Original Research

Potential antibacterial activity of ethanolic Fig and Olive leaves extract against *Enterococcus Faecalis*

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Abstract

There has been an increasing awareness about the importance of herbal plants in therapeutic uses which are safe, efficient and induce little side effects This study was based on the evaluation of antibacterial activity of ethanolic extracts of the leaves of *Oleaeuropaea* (Olive tree) and *Ficuscarica* (Fig tree), alone and in combination, against enterococcus faecalis (*E. faecalis*) strain using well diffusion method and minimum inhibitory concentration. The results revealed that the *Oleaeuropaea* leave extract is more potent than the *Ficuscarica* leave extract against *E. faecalis*, as the zone of inhibition was 23 mm and 3 mm, respectively. By mixing both extracts, the results showed a slight synergistic effect, as the zones of inhibition were 20 mm, 24 mm, 24 mm and 25 mm for 1:1, 1:3, 1:6 and 1:9 ratios of the *Oleaeuropaea* leave and the *Ficuscarica* leave extracts, respectively. The minimum inhibitory concentration for *E. faecalis* growth was 200 mg/ml for the *Oleaeuropaea* leave alone, *Ficuscarica* and mixed with *Ficuscarica*. The present findings conclude a potent antibacterial activity of the olive and fig leave, and, their potential as a source of drug in the treatment of *E. faecalis* infections is suggested.

Keywords: Antibacterial activity, *E. faecalis, Ficuscarica*, fig, *Oleaeuropaea*, olive

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Introduction

Enterococci, particularly antibiotic resistant enterococci have emerged as important nosocomial pathogen around the world [1, 2]. The most frequent clinical enterococcal infections are urinary tract infections [3] and have been related to infectious endocarditis and surgical wound infection [4]. *Enterococcus faecalis* are considered important difficult-to-treat pathogens, due to their intrinsic resistance to several antimicrobial agents and their propensity to acquire resistance [5]. In recent years, there has been an increasing awareness about the importance of herbal medicines which are easily available, inexpensive, safe, efficient, and induce little side effects [6]. The *Ficuscarica* is one of the oldest herbal trees that was used as herbal medicine [7]. *Oleaeuropaea*is cultivated from ancient time in Mediterranean regions [8]. The phenolic compound (Oleuropein) represents the highest amount of olive leaves (up to 60 - 90 mg/g dry leaves) and so reflect the antibacterial activity of different leaves extracts [9]. Mohamed and others [10] studied aqueous Fig (Ficuscarica L.) leaves' extract (FLE), Olive (Oleaeuropaea L.) leaves' extract (OLE) and their mixture (MLE) to extend the shelf life of pasteurized milk. The potential antimicrobial activity of OLE, FLE, and MLE were evaluated against food borne pathogens involving three Gram - positive bacterial strains (*Staphylococcus aureus*, *E. faecalis*, *and Bacillus cereus*) and three Gram-negative bacterial strains (*Escherichiacoli*, *Salmonella entericaserovarTyphi*, *and Pseudomonas aeruginosa*). The study found that FLE was the most active extract

inhibiting bacterial growth of Gram - negative bacterial strains (*E. coli, S. typhi, and P. aeruginosa*) as well as S. aureus. FLE has a relatively weak antimicrobial against both spores forming bacteria (*B. cereus*) and thermoduric bacterial strain (*E. faecalis*). However, OLE was very effective agent against Gram-positive bacterial strains (*B. cereus, E. faecalis, and S aureus*). MLE was potent as an extract by inhibiting bacterial growth of all tested strains [10]. However, ethanolic extract of fig and olive leaves were not evaluated.

It has been reported that the antimicrobial activity of aqueous and ethanolic fig leaves extracts from five different regions in Morocco against sixteen pathogenic bacterial strains including *S. aureus* (SA) and (MRSA). The results of this study reported that the aqueous extracts had a better activity against gram - positive bacteria including (SA) and (MRSA) than ethanolic extracts [11]. Although *E. faecalis* was not included in this study. It has also been found that the antibacterial effect of olive leaves extracts was higher than that of the stem extracts, and that the petroleum ether extract of the olive leaves and stems reported no activity against SA and MRSA, while ethanolic extract of olive leaves caused high inhibition zone against both of them [12]. Moreover, leaf extract was not broad-spectrum in action, showing appreciable activity only against *H. pylori*, *C. jejuni*, *S. aureus* and MRSA [13]. The aim of this study was to evaluate the antibacterial activity of locally collected *Oleaeuropaea* and *Ficuscarica* leaves ethanolic extracts and their synergistic action against *E. faecalis*.

Materials and methods

Medicinal plant materials and preparation: The leaves of two medicinal plants, namely *Oleaeuropaea* and *Ficuscarica* were collected from Al-Assaba area in western region of Libya. The medicinal plants were identified and confirmed by Department of Botany, Faculty of Science, Aljabal Algharbi University. The leaves of the medicinal plants were collected in early morning, then cleaned with tap water to remove dusts and dried at shadow for 15 days till they became crisp. After drying, the leaves were powdered finely using a blender.

Plant ethanolic extraction: Extraction procedure were carried out at the microbiology laboratory at Al-Assaba General Hospital. Briefly, 50 grams of each finely powdered plant were separately dissolved in a flask containing 500 ml ethanol 96% for 48 hr using hot plate magnetic stirrer. The samples were filtered using filter paper Whatman filter paper No 1. The filtrates were collected and evaporated to dryness using hot air oven at 40 ºC and the residue was kept in the refrigerator at 4 ºC until use as described [14].

Bacterial isolate: Bacterial strain of *E. faecalis* (Gram - positive cocci) was isolated from out-patient in Microbiology Department of Al-Assaba General Hospital. The aforementioned bacterial isolate was subjected to gram staining, growth on selective media and some biochemical tests, using Bergy´s manual of determinative bacteriology charts to verify the bacterial isolates [15]. The pure culture bacteria were streaked on nutrient agar plates and incubated at 37 ºC for approximately 24 hr to obtain isolated, actively growing colonies.

Antibacterial activity assay: Antimicrobial activity of both leaves extracts were researched by well diffusion method on Mueller-Hilton agar (Oxoid CM337) [16]. Both leaves extracts were dissolved in 2:4 Dimethyl Sulfoxide (DMSO) and water, respectively. All assays were carried out under aseptic conditions and performed trice to check the results. Suspension of the tested microorganisms (10^8 CFU/µL) was spread on the solid media plates [17]. Then the 6-mm diameter wells were punched into the Muller- Hinton agar using sterile well cutter, 25µl of the desired extract (200 mg/ml) from *Ficuscarica* and *Oleaeuropaea* ethanolic extract was added and placed on the inoculated agar and they were incubated at 37 °C for 24 hrs. The antimicrobial activities were evaluated by measuring the zones of inhibition against the test organisms.

Determination of the minimum inhibitory concentration (MIC): The antimicrobial activity of the both plant leaves extracts were determined using the broth micro-dilution assay as previously described with slight modifications [18]. Six different concentrations were tested in the microdilution method starting with 200, 100, 50, 25, 12.5 and 6.25 mg/ml. Briefly, a stock solution was prepared by dissolving 200 mg of each extract in one ml of the solvent containing dimethyl sulfoxide and water in a ratio of 2:4 v/v, respectively. 100 µl of nutrient broth only were dispensed in the first well to serve as a first negative control, then 100 µl of DMSO and water (2:4) added to the second as well as the other control, then 200 µl of each 200 mg per ml extract solution were added to the other well and serial dilution was performed by taking 100 µl from the extract and transferred to other wells until reaching to last concentration 6.25 mg/ml. Aliquot of 100 µl *E. faecalis* bacterial broth 10^8 cfu/ml previously prepared was added to each well. Furthermore, the previous prepared 200 mg both extracts were mixed together in a ratio of 1:1, 1:3, 1:6 and 1:9 fig/olive (F:O), respectively, and serial dilution was done in the same manner. The plates were incubated for 24 hr at 37 ºC, the MIC was detected by the lack of turbidity in the wells, for the confirmation of growth inhibition, the subcultures from no-growth wells were incubated for 24 hr at 37 ºC.

Statistical analysis: Each experiment was repeated three times. All data were presented as Mean ± SEM. IBM

SPSS version 20 software was used for the analysis and the results were tested by one-way analysis of variance (ANOVA) followed by Dunnett's test (2-sided) and $p < 0.05$ was considered statistically significant.

Results

In **Figure 1**, there is a significant variation between *Ficuscarica* and *Oleaeuropaea* ethanolic leaves extracts at concentration of 200 mg/ml on the growth of *E. faecalis* ($p < 0.05$).*Oleaeuropaea* leaves extract was the more effective for the inhibition of *E. faecalis* than the *Ficuscarica* leaves extract by 7 folds. Zone of inhibition were 23 mm and 3 mm, respectively, (**Figure 1**). In contrast, there were no statistically significant differences for the efficacy of synergistic activity of *Ficuscarica* and *Oleaeuropaea* leaves extracts on *E. faecalis* growth. The ratio 1:1, 1:3, 1:6 and 1:9 showed convergent zone of inhibition i.e. 20 mm, 24 mm, 24 mm and 25 mm, respectively, (**Figure 1**). The minimum inhibitory concentration exhibited by ethanolic extract of *Ficuscarica* and *Oleaeuropaea* leaves on growth of *E. faecalis* showed in **Table 1**. *Oleaeuropaea* and *Ficuscarica* leaves extract inhibited *E. faecalis* at concentration of 200 mg/ml, there was also inhibition action against *E. faecalis* when *Ficuscarica* mixed with *Oleaeuropaea* leaves in all ratio of F:O at concentration of 1:1 (100/100 mg/ml), 1:3 (50:150 mg/ml), 1:6 (80:120 mg/ml) and 1:9 (20:180 mg/ml).

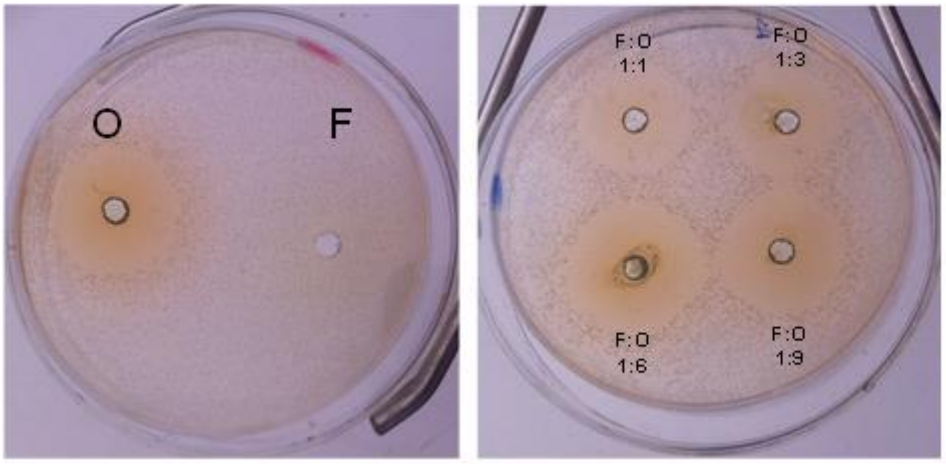

Figure 1: Zone of inhibition effect of fig (F), olive (O) and ratio of F : O 1 : 1, 1 : 3, 1 : 6 and 1 : 9 on growth of *E. faecalis*

Table 1: MIC exhibited by ethanolic extract of tested plants on growth of *E. faecalis*, non-growth (-) and growth (+)

Test plants extract	Tested concentrations (mg/ml) Serial dilution					
	200	100	50	25	12.5	6.25
Oleaeuropaea	-	+	+	+	+	+
Ficuscarica	-	+	+	+	+	+
Ratio (mg/ml)	**Serial dilution**					
F:O 1:1 (100:100)	-	+	+	+	+	+
F:O 1:3 (50:150)	-	+	+	+	+	+
F:O 1:6 (80:120)	-	+	+	+	+	+
F:O 1:9 (20:180)	-	+	+	+	+	+
DMSA +	**NB+**					

Discussion

According to World Health Organization report about 80% people of the world used traditional medicines for health care [19] .Thus, in this study, two plants are chosen which are commonly used in Libya to be exhibited synergistic activity against *E. faecalis*. Ethanolic extract was used as it was more active and safe, when compared with other organic solvents used in other literature review. Olive leaves extract exhibited stronger inhibitory action against (*E. faecalis*) than fig leaves extract by seven folds (zone of inhibition were 23 and 3 for olive leaves and fig leaves, respectively) while the extracts together showed relatively differences in inhibitory zones which reflected that there was a slightly synergism action between both extracts. In this study, ethanol extraction was conducted because of that this extraction was more active than other organic solvents as mention in the previous study [18]. The only limitation of present study was the use of one species of gram-positive bacteria *in vitro*, in addition of using one an organic solvent for extraction procedure. Furthermore, we used leaves of tested plants but not fruits. Therefore, all these shortcomings should be considered in further studies and should be supported by *in vivo*. Our findings highlight that olive extraction had significant effect on *E. faecalis*. The high synergistic action of fig and olive leaves extraction (100:100 mg/ml) against *E. faecalis* done in this study may be useful observation for traditional medicine to decrease side effect of high concentration of olive extraction alone (200 mg/ml) on growth of *E. faecalis*. In our previous finding, Abeed and others [20] showed that there were high synergistic action of fig and olives leaves extraction (100:100 mg/ml) against SMRSA. Similarly, olive leaves extracts play moderately active against S. typhimurium and highly active against the other bacteria including *E. faecalis* (ATCC 29212) [21]. Moreover, these observations are consistent with the previously findings reported that aqueous olive and fig mixture leaves extract was inhibiting bacterial growth of all tested strains including *E. Faecalis* [10].

Conclusion

The results provide that using of olive extract, fig extract or a mixture of olive and fig extract had antibacterial activity against *E. faecalis* which can be used as antimicrobial agent for treatment infected surgical wound. *E. faecalis* has also been related to oral diseases, such as caries.

Therefore, the current finding highlights that these leaves extracts could be applied as toothpaste or mouth wash to protect from *E. faecalis* infection.

Author's contribution

A. K. Elbaz and A.A. Abeed have designed the study, drafting the manuscript, and revised it for important intellectual context. A. K. Elbaz collected data. O.K. Sawesi and A. K. Elbaz performed the analysis and interpretation of data. All authors have proofread and approved the final version of the manuscript.

Ethical issues

Including plagiarism, Informed Consent, data fabrication or falsification and double publication or submission have completely been observed by authors.

Conflict of interest

The authors have declared that no competing interest.

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