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**Original Research Article**

# Screening of *Elaeocarpus floribundus* fruit extracts for bioactive phytocomponents and antibacterial activity against food-borne bacteria

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## ABSTRACT

**Background:** Medicinal plants possess several active components having antimicrobial activity. This study was undertaken to explore the antibacterial activity of Indian olive, *Elaeocarpus floribundus*, fruit extracts against potential food-borne bacterial isolates.
**Methods:** The ethanolic extracts of olive seed (OSE) and mesocarp-epicarp (OMeE), and the aqueous extracts of olive seed (AqOSE) and mesocarp-epicarp (AqOMeE) were prepared, and analysed qualitatively for phytochemicals. The antibacterial activity of the extracts against food-borne pathogenic bacteria: *Bacillus sp.*, *Bacillus cereus*, *Enterococcus sp*. and *Corynebacterium sp*., was determined by agar-well diffusion method, and minimum inhibitory concentration (MIC) values by agar dilution method.
**Results:** The concentration dependent activity of the extracts against the bacteria was recorded with zone diameter of inhibition 6 - 28 mm for ethanolic extracts, and 7 - 23 mm for aqueous extracts. The ethanolic extracts were confirmed positive for the presence of cardiac glycosides, anthraquinone glycosides, steroids, terpenoids and quinones, while cardiac glycosides, anthraquinone glycosides, steroids, terpenoids, quinones and phenol were detected in the aqueous extracts. The MICs of OSE and OMeE ranged 9.375-12.5mg/ml, and 1.875 - 3.125 mg/ml, respectively, for the test bacteria.
**Conclusions:** The olive fruit extracts contained various bioactive components, and had excellent antibacterial activity against food-borne bacteria. The plant might be useful in the preparation of non-antibiotic antibacterial agents and in the storage of food as well.

**Keywords:** Antibacterial activity, Food-borne bacterial pathogens, Minimum inhibitory concentration, Olive fruit, Phytochemicals

## INTRODUCTION

Due to the injudicious use of synthetic antibiotics against the treatment of various infections caused by the bacterial pathogens, multidrug resistance (MDR) developed, among them; this fact led treatment failure of antibiotics.[1,2] Considering the problem antibiotic treatment failure of MDR bacterial infection, development of alternative treatment protocol is essential. Several authors studied the antibacterial activity of various plant extracts against gram-positive as well as gram-negative bacteria, from various parts of the world, in order to find new sources of antibacterial agents.[3,4]

India is recognised as one of the luxurious emporia of thousands of plant species used in the indigenous treatment, and as the food sources.[5,6] The olive plant, *Elaeocarpus floribundus*, which is known as 'Jalpai' in

Bengali belongs to the family Elaeocarpaceae, and famous for its fruits. The plant is evergreen and naturally found in South and East Asian regions, including India.[7] The scientific investigation of the phenolic components from the olive fruits, olive oil and olive leaves are available in the literatures for their antioxidant properties.[7-12] The *in vitro* antimicrobial activity of olive leaves has been reported by earlier researchers.[7,13] Khalil et al reported that the aqueous olive leaf extract had the ability to produce stable silver nanoparticles possessing antibacterial activity against drug resistant pathogens.[14,15] The *E. floribundus* known as *medangteja* (in Malay), a mixture of bark and leaves has been reported to be used as a mouthwash for irritated gums; the fruits and leaves of the olive (*Olea europaea* L.) have been reported to contain a series of compounds responsible for defence against microbial infections.[16]

Various life-threatening infections caused by several kinds of food-borne bacterial pathogens showing resistance to the conventionally used antibiotics remains the major concerns of clinicians as well as the microbiologists.[17] Therefore, the current study has been undertaken to screen the bioactive components present in different extracts of locally available *E. floribundus* fruits, and to evaluate the growth inhibition property against food-borne pathogenic bacteria, in order to recognize those agents as the potential source of antibacterial compounds to be used against such infection.

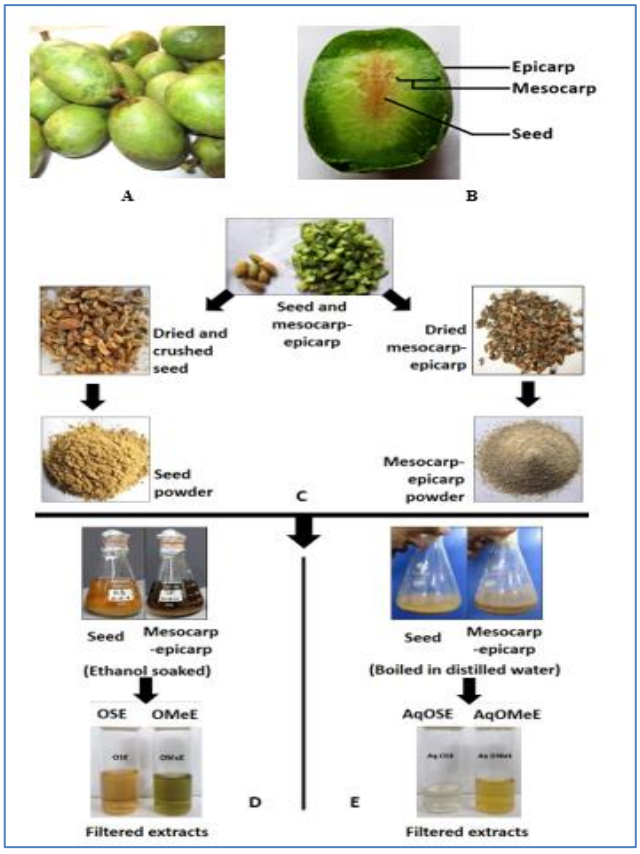
## METHODS

### *Collection of plant materials and extract preparation*

The mature olive, *E. floribundus*, fruits were collected from naturally grown wild plants at Hemtabaad block of Uttar Dinajpur district, West Bengal state, India, and seeds and mesocarp-epicarp parts were separated after washing with distilled water. The plant materials werethen shed-dried and grinded was using an electronic grinding machine. The ethanolic extracts of the grinded fruit parts (25 g in 200 ml of ethanol) were prepared following the method described earlier.[18] For the preparation of aqueous extracts, 10 g of grinded materials, seed as well as mesocarp-epicarp parts, were boiled separately in 60 ml of double distilled water for 30 min. Various steps in the process of extract preparation are represented in Figure 1. The prepared extracts were stored in the refrigerator, at 4$^{o}$ C. The concentration of the aqueous olive seed extract (AqOSE) and mesocarp-epicarp extract (AqOMeE) was 166 µg/µl, while for the ethanolic seed extract (OSE) and mesocarp-epicarp extract (OMeE) was 125 µg/µl.

### *Determination of bioactive compounds*

The bioactive components, such as flavonoids, steroids, terpenoids, quinoneandphenol, cardiac glycosides, anthraquinone glycosides and saponins, present in the plant extracts have been detected following protocols mentioned elsewhere, with slight modification as described below.[19-22]




OSE: ethanolic olive seed extract; OMeE: ethanolic olive mesocarp-epicarp extract; AqOSE: aqueous olive seed extract; AqOMeE: aqueous olive mesocarp-epicarp extract

**Figure 1: Preparation of extract from different parts of olive fruit. A: fresh fruit; B: different parts of the fruit. C: preparation of granulated forms of fruit parts; D: prepared ethanolic extract; E: prepared aqueous extract.**

The turning of yellow colouration, developed in the mixture of few drops of NaOH (1%) solution and the plant extract (1 ml), to colourless on addition of few drops of HCl (1%) to it, was indicative for the presence of flavonoids. The extract (1 ml) was treated with glacial acetic acid (2 ml) containing a drop of $FeCl_3$ solution, brown ring development at the interface of the mixture on addition of concentrated $H_2SO_4$ (1 ml) indicated the presence of cardiac glycosides.

For anthraquinone glycosides, $H_2SO_4$ (1ml; 5 %) was mixed gently to the extract (1 ml) with chloroform (2 ml), and the upper part was discarded after few minutes (4-5 min) of keeping the mixture undisturbed. On addition of ammonium solution (2 ml) to the lower phase of the mixture reddish pink or yellow colour developed indicating the presence of anthraquinone glycosides. Chloroform (5 ml) was mixed to 0.5ml of the extract, yellowish brown ring formed at the interface on addition

of concentrated $H_2SO_4$ (5 ml) to the mixture, was confirmatory to steroids. The mixture of the extract (2.5 ml) and chloroform (1 ml) produced reddish brown colour, which turned into colourless on addition of concentrated $H_2SO_4$ (1.5 ml) indicating the presence of terpenoids. For quinone, deep reddish-brown ring developed after addition of few drops of concentrated $H_2SO_4$ into test extract (1 ml). To detect phenol, 1 ml of extract was mixed with 2% NaOH solution and then few drops 10% HCl was added that formed a colourless solution. Retention of froth, on incubation at water bath (80 ºC) for 3-4 min, developed in the mixture of the plant extract and distilled water on shaking, was the indicative for the presence of saponins.

***Determination of antibacterial activity of plant extracts***

The antibacterial activity of fruit extracts was determined by agar-well diffusion method, using nutrient agar plates, following the protocol described elsewhere, against some food borne bacterial strains: *Bacillus* sp. A1AC, *Bacillus* sp. A3C1, *Bacillus* sp. G2D1b, *Bacillus cereus* (B4A), *Bacillus cereus* F61, *Bacillus cereus* IA2A3, *Enterococcus* sp. G2D1 and *Corynebacterium* sp. A2B1.[18] The concentrations of the extracts used were 15, 25 and 50 µl/well (i.e. equivalent to 1.875, 3.125 and 6.25 mg/well, respectively, for OSE and OMeE, and 2.49, 4.15 and 8.3 mg/well for AqOSE and AqOMeE. The ZDI (zone diameter of inhibition) values obtained due to the action of the extracts, for the test bacterial isolates, were interpreted for resistance and sensitivity of the isolates.[23]

***Determination of minimum inhibitory concentration***

The minimum inhibitory concentration (MIC) values of OSE and OMeE for the test bacterial isolates was determined by agar dilution method, using the concentrations of1.875-12.5mg/ml, for both the extracts, and the results were interpreted as described elsewhere.[23,24]

## RESULTS

The antibacterial activity of *E. floribundus* fruit extracts (mesocarp-epicarp, and seed) has been represented in Figure 2 and Figure 3. The ethanolic extracts of *E. floribundus* fruit part (mesocarp-epicarp, and seed) and aqueous extracts of mesocarp-epicarp showed antibacterial activity against the test bacteria, while the seed aqueous extracts had no activity.

**Table 1. Minimum inhibitory concentration (MIC) values of OSE and OMeE against food borne bacteria.**

| Bacterial isolates | Extract name | MIC (mg/ml) |
|---|---|---|
| *Bacillus sp.* (A1AC) | OSE | 12.5 |
| | OMeE | 3.125 |
| *Bacillus sp.* (A3C1) | OSE | 9.375 |
| | OMeE | 2.5 |
| *Bacillus sp.* (G2D1b) | OSE | 9.375 |
| | OMeE | 1.875 |
| *Bacillus cereus* (B4A) | OSE | 12.5 |
| | OMeE | 3.125 |
| *Bacillus cereus* (F61) | OSE | 12.5 |
| | OMeE | 3.125 |
| *Bacillus cereus* (IA2A3) | OSE | 10 |
| | OMeE | 3.125 |
| *Enterococcus sp.* (G2D1) | OSE | 12.5 |
| | OMeE | 3.125 |
| *Corynebacterium sp.* (A2B1) | OSE | 10 |
| | OMeE | 3.125 |

OSE: olive seed ethanolic extract; OMeE: 'olive mesocarp-epicarp ethanolic extract.'

The mesocarp-epicarp had ZDIs 6-28mm, for the ethanolic extracts, and 7-23mm, for the aqueous extracts; the ethanolic seed extract had ZDIs 6-15 mm.

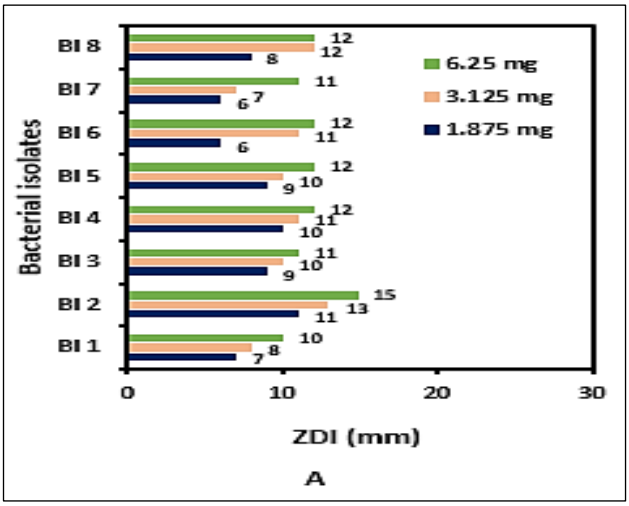
**Table 2: Bioactive components detected in various extracts of olive fruits.**

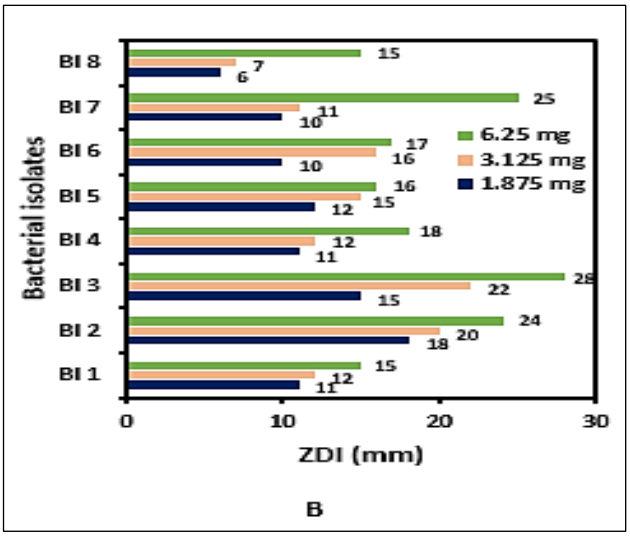
| Bioactive compounds | Ethanolic extracts | | Aqueous extracts | |
|---|---|---|---|---|
| | OSE | OMeE | AqOSE | AqOMeE |
| Flavonoids | - | - | - | - |
| Cardiac glycosides | + | + | + | + |
| Anthraquinone-glycosides | + | + | + | + |
| Steroids | + | + | + | + |
| Terpenoids | + | + | + | + |
| Quinones | + | + | + | + |
| Phenol | - | - | + | + |
| Sapponins | - | - | - | - |

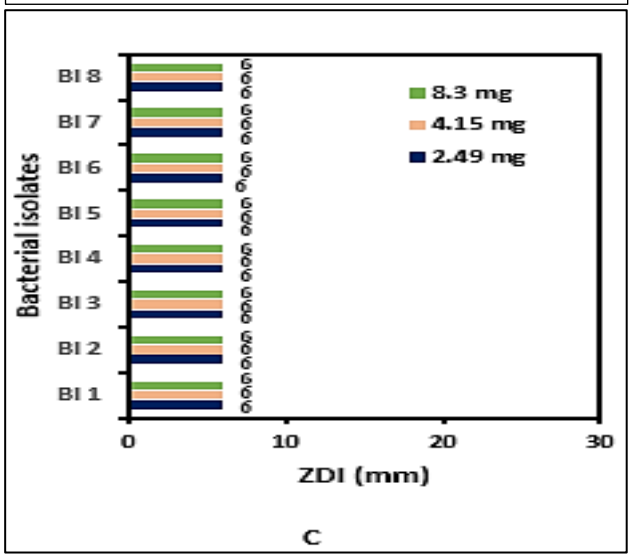
AqOSE: aqueous olive seed extract; AqOMeE: aqueous olive mesocarp-epicarp extract; OSE: ethanolic olive seed extract; OMeE: ethanolic olive mesocarp-epicarp extract; '+' : Positive; '-' :Negative.

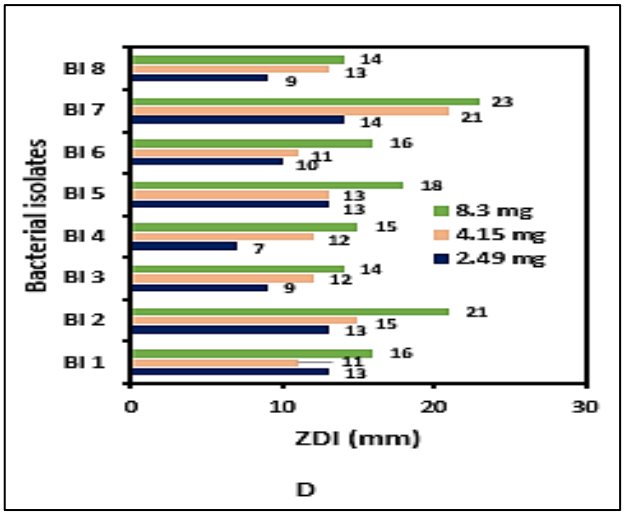
The MICs of OSE and OMeE for the test bacteria ranged 9.375 - 12.5 mg/ml and 1.875 - 3.125 mg/ml, respectively (Table 1).

The bioactive components detected in the ethanolic and aqueous extracts of mesocarp-epicarp and seed of *E. floribundus* are represented in Table 2.



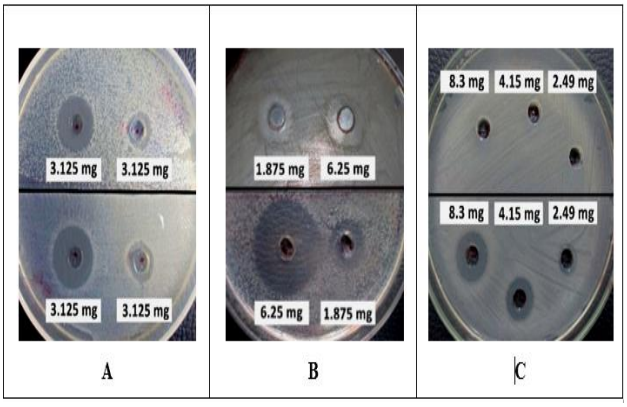




BI: bacterial isolates; 1: *Bacillus sp.*A1AC; 2: *Bacillus sp.*A3C1; 3: *Bacillus sp.* G2D1b; 4: *Bacillus cereus* B4A; 5: *Bacillus cereus* F61; 6: *Bacillus cereus* IA2A3; 7*: Enterococcus sp.*G2 D1; 8: *Corynebacterium sp.* A2B1.

**Figure 2: A. Antibacterial activity in terms of zone diameter of inhibition (ZDI) of olive fruit parts extracts: (A) ethanolic olive seed extract; OSE, (B) ethanolic olive mesocarp-epicarp extract; OMeE, (C)aqueous olive seed extract; AqOSE, (D) aqueous olive mesocarp-epicarp extract; AqOMeE.**




A: OMeE (3.125 mg/well) on left upper part and OSE (3.125 mg/well) on right upper part against *B. cereus* IA2A3 strain, and OMeE (3.125 mg/well) on left lower part and OSE (3.125 mg/well) on right lower part against *Corynebacterium sp.* A2 B1 strain; B: OSE (left well: 1.875 mg; right well: 6.25 mg) on the upper part against *Bacillus cereus* F61 strain and OMeE (left well: 6.25 mg; right well: 1.875 mg) on the lower part against *Bacillus sp.*G2D1b strain; C: AqOSE on the upper part and AqOMeE on the lower part against *Corynebacterium sp.* A2B1 strain (left well: 8.3 mg; middle well: 4.15 mg; right well: 2.49 mg, on both upper and lower parts).

**Figure 3: Agar-well diffusion method demonstrating the antibacterial activity of olive fruit parts extracts on culture plate.**

## DISCUSSION

The emergence of multidrug resistance among bacterial pathogens, including the food-borne ones, caused due to

the rampant use of antibiotics therapeutically, as well as their (antibiotics) nutritional application in agriculture, renders global antibiotic resistance difficulty.[17] As such the search of new agents in combating bacterial antibiotic resistance for the recovery of food-borne infection, at least in the developing country like India, is an urgent task. The food-borne microorganisms usually and effortlessly transmit from the foods of daily usage contaminated from the hands of infected persons with unhygienic practices to the gastrointestinal tracts of healthy persons.[25] Most of the cases of food poisoning occurs every year are due to bacterial pathogens, such as *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringes*, *Clostridium botulinum*, *Campylobacter* sp., *Salmonella* spp., entropathogenic *Escherichia coli* and *Vibrio parahaemolyticus* that are found generally in raw foods.[26] Therefore, scientific studies in search of new antimicrobials, in order to combat the bacterial antibiotic resistance as well as to control the food borne infection, are imperative, in such an emergent situation.

In the current study, the food-borne isolates of *Enterococcus* sp., *Corynebacterium* sp. and *Bacillus* spp. were subjected to their susceptibility against *E. floribundus* fruit extracts, and the top ZDI value was recorded as 28 mm. Medina et al. reported the efficacy of the aqueous extracts of virgin olive oil against food-borne pathogens, such as *Salmonella enteritidis* and *Listeria monocytogenes.*[27] Zaman validated the antibacterial capacity of various extracts of *E. floribundus* leaves against *Staph. aureus*, *B. cereus*, *B. megaterium*, *B. subtilis*, *E.coli*, *V. parahemolyticus*, *Shigella dysenteriae*, *Salmonella enterica* serovar para Typhi and *Pseudomonas aeruginosa*, following disc diffusion method, and recorded the ZDIs of 22 mm.[7] Karaosmanoglu et al. verified the bactericidal activity of olive extract against three food-borne pathogenic bacteria: *E. coli* O157:H7, *L. monocytogenes* and *S. enteritidis*.[28] Sousa et al reported that the table olive had excellent growth inhibitory action against gram-positive (*B. cereus*, *B. subtilis*, and *Staph. aureus*) as well as gram-negative bacteria (*P. aeruginosa*, *E. coli* and *K. pneumoniae*) at concentration 50 µg/ml.[29] The olive leaves (*O. europaea*) acetone extract, as per the report of Korukluoglu et al., had great inhibition activity against gram-positive: *S. enteritidis*, *B. cereus*, *Enterococcus faecalis*, *Streptococcus thermophilus*, and gram-negative: *K. pneumoniae*, *E. coli*, bacterial pathogens.[30] Some other plants belonging to the family *Elaeocarpaceae* demonstrated antibacterial activity, as has been reported by earlier authors, such as the aqueous and/or ethanolic extracts of several species of *Elaeocarpus* (*E. munronii*, *E. serratus* var. Serratus, *E. serratus* var. *Weibelii*, *E. tuberculatus*, *E. variabilis*, *E. sphaericus* and *E. serratus*) leaves inhibited the growth of different pathogenic bacteria, including *Staph. aureus*, *Shigella flexneri* and *B. subtilis* activity.[31-33]

In order to corroborate the results of antibacterial activity of the plant extracts, MICs were determined by many earlier authors. In our earlier studies, the antibacterial activity of *Mimusops elengi* and *Azadirachta indica* seed extracts have been demonstrated.[34,35] *A. indica* seed extract had MICs 200 – 300µg/ml, and *Bacopa monniera* leaf extract had MICs 300 – 600µg/ml, against MDR *S. enterica* serovar paraTyphi isolates.[35] Sudjana et al determined MICs of a commercial extracts derived from *O. europaea* (olive) leaves by various method against pathogenic bacteria like *Helicobacter pylori*, *Campylobacter jejuni*, *Staph. aureus* and meticillin-resistant *Staph. aureus* (MRSA), and reported the values 0.31–0.78%.[36] In the current study, ethanolic extracts of *E. floribundus* seed and mesocarp-epicarp fruit parts were used to determine the MICs against some food-borne bacteria, and the values ranged 9.375 - 12.5 mg/ml, for seed ethanolic extracts, and 1.875 - 3.125 mg/ml, for ethanolic mesocarp-epicarp extracts.

The capacity of antibacterial activity of plant extracts is due to the presence of various phytocomponents active against the test bacterial pathogens. Specific phenolic compounds were identified and quantified from olive leaves extracts possessing effective antimicrobial capacity.[37-41] The bactericidal efficacy of seven phenolic compounds from different table olives (in Portugal, namely, natural black olives “Galega” and black ripe olive “Negrinha de Freixo”) have been demonstrated against gram-positive (*B. cereus*, *B. subtilis*, *Staph. aureus*) and gram-negative bacteria (*P. aeruginosa*, *E. coli*, *K. pneumoniae*) bacteria.[37] Medina et al reported the potentiality of olive fruit oils in the growth inhibition of foodborne bacteria, such as *L. monocytogenes*, *Staph. aureus*, *S. enterica*, *Yersinia* sp., and *Shigella sonnei*.[42] Sharvani*et al.* revealed that the aqueous leaf extracts of different species of *Elaeocarpus* (*E. munronii*, *E. tuberculatus* and *E. variabilis*) possess several bioactive compounds like flavonoids, glycosides, steroids, tannins and terpenoids.[31] In the current study, phytocomponents, like cardiac glycosides, anthraquinone glycosides, steroids, terpenoids, quinone and phenol have been detected in the test extracts prepared from different parts of olive fruits, and the extracts (OMeE, OSE and AqOMeE) had excellent antibacterial activity; however, AqOSE had no activity against the target bacterial pathogens.

## CONCLUSION

The ethanolic extracts of various parts of olive fruits had excellent antibacterial activity in terms of ZDIs against a spectrum of food-borne bacteria, while only the mesocarp-epicarp aqueous extract was found excellent in terms of ZDIs against the test bacteria. The olive fruit aqueous and ethanolic extracts had a wide range of bioactive phytocomponents, as detected qualitatively. The olive fruits, thus, might be useful in the preparation of non-antibiotic antibacterial agents to be administered against bacterial infection and in the storage of food as well. However, further studies are mandatory for the

pharmacokinetics of the extracts and dose determination, in clinical practices.

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