBC). The value of the MBC as a clinical test has not been established, but it may be useful in special instances where very precise knowledge of the ability of a given anti-microbial agent to kill a specific clinical isolate is critical, as in the therapy of bacterial endocarditis. The dilutions can be made in broth or in agar.

Test Organisms⁹:

-Gram-positive bacteria- *Staphylococcus aureus* and *staphylococcus, epidermidis*

-Gram-negative bacteria- *Klebsiella* and *E.coli*

-Fungus- *Candida albicans* and *Aspergillus niger*

Assessment of Antimicrobial Activity by Agar Disc Diffusion Method:

The screening of antimicrobial activity of Ardraka Avaleha was carried out using agar well diffusion method. The antimicrobial strains were inoculated in nutrient broth and incubated at 37°C overnight. The culture was then adjusted to 0.5 McFarland turbidity standards. Lawn culture of the test organism was made on the Brain Heart Infusion agar plates using a sterile cotton swab, and the plates were dried for 15min. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations (75µg / ml, 50 µg / ml, 25 µg / ml, 10µg/ml, 5µg/ml). Then the extract was introduced into the wells with the help of micropipettes. The culture plates were made stand on the working bench for 30 min for pre-diffusion

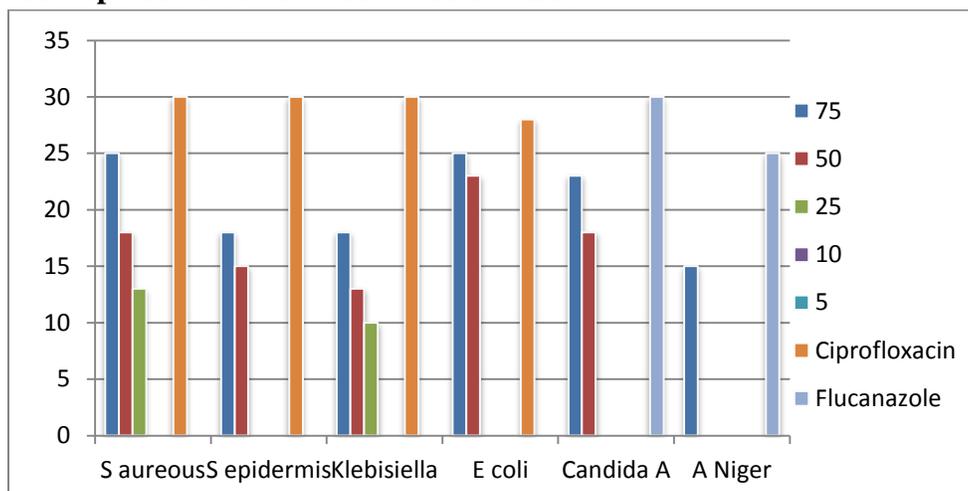
and were incubated in an upright position at 37°C for 24 hrs. After 24 hrs, antimicrobial activity was determined by measuring the diameter of zones of inhibition. Standard antimicrobial discs of Ciprofloxacin and

Fluconazole were used as positive control. All the tests were done in triplicate to minimise the error.

Result of Disc Diffusion method:

Organisms	75µg/ml	50	25	10	5	Ciprofloxacin	Fluconazole
<i>S aureus</i>	25mm	18mm	13mm	R	R	30mm	-
<i>S epidermis</i>	18mm	15mm	R	R	R	30mm	-
<i>Klebisiella</i>	18mm	13mm	10mm	R	R	30mm	-
<i>E.coli</i>	25mm	23mm	R	R	R	28mm	-
<i>Candida</i>	23mm	18mm	R	R	R	-	30mm
<i>A niger</i>	15mm	R	R	R	R	-	25mm

Graphical representation of Disc Diffusion results



Assessment of Antimicrobial Activity by MIC Method:

Minimum inhibition concentration (MIC) procedure⁷¹ also called as Serial dilution method. In this a stock solution of Antimicrobial agent to be tested is prepared in distilled water. Two fold dilutions of this solution is prepared in Brain Heart Infusion broth. A standard suspension of the

organism is inoculated into the medium with one Antimicrobial agent- free medium as control. The inoculated media are inoculated at 35⁰-37⁰C for 18-24 hrs and examined for growth. MIC is taken as the lowest concentration of Antimicrobial agent which completely inhibits the growth.

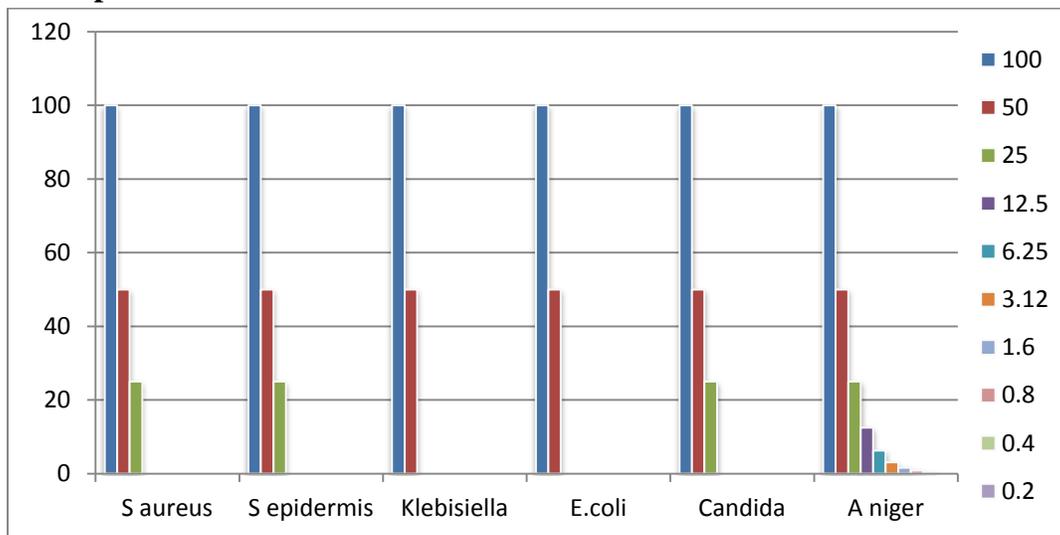
Result of MIC method

Organisms	100mg/ml	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
<i>S aureus</i>	S	S	S	R	R	R	R	R	R	R
<i>S epidermis</i>	S	S	S	R	R	R	R	R	R	R
<i>Klebisiella</i>	S	S	R	R	R	R	R	R	R	R
<i>E.coli</i>	S	S	R	R	R	R	R	R	R	R

<i>Candida</i>	S	S	S	R	R	R	R	R	R	R
<i>A niger</i>	S	S	S	S	S	S	S	S	S	R

Note: S- Sensitive, R- Resistant, NG- No Growth

Graphical representation of MIC results



Interpretation:

For Disc diffusion method-

The zone of inhibition is 30mm for the standard drug Ciprofloxacin against *S aureus*, *S epidermis* & *Klebsiella* and 28mm for *E.Coli*. The zone of inhibition is 25mm for the standard drug Fluconazole against *Candida* and 28mm for *A niger*. The zone of inhibition of *S aureus* is 25mm, 18mm, 13mm and resistant with the next two dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually reduces as the dilution decreases and it becomes resistant at 10 and 5ug/ml. The zone of inhibition of *S epidermis* is 18mm, 15mm and resistant with the next three dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually reduces as the dilution decreases and it becomes resistant at 25, 10 and 5ug/ml. The zone of inhibition of *klebisilla* is 18mm, 13mm &

13mm and resistant with the next two dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually reduces as the dilution decreases and it becomes resistant at 10 and 5ug/ml. The zone of inhibition of *E.coli* is 25mm & 23mm and resistant with the next three dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually reduces as the dilution decreases and it becomes resistant at 25, 10 and 5ug/ml. The zone of inhibition of *Candida* is 23mm & 18mm and resistant with the next three dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually reduces as the dilution decreases and it becomes resistant at 25, 10 and 5ug/ml. The zone of inhibition of *A niger* is 15mm and resistant with the next four dilutions. Which means the activity of the test drug is more when the dilution is 75ug/ml and gradually

reduces as the dilution decreases and it becomes resistant at 50, 25, 10 and 5µg/ml. Ciprofloxacin and Flucanazole have the fixed dilution ratio - 30µgm/ml ie 0.03mg of standard drug is diluted in 1ml. Test drug has been diluted at 75 µgm/ml, 50 µgm/ml, 25 µgm/ml, 10 µgm/ml & 5 µgm/ml. When calculated with the human dose of Avaleha for 50gm the dilution needed will be 3,00,000 µgm/ml. The test drug has been found sensitive at 50 & 75 µgm/ml itself, which shows that it will be sensitive at higher dilution also.

For MIC method-

In MIC the interpretation is done based on the turbidity. Lesser the turbidity more the activity of drug & more the turbidity lesser the activity of drug. In this the ten dilutions are made starting from the 100mg/ml to 0.2mg/ml. The test drug (Ardraka Avaleha) is more active with 100, 50 and 25mg/ml and gradually becomes resistant i.e. the solution is clear with higher dilution and gradually becomes turbid. The test drug is more sensitive for *Aspergillus niger* even with the lesser dilution like 0.4mg/ml.

DISCUSSION

Ardraka Avaleha was prepared as per the general method of preparation explained in *Sharangdhara Samhita*. The ingredients of *Ardraka Avaleha* are having the properties like *Balya*, *Rasayana* and *Kaphahara*. *Loha bhasma* is *Ayupradata*, *Bala veeryakarta* and is a *shrestha Rasayana* for human beings. It is indicated in diseases like *kasa*, *shwasa*, *aruchi*, *gulma*, *jwara* etc.

Anti microbial effect of *Ardraka Avaleha* by Disc diffusion method was found sensitive at 25, 50 and 75 µgm/ml with 13, 18 and 25mm of zone of inhibition respectively

when compared with the Ciprofloxacin having 30mm of zone of inhibition.

Ardraka Avaleha found sensitive at 50 and 75 µgm/ml with 18 and 23mm of zone of inhibition respectively when compared with the Fluconazole having 30 and 25mm of zone of inhibition.

Ardraka Avaleha by MIC method was found more active with 100 and 50mg/ml and gradually becomes resistant i.e. the solution is clear with higher dilution and gradually becomes turbid. *Ardraka Avaleha* was more sensitive for *Aspergillus niger* even with the lesser dilution like 0.4mg/ml.

CONCLUSION

Ardraka Avaleha is one of the formulation explained in Yoga Ratnakar in Kasa rogadohikar showed significant effect in both Gram positive and Gram negative bacteria namely *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*. It also showed effect against fungal strains viz. *Candida albicans* and *Aspergillus niger* even with the lesser dilution like 0.4mg/ml.

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Source of support: Nil

Conflict of interest: None Declared

Cite this article as

Dr Veena Nandennavar : In-vitro Antimicrobial Activity of Ardraḳa Avaleha ayurpub; V(5): 1568-1575

Pharmaceutical Study



Pictures of Disc Diffusion Method



Pictures of MIC Method

