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Indian olive, *Elaeocarpus floribundus* fruits in combating MRSA infection

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ABSTRACT

Objective: To explore the antibacterial activity of *Elaeocarpus floribundus* fruits aqueous extracts against methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolates.
Methods: The fresh olive fruits were collected, dried and milled in order to prepare aqueous extracts of olive seed and olive mesocarp-epicarp. The extracts were tested against clinical isolates of MRSA by agar well diffusion method, and the diameter of inhibition zone was recorded, and antibacterial capacity of the extracts (AU/mL) was determined.
Results: The MRSA isolates were sensitive to aqueous extract of olive mesocarp-epicarp with diameter of inhibition zone ranging from (11.670 ± 0.577) to (15.330 ± 0.577) mm; the olive aqueous seed extract had no anti-MRSA activity. The antibacterial capacity of the extracts against the tested MRSA isolates ranged from 733.33 to 800 AU/mL.
Conclusions: The olive fruits can be used as a new source of plant based non-antibiotic biotherapeutic agents for the treatment of MRSA infection.

1. Introduction

The rapid emergence and spread of antibiotic resistant bacteria, causing life-threatening illnesses in humans, necessitated the searching of new biotherapeutics alternative to the synthetic antibiotics, in order to combat the antibiotic resistance of such pathogenic microorganisms and disorders caused by them[1,2]. The olive plant, *Elaeocarpus floribundus* (*E. floribundus*) (family: Elaeocarpaceae), is famous for its fruits, and is also an excellent medicinal plant native to India. The various parts of the plant (leaf, fruit, bark, and root) are used to cure several diseases. The plant possesses different biological activities including antioxidative and antibacterial properties because of the presence of an array of bioactive phytochemicals, mainly the phenolic compounds in the plant parts[3,4]. The *E. floribundus* leaves and stem bark extracts have been reported to show antioxidative activity with IC_{50} value of 7.36–9.37 μg/mL[3]. The antibacterial activity of *E. floribundus* leaf extract has been authenticated against Gram-positive and Gram-negative human pathogenic bacteria, having diameter of inhibition zone (ZDI) of 18–22 mm[5]. The olive extracts are natural sources of reducing as well as stabilizing agents for the synthesis of silver nanoparticles with antibacterial activity against spoilage and pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*[6]. It has been reported that the ethanolic as well as aqueous extracts of seed and mesocarp-epicarp of olive (*E. floribundus*) fruits had growth inhibitory activity against food-borne bacteria[7]. However, no scientific report has been made on anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of extracts of olive fruits parts, at least to our knowledge. The above background prompted us to determine the antibacterial activity of aqueous extracts of seed and mesocarp-epicarp from an indigenous edible fruit, olive, *E. floribundus* against clinical MRSA isolates.

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2. Materials and method

The aqueous extracts of seed and mesocarp-epicarp of *E. floribundus* fruit were prepared according to the method mentioned earlier[7], and tested against MRSA by agar well diffusion method[1] using nutrient agar medium (Hi-Media, India). The ZDI (nearest whole) on the test agar plate, around each of the wells, due to the antimicrobial action of the extracts at various concentrations (2.49, 4.15 and 8.3 mg/well), was measured and interpreted following the criteria mentioned earlier[1]. The bacterial growth inhibition capacity (AU/mL) of the plant extracts was evaluated as a measure of bioactive components present in the extract[1], using the formula[8]:

$$\text{AU/mL} = \frac{\text{ZDI} \times 1\,000}{\text{Volume taken in the well (μL)}}$$

The antibacterial test results were expressed as mean ± SD, and were assessed by One-way ANOVA using Mcrosoft Excel 2010, and comparison of the mean values was done using the Tukey's test, and statistical significance was set at $P < 0.05$.

3. Results

The antibacterial activity of aqueous extracts of seed and mesocarp-epicarp against clinical MRSA isolates determined by agar well diffusion method is represented in Figure 1.

The mesocarp-epicarp extract had concentration-dependant activity against the tested MRSA isolates (Table 1). The mesocarp-epicarp extract, at 2.49 and 4.15 mg/well, was moderately active with ZDIs of (11.670 ± 0.577) mm (range: 11–12 mm) and (13.330 ± 0.577) mm (range: 13–14 mm), respectively, while at 8.3 mg/well the extract was highly active with ZDI of (15.330 ± 0.577) mm (range: 15–16 mm). The seed extract showed no bacterial growth inhibition with ZDI of 6 mm, which is the diameter of the well on the test plate against the tested MRSA isolates. The antibacterial activity of seed extract against MRSA was significantly different from that of mesocarp-epicarp extract at all the concentration levels (2.49, 4.15 and 8.3 mg/well).

Table 1
Antibacterial action of seed extract and mesocarp-epicarp extract at various concentrations against MRSA clinical isolates (mm).

Bacterial isolates	ZDI (mm)		
	Seed extract*	Mesocarp-epicarp extract	
	Less active (ZDI: ⩽10 mm)	Moderately active (ZDI: 11–14 mm)	Highly active (ZDI: ⩾15 mm)
MRSA1	6[a]	12[a]–13[b]	15[c]
MRSA2	6[a]	11[a]–13[b]	16[c]
MRSA3	6[a]	12[a]–14[b]	15[c]

*: Not active at all concentrations; [a]: 2.49 mg/well; [b]: 4.15 mg/well; [c]: 8.3 mg/well. The anti-MRSA activity of seed extract is significantly different from that of mesocarp-epicarp extract at all the concentrations a, b, c with *P* values of 0.0017, 0.0010 and 0.0006, respectively.

The antibacterial capacity of mesocarp-epicarp extract observed at its lowest volume (15 μL) against the clinical MRSA isolates was (777.77 ± 38.49) AU/mL with the range of 733.33–800 AU/mL (Figure 2).

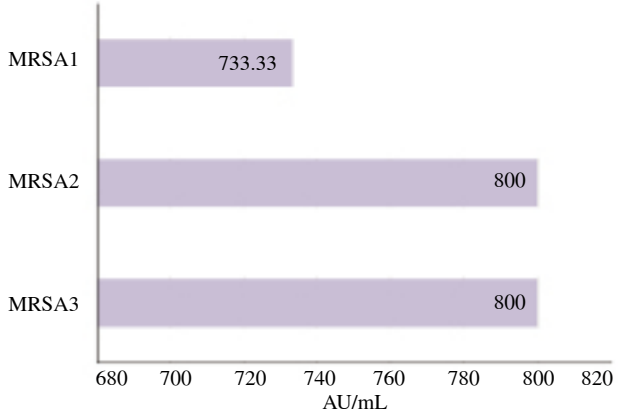

Figure 2. Growth inhibitory capacity of olive mesocarp-epicarp aqueous extract against clinical MRSA isolates.

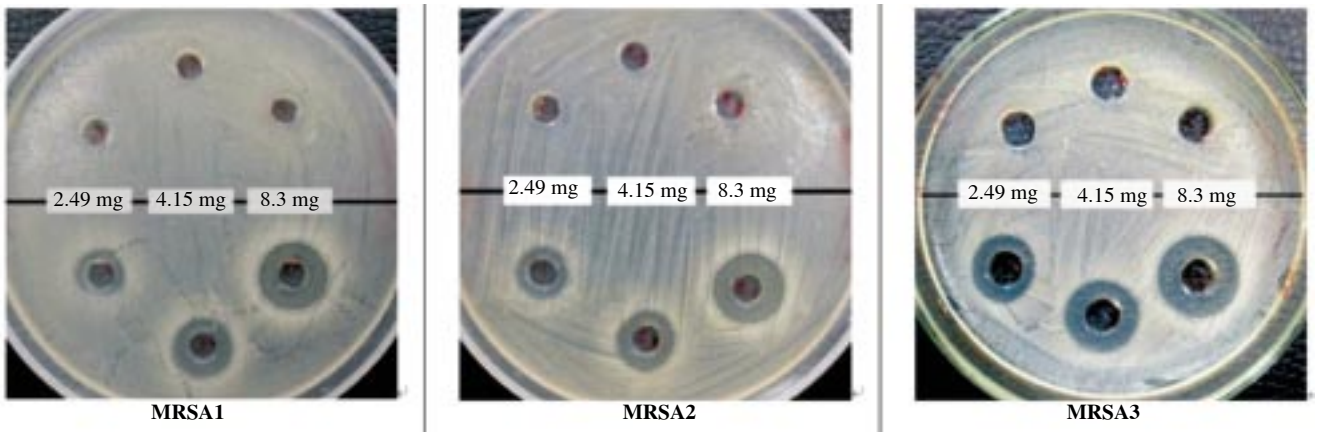

Figure 1. Agar-well diffusion method demonstrating the anti-MRSA activity of olive seed aqueous extract on the upper half of the culture plates and olive mesocarp-epicarp aqueous extract on the lower half of the culture plates.
Clear halos surrounding the wells on each of the plates indicate the bacterial growth inhibition by olive mesocarp-epicarp aqueous extract; olive seed aqueous extract was not able to produce any halo.

4. Discussion

This study evaluated an indigenous edible olive fruit having activities against various human illnesses, and provided the scientific basis of using olive fruits as medicine against antibiotic resistant bacterial infection. The emerging and reemerging antibiotic resistances among human pathogenic bacteria possessing the capacity to cause an array of life-threatening diseases in developing as well as developed countries raised the necessity to incessantly search for biotherapeutics, including plant based non-antibiotic antimicrobials, against such pathogenic infections. The olive leaves extract has been reported to show antimicrobial activities against food-borne bacterial pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes* (Gram-positive) and *Escherichia coli* and *Salmonella* spp. (Gram-negative)[9]. Olive (*Olea europaea*) leaves and stem bark ethanol and ethyl acetate extracts had antimicrobial activity against MRSA and *Staphylococcus aureus* with ZDIs of 14–18 mm and 23–25 mm (leaf extract), and 13–15 mm and 20–22 mm (stem bark extract), respectively[10]. Sudjana *et al.*[11] reported anti-MRSA activity of olive leaves extract with minimum inhibitory concentration of 0.78%, and suggested that the olive products might be potential in conveniently altering the gastrointestinal floral composition. The antibacterial potential of *E. floribundus* leaf extract has been reported against Gram-positive bacteria of *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus megaterium* and *Bacillus subtilis*, and Gram-negative bacteria of *Escherichia coli*, *Vibrio parahemolyticus*, *Shigella dysenteriae, Salmonella enterica* serovar Paratyphi and *Pseudomonas aeruginosa*, and human pathogenic bacteria with ZDI of 18–22 mm[5]. In the current study, the mesocarp-epicarp extract had bacterial growth inhibitory activity against the tested MRSA isolates, and thus, the Indian olive, *E. floribundus*, has experimentally been proven as the source of non-antibiotic plant based biotherapeutics to be applied against MRSA infection in humans.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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