



## ANTIBACTERIAL ACTIVITY OF *OCHROCARPUS LONGIFOLIUS* EXTRACT AGAINST *S.AUREUS* AND MRSA STRAINS

K. Salkar\*, C. Chotalia and R. Salvi

Phytomedicines Department, Piramal Enterprises Limited, Mumbai, Maharashtra, India.

\*Corresponding Author: K. Salkar

Phytomedicines Department, Piramal Enterprises Limited, Mumbai, Maharashtra, India.

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### ABSTRACT

**Objective:** The aim of our study was to evaluate the anti-bacterial potential of a plant extract to develop further a plant based anti-bacterial agent. **Methods:** *Ochrocarpus longifolius* was studied for its Antibacterial potential. Seven different extracts of this plant were tested by agar-dilution method to obtain MIC values. The plant was tested against standard strains, clinical isolates of *S.aureus* and MRSA strains. **Results:** MIC value in the range of 1 to 4 mcg/ml was obtained for the plant extracts. Similar type of activity was obtained for clinical isolates and MRSA strains of *S.aureus*. **Conclusion:** *Ochrocarpus longifolius* possess potent antibacterial activity and can be developed further into a new antibacterial agent.

**KEYWORDS:** *Ochrocarpus longifolius*, Antibacterial, *S.aureus*, MRSA, resistance.

### INTRODUCTION

The indiscriminate use of antibiotics and modern lifestyle has led to the development of antibiotic resistance in current days. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity, and the plant extracts were found to have potential against microorganisms.

The normal microbial flora of human skin like *Staphylococcus aureus* has developed into various resistant species like MRSA (Methicillin Resistant *Staphylococcus aureus*). MRSA was once thought to be problematic only in healthcare settings, but now community associated MRSA infections are becoming more common. MRSA have become resistant to the multiple classes of antibiotics including beta lactams, macrolides, and quinolones, as well as the glycopeptide vancomycin, the common drug of last resort.<sup>[1]</sup>

*Staphylococcus aureus* a Gram positive bacterium, has a peculiar ability to colonize the skin of patients with eczema and Atopic Dermatitis (AD) and is consistently found in eczematous skin lesions in these patients. The skin lesions of 80–100% of patients with eczema and AD are colonized with *S. aureus*. In contrast, *S. aureus* can be isolated from the skin of only 5–30% of normal individuals, mainly from intertriginous areas.<sup>[2,3]</sup> A correlation between the severity of the eczema and colonization with *S. aureus* has been demonstrated, and

it has been determined that bacterial colonization is an important mechanism aggravating skin lesions. It has been shown that lesional skin of patients with eczema and Atopic Dermatitis (AD) was more frequently colonized with *S. aureus* than was nonlesional skin. The more severe the eczema, the higher the colonization rate of *S. aureus*.<sup>[2,3,4]</sup>

In our regular screening program we screen indigenous plant flora for various ailments. In one such study we identified a plant, *Ochrocarpos longifolius* which was active against *S.aureus* and MRSA strains.

*Ochrocarpos longifolius* Benth. & Hook.f. ex T.Anderson, commonly known as Nagappu, Nagesarpu (Siddha/Tamil) and Laal-Naagakeshar, Surangi (Folk) belongs to family *Clusiaceae* and is found in the evergreen Western Ghats southwards from Konkan to Malabar and Coimbatore.<sup>[5,6]</sup> *Ochrocarpus longifolius* was shown to have antifungal and anthelmintic activity.<sup>[7,8]</sup> Flowers buds are aromatic, possess carminative and astringent properties and used for hemorrhoids and dyspepsia. They are also used for gastritis, leucoderma, headache and snake and scorpion-bite.<sup>[9,10]</sup> The usage of flower buds is mentioned in Bhavaprakash Nighantu and in Sushruta Samhita.

The plant was studied in various *in vitro* models for activity against *S. aureus* strains including the clinical isolates and antibiotic resistant strains of *S. aureus* (MRSA). We obtained very positive results in our preclinical studies in which the plant extract showed

MIC values of 1 to 4 mcg/ml against various strains of *S. aureus*. The results of these pre-clinical studies of *Ochrocarpus longifolius* extract are presented over here.

## MATERIALS AND METHODS

### Organisms

*Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA) 3710, (MRSA) ATCC 3351 were used as test organisms. Clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were obtained from various hospitals of Mumbai-India. Soyabean casein digest medium was used for all the assays and Brain Heart Infusion Agar (BHI) was used for culture maintenance.

### Inoculum preparation

A loopful of culture from 24-hour-old slant (37°C) was suspended in saline and vortexed for 15 seconds. The inoculum was adjusted to the optical density (absorbance) of 0.08 to 1.00 at 625 nm using a spectrophotometer. This yields a stock suspension of  $1 \times 10^8$  cells/ml (correlation of absorbance and cell density was established previously using standard growth curves). Further it was diluted 1:100, to yield a stock suspension of  $1 \times 10^6$  cells/ml.

### Sample preparation

A series of two-fold dilution of extract (Methanolic extract of *O. longifolius*) was prepared, so as to get 20-times higher concentration of the final concentrations required. Calculated amount of stock solutions of extract was added to 20 ml of sterile, melted Soyabean Casein Digest (TSA) agar medium and poured into sterile pre-

labeled petri plates so as to get a series of serial two fold dilution of the extract in the medium. The final concentrations of extract in the plate medium ranged from 0.03 to 4 mcg/ml. A growth control plate without any extract and a solvent control plate were included in the study. Antibiotic standards Oxacillin and Linezolid were used as positive controls.

### Assay

The culture suspension (final stock suspension of  $10^6$  cells/ml) prepared by above method was spotted in 10µl amount on the solidified plates so as to transfer  $10^4$  cells per spot. Spots were allowed to dry at room temperature and then the plates were incubated at 37°C for 24 hours.

### Endpoint criteria

At the end of incubation period plates were observed and recorded for presence (+) or absence (-) of growth. The MIC was defined as the lowest concentration of extract giving no visible growth or causing almost complete inhibition of growth in the plates as judged by the naked eyes, disregarding a single colony or thin haze within the area of the inoculated spot. Each test was repeated in triplicate on two separate occasions.

## RESULTS AND DISCUSSION

*Ochrocarpus longifolius* was studied for its antibacterial activity against various strains of *Staphylococcus aureus* by agar-dilution method. The seven different extracts showed good activity against *Staphylococcus aureus* normal and resistant strains with the MIC value of 0.5 to 4 mcg/ml (table 1).

**Table 1: MIC of *Ochrocarpus longifolius* extract and Linezolid against *S. aureus* and MRSA.**

Sr. No.	Extracts	MIC (mcg/ml)		
		<i>Staphylococcus aureus</i>	M-1 (MRSA) 3710	M-2 (MRSA) ATCC 33591
1	OL-170409	2	2	2
2	OL-200409	4	4	4
3	OL-290409	2	2	2
4	OL-130509	0.5	0.5	0.5
5	OL-140509	1	2	2
6	OL-110509	1	1	1
7	OL-150909	1	1	1
8	Std.-Oxacillin	0.12	> 4	> 4
9	Std.-Linezolid	0.25	0.25	0.25

Key: OL-*Ochrocarpus longifolius* extract.

The MIC value was comparable with the standards used. The plant was consistent with respect to the antimicrobial activity, as evident from the results of these different extracts. The standard antibiotic –Linezolid showed MIC value in the range of 0.5 to 1.25 mcg/ml, thus MIC value of *Ochrocarpus longifolius* is very much comparable to the latest synthetic antibacterial agent. The plant extracts were also tested for their antibacterial potential using clinical isolates of *S. aureus*. The clinical isolates were obtained from hospitals of Mumbai, India

(KEM and Lilavati hospital). The plant extracts were tested against total 24 clinical isolates, MIC value in the range of 0.5 to 4 mcg/ml was obtained for these clinical isolates (table 2, figure 1).

Table 2: MIC of *Ochrocarpus longifolius* extract and Linezolid against clinical isolates of *S.aureus*.

S.aureus-Clinical isolates No.	MIC (mcg/ml)							
	OL-170409	OL-200409	OL-290409	OL-130509	OL-140509	OL-110509	OL-150909	Linezolid
KEM-MRSA-1	2	4	2	1	1	1	1	1
KEM-MRSA-2	2	4	2	1	1	1	1	1,25
KEM-MRSA-3	2	4	2	1	1	1	1	1,25
KEM-MRSA-4	2	4	2	1	1	1	1	1,25
KEM-MRSA-5	2	4	2	1	1	1	1	1,25
MRSA-3-Lilavati	1	2	1	0.5	0.5	0.5	0.5	0.5
C1-MRSA-2	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-3	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-5	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-7	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-8	2	4	2	1	1	1	1	1,25
C1-MRSA-9	2	4	2	1	1	1	1	1,25
C1-MRSA-10	2	4	2	1	1	1	1	1,25
C1-MRSA-13	2	4	2	1	1	1	1	1,25
C1-MRSA-16	2	4	2	1	1	1	1	1,25
C1-MRSA-17	2	4	2	1	1	1	1	1
C1-MRSA-20	2	4	2	1	1	1	1	1
C1-MRSA-21	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-22	1	2	1	0.5	0.5	0.5	0.5	1
C1-MRSA-23	1	2	1	0.5	0.5	0.5	0.5	0.5
C1-MRSA-24	1	2	1	0.5	0.5	0.5	0.5	0.5
C1-MRSA-25	1	2	1	0.5	0.5	0.5	0.5	0.5
C1-MRSA-30	1	2	1	0.5	0.5	0.5	0.5	0.5
C1-MRSA-35	2	4	2	1	1	1	1	1

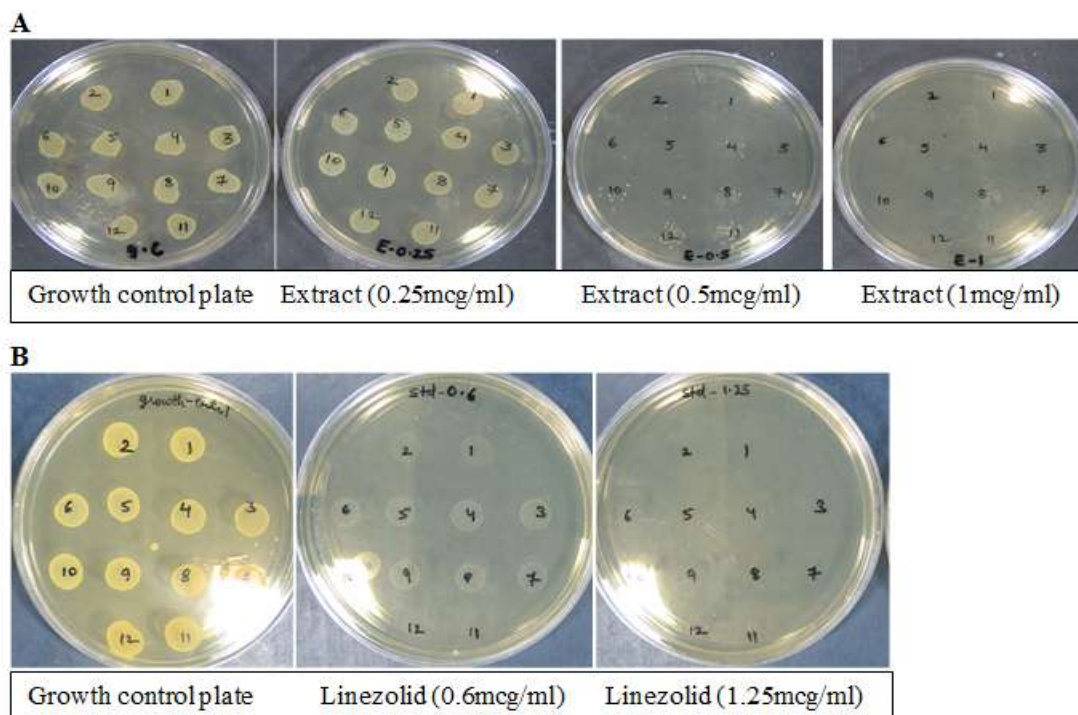


Figure 1: Agar dilution plates of plant extract and standard inhibiting clinical isolates of MRSA.

Key: the numbers 1 to 12 indicate the spots of *S.aureus* strains.Legend: A- Agar dilution plates of *Ochrocarpus longifolius* extract inhibiting clinical isolates of MRSA at conc. of 0.5 & 1 mcg/ml, B-Agar dilution plates of std. Linezolid inhibiting clinical isolates of MRSA at conc. of 1.25 mcg/ml.

From the compiled values it is evident that *Ochrocarpus longifolius* extract has potent anti bacterial activity comparable with the standard Linezolid. Being a plant extract with such remarkable activity of 0.5 to 4 mcg/ml definitely accounts for further development of this plant into a topical formulation.

Vast research is going on worldwide using plants as a source of new antibacterial agents. S. Mandal et al reported MIC of ethanolic extracts of cinnamon (*Cinnamomum zeylanicum*; CIN), clove (*Syzygium aromaticum*, CLV) and cumin (*Cuminum cyminum*, CMN) against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA), from Kolkata, India. The MICs, for the isolates, were in the range of 64-256, 64-512 and 128-512 µg/ml, respectively.<sup>[11]</sup>

Elisha et al screened 9 different plants and reported MIC in the range of 0.08 to 0.45 mg/ml against *S.aureus*.<sup>[12]</sup> Isabelle B. reported antibacterial activity against MRSA for essential oils (EOs) from the species *T. fontanesii* and *T. numidicus* and for *M. pulegium* from Algeria. EOs were found to be strongly bactericidal (MIC from 0.3 µL/mL to 4.7 µL/mL) which suggests an additional option to treat MRSA infections.<sup>[13]</sup> Four plant species of *Ageratum conyzoides*, *Phyllanthus emblica*, *Camellia sinensis* and *Mentha longifolia* showed good activity against MRSA in a study carried out by P. Agarwal et al.<sup>[14]</sup>

A.Gupta et al conducted a detail study on Anti-*S.aureus* activity of *Curcuma longa* extract and showed that the plant is active against various strains of *S.aureus*.<sup>[15]</sup> Curcumin a compound isolated from *C.longa* is widely studied for its antibacterial potential against *S.aureus* and MRSA, the MIC values is in the range of 18 to 256 mcg/ml as reported by various scientists.<sup>[16]</sup>

In view of these studies, our plant extract *Ochrocarpus longifolius* can be considered as highly potent antibacterial plant with the MIC in the range of 0.5 to 4 mcg/ml against *S.aureus* and MRSA strains. It can be concluded that the plant extract has good potential to be developed as an antibacterial agent. Further investigations are in progress for the plant species to potentiate its development as an antibacterial agent for *S.aureus*.

MRSA infections in both the hospital and community setting are commonly treated with non-β-lactam antibiotics, such as clindamycin (a lincosamine) and cotrimoxazole (also commonly known as trimethoprim/sulfamethoxazole). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics, such as linezolid, because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin).<sup>[17,18]</sup> There are number of problems with these antibiotics, such as the need for intravenous

administration (there is no oral preparation available), toxicity, and the need to monitor drug levels regularly by blood tests. There are also concerns that glycopeptide antibiotics do not penetrate very well into infected tissues (this is a particular concern with infections of the brain and meninges and in endocarditis). Glycopeptides must not be used to treat methicillin-sensitive *S. aureus* (MSSA), as outcomes are inferior.

The treatment options including available drugs offers limited benefits as the development of resistance showed by bacteria. Hence, there is a need for improved and alternative medicaments for the prevention and treatment of infectious diseases caused by *S. aureus* and MRSA. In such scenario plant based antibacterial agents like *Ochrocarpus longifolius* can offer new and natural options for the patients suffering from such infections with equivalent benefits at low cost and with better safety profile.

## CONCLUSION

*Ochrocarpus longifolius* showed potent anti-bacterial activity profile and can be developed into a topical agent for bacterial and other skin infections. In brief, the topical dosage forms developed with a natural extract with known and proven anti-infective activities and minimal toxicity will impact both therapeutic and pharmaceutical areas. In addition to this, related areas of hospital related infection caused by MRSA will also benefit from *Ochrocarpus* based formulations.

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