

Original Research Article

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## Screening of Ayurvedic Drugs against Multidrug Resistant *Staphylococcus aureus*

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### ABSTRACT

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Emergence of resistance against commonly used antibiotics has become a serious global concern. The emergence of antimicrobial or multidrug resistance to standard antibiotics associated with *Staphylococcus aureus* causes a benefit of interest to ayurvedic drugs as an effective means of control. Acetone, ethanol, methanol, and aqueous hot and cold extracts of nine ayurvedic churnas viz Manjishta, Kadunimchal, Neempati, Behada, Arjusal, Trikatu, Dhataki, Jati and Marich were tested for their antibacterial action against three multidrug resistant *Staphylococcus aureus* strains isolated from clinical samples. Ethanol and acetone extracts of Kadunimchal, behada & Jati were found bactericidal against all three strains. Antibiogram studies revealed the antibacterial pattern of many churnas against the MDR *Staphylococcus aureus* strains and potential use of ayurvedic formulations against infections caused by *Staphylococcus aureus*.

### Introduction

Resistance to antimicrobial agents is a global problem especially in developing countries, where there is high burden of infectious diseases, where the replacement of old antibiotics with new, is more expensive (Kaper *et al*, 2004). Emergence of resistance against commonly used antibiotics has become a serious global concern. Management of infections caused by bacteria is compromised by spread of bacterial resistance to antimicrobial agents. Resistant organisms exert a great challenge in the treatment of bacterial infections which leads to prolonged duration of illness, treatment failure, great risk of death and significant economic loss to the patient and nation. *S.aureus* is significant in its capability

to acquire resistance to any antibiotic (Chambers and Deleo, 2009). Diseases and Infections caused by antibiotic-resistant strains of *S. aureus* have reached epidemic proportions around the globe (Morales *et al.*, 2012; Rosenberger *et al.*, 2010; Singh and Rajurrkar, 2016).

The overall burden of staphylococcal disease, particularly which caused by methicillin-resistant *S. aureus* strains (MRSA), is increasing in many countries in both healthcare and community settings (EARSS, 2007; Kaplan *et al.*, 2005). Carriers of it are at a higher risk of infection and are presumed to be an important source of the spread of *S.aureus* strains among individuals.

There is emergency to replace therapies for the treatment of drug resistant infections and to focus on least use of antibiotics to reduce the development of antibiotic resistance. Ayurveda is a traditional medicine which uses natural therapy. In contrast the allopathic medicine, which primarily uses synthetic chemicals designed for specific target receptors and provides symptomatic relief (Parasuraman *et al.*, 2014). Phytochemicals from ayurvedic drugs have been used for their effective antimicrobial activity since ancient times, and there is an increasing trend for the development of plant-based natural products for the prevention and treatment of pathogenic diseases (Moradigarav *et al.*, 2017). One of the strategies for effective resistance management is the use of antimicrobial agent-phytochemical combinations that will neutralize the resistance mechanism, enabling the drug to still be effective against resistant microbes.

In modern science there was least research done on ayurvedic drugs as compared to allopathic medicines. So, there was need to focus on ayurvedic drugs and develop new strategy to combat multidrug resistance by using ayurvedic drugs, to increase their sensitivity, and to treat infectious diseases with the help of effective formulations. There are thousands of ayurvedic drugs available in the market and have wide scope to explore for their antibacterial activity. Present research work was aimed to search effective ayurvedic drugs against multidrug resistant *Staphylococcus aureus* strains isolated from Akola region of Maharashtra.

## Materials and Methods

### Collection of Samples

Pus samples of the patients suffering with various infections from renowned hospitals located at Akola, Maharashtra were collected aseptically in specimen containers, transported to laboratory and used for isolation of pathogens.

### Isolation and identification

Pus samples were inoculated in enrichment medium separately and incubated at 37°C for 24 h. A loopfull from enrichment medium was streak inoculated on selective nutrient medium such as mannitol salt agar, nutrient agar and salt milk agar separately and incubated at 37°C for 24 h. Well isolated colonies were picked up and studied for morphological, cultural, biochemical

characteristics involving coagulase test which were used for identification of isolates by referring Bergey's Manual.

### Study of antibiogram and screening of ayurvedic drugs

Identified isolates were studied for their sensitivity/resistant pattern to commonly used three antibiotics viz Gentamicin, Rifampicin and Vancomycin by performing antibiotic sensitivity test as per Kirby Bauer method using Muller Hinton medium and other 19 antibiotics by automated bioMerieux vitek 2 system. Total nine ayurvedic drugs viz Manjishta, Kadunimchal, Neempati, Behada, Arjusal, Trikatu, Dhataki, Jati and Marich having traditional applications for infectious diseases were purchased from local market and used to prepare methanol, ethanol, acetone, aqueous hot and aqueous cold extracts. These extracts were then screened by testing for antibacterial activity against identified *S.aureus* by performing antibiotic sensitivity test as per Kirby Bauer method using Muller Hinton medium.

## Results and Discussion

### Isolation, characterisation and identification

In addition to colonial characteristics, Gram positive coccil appearance and development of yellowish pigment on selective media (Fig:1), specific test viz coagulase positive test (Fig 2) by isolates confirmed these isolates as *Staphylococcus aureus* by referring Bergey's Manual (Table 1). Total three strains were identified as *Staphylococcus aureus*.

### Antibiotics sensitivity/ resistance pattern of identified strains

Antibiogram of identified *Staphylococcus aureus* against Gentamicin, Rifampicin and Vancomycin (Table 2) revealed that all three strains were found sensitive to all these antibiotics. It was also observed from the antibiogram performed by bioMerieux (Table 2 & Fig 3) that out of 19 tested antibacterial agents, seven, four & nine antibiotics were reported non inhibitory to strain 1, 2 & 3 respectively. Antibiotics viz Gentamicin, Linezolid, Teicoplanin, Vancomycin, tigecycline, Nitrofurantoin, Rifampicin & Sulfamethoxazole were recorded as inhibitory to all three strains of *Staphylococcus aureus*. On the other hand, Ciprofloxacin, Levofloxacin &

Erythromycin only were reported noninhibitory. The antibiogram study indicates that the given strains are MDR resistant.

### Antibacterial activity of churnas against *Staphylococcus aureus* strains

It is evident from the table of antibacterial activity of churnas against *Staphylococcus aureus* strains (Table 3 & Fig 4) that ethanol and acetone extracts of Kadunimchal, behada & Jati were found bactericidal against all three strains. Methanol extract of behada, arjunsal & Jati were reported as inhibitory to all three strains. Aqueous hot extract of marich was found inhibited all three strains, while hot aqueous of behada inhibited strains 2 & 3, similarly cold extract of marich and behada inhibited strain 1 & strain 2. Ethanol, acetone and water extracts of Trikatu were found non antibacterial against all three strains. Ethanol extract of manjishtachurna showed significant zone against strain 2. As well as ethanol extract of neempati and behadachurna showed significant zone against strain 3. Thus, it is clear from all these results of antibacterial activity that ayurvedic drugs have potential capacity to inhibit the growth of MDR resistant strains of *Staphylococcus aureus*.

There are many reports in the literature regarding the antibacterial activity of common antibiotics and traditional herbal preparations against MDR resistant strains of *Staphylococcus aureus*. *Staphylococcus aureus* isolates have showed resistance of 90% to Ceftriazone, Tetracycline, and Ampicillin and in case of Cloxacillin

antibiotic it has showed resistance up to 85% (Godebo *et al.*, 2013; Mengesha *et al.*, 2014). Basu *et al.*, (2005) evaluated antibacterial activity of *Rubia cordifolia* Linn, *Ventiligo madraspatana* Gaertn and *Lantana camara* Linn by agar well diffusion method.

In their study they reported maximum antibacterial activity of chloroform and methanol extracts of *R. cordifolia* (Manjishta) against *B.subtilis* and *S.aureus* as compared to the standard drugs penicillin G and streptomycin. Debnath *et al.*, (2013), worked on antifungal and antibacterial activity of bark of *Termanalia arjuna* against MDR clinical isolates.

Their study revealed therapeutic use of *T. arjuna* for treatment of microbial diseases and the polar fractions of bark used to developed novel antimicrobial agents. Dubey *et al.*, (2014), worked on in vitro antibacterial activity and GC-MS analysis of *Woodfordia fruticosa* (Dhataki) and reported potential of methanol extract to control MDR bacteria. Gupta *et al.*, (2019) studied antibacterial activity of ethanolic, methanolic, chloroform, acetone and aqueous leaf extracts of *Embilica officinalis*, *Termanalia bellirica*, *Termanalia chebula* and their triphala formulation against pathogenic microorganisms and found excellent antibacterial activity against gram positive and gram-negative bacteria by ethanolic extracts. Altayb *et al.*, (2022), studied in vitro and in silico antibacterial activity of methanolic extract of *A. indica* (Neem) against pathogenic *S. aureus* and reported 10 mm zone of inhibition at the lowest concentration 3.125%. Our results are also on the same line of findings as by these researchers.

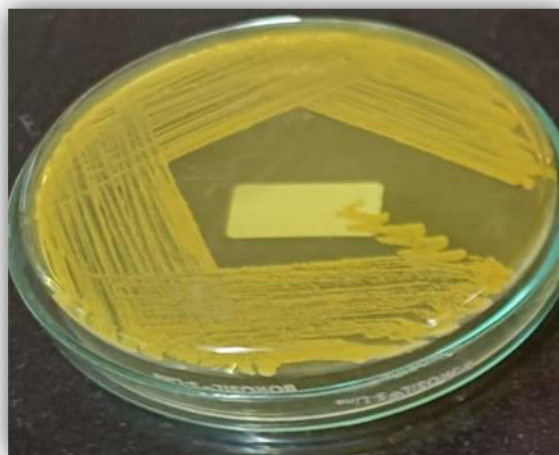
**Table.1** Characteristics of isolates and identification

Sr.No	Isolate	Pigmentation on			Gram character Gram staining	Coagulase Test		Isolate identified as
		Mannitol Salt agar	Nutrient agar	Salt milk agar		Slide	Tube	
1	S A 1	Yellowish	Whitish yellow	Yellowish	+ Cocci	+	+	<i>Staphylococcus aureus</i> strain I
2	SA 2	Yellowish	Whitish yellow	Yellowish	+ Cocci	+	+	<i>Staphylococcus aureus</i> strain II
3	SA 3	Yellowish	Whitish yellow	Yellowish	+ Cocci	+	+	<i>Staphylococcus aureus</i> strain III

**Table.2** Antibiogram of *S. aureus*

Sr.No.	Method Used	Antimicrobial agent	Susceptibility pattern of <i>S.aureus</i> strain		
			SA1	SA2	SA3
1	Kirby Bauer method	Gentamicin	S(11mmZOI)	S(13mmZOI)	S(14mmZOI)
2		Rifampicin	S(17mmZOI)	S(22mmZOI)	S(23mmZOI)
3		Vancomycin	S(14mmZOI)	S(14mmZOI)	S(14mmZOI)
4	BioMerieux vitex 2.	Cefoxitin Screen	R	S	R
5		Benzylpenicillin	R	S	R
6		Oxacillin	R	S	R
7		Gentamicin high level (Synergy)	S	S	S
8		Gentamicin	S	S	S
9		Ciprofloxacin	R	R	R
10		Levofloxacin	R	R	R
11		Clindamycin	S	R	R
12		Erythromycin	R	R	R
13		Clindamycin	S	S	R
14		Linezolid	S	S	S
15		Daptomycin	S	S	R
16		Teicoplanin	S	S	S
17		Vancomycin	S	S	S
18		Tetracycline	R	S	S
19		Tigecycline	S	S	S
20		Nitrofurantoin	S	S	S
21		Rifampicin	S	S	S
22		Trimethoprim/ Sulfamethoxazole	S	S	S

**Figure.1** Yellowish pigmentation by *S.aureus* on mannitol salt agar



**Table.3** Antibacterial activity of ayurvedic churna extracts

Sr.No	Churn	Extracts	Antibacterial action on <i>S.aureus</i> (Diameter Zone of inhibition in mm)		
			SA1	SA2	SA3
1	Manjishta	Methanol	-	-	-
		Ethanol	7	13	-
		Acetone	-	-	-
		Aqueous cold/hot	-	-	-
2	Kadunimchal	Methanol	-	-	-
		Ethanol	10	10	10
		Acetone	12	11	10
		Aqueous cold/hot	-/-	-/10	-/-
3	Neempati	Methanol	-	-	-
		Ethanol	11	-	11
		Acetone	-	-	-
		Aqueous cold/hot	-/-	-/-	-/-
4	Behada	Methanol	7	7	8
		Ethanol	10	8	10
		Acetone	11	10	8
		Aqueous cold/hot	-	7 / 7	-/9
5	Arjunsal	Methanol	7	10	10
		Ethanol	-	7	8
		Acetone	10	11	12
		Aqueous cold/hot	-/-	-/-	-/-
6	Trikatu	Methanol	7	-	8
		Ethanol	-	-	-
		Acetone	-	-	-
		Aqueous cold/hot	-/-	-/-	-/-
7	Dhataki	Methanol	-	7	11
		Ethanol	-	7	7
		Acetone	-	6	-
		Aqueous cold/hot	-/-	-/-	-/-
8	Jati	Methanol	6	6	7
		Ethanol	8	7	7
		Acetone	7	6	6
		Aqueous cold/hot	-/-	-/-	-/-
9	Marich	Methanol	-	-	8
		Ethanol	-	-	-
		Acetone	8	8	-
		Aqueous cold/hot	7 / 7	-/7	-/7



Figure.2 Slide & Tube coagulase test

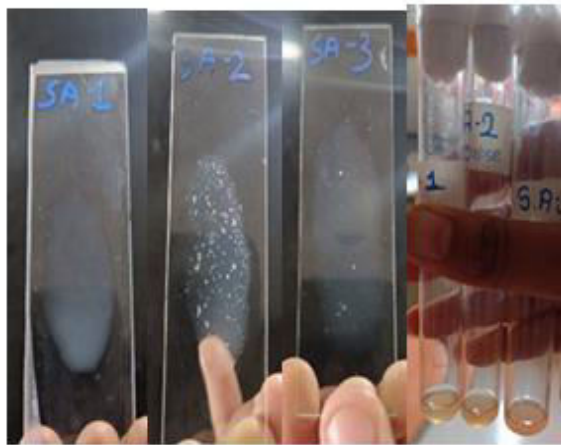


Figure.3 Antibiotic sensitivity pattern of *Staphylococcus aureus* strains



Figure.4 Bactericidal activity of churna against *Staphylococcus aureus*



Results of present research findings clearly support the previous work that, ayurvedic preparations/extracts have potential as an antibacterial activity against pathogenic *Staphylococcus aureus* & can be used as to treat infectious diseases caused by MDR *Staphylococcus aureus*.

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### Author Contributions

Lokhande Ankita: Investigation, formal analysis, writing—original draft. Deshpande Aarti: Validation, methodology, writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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