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Synergistic Antibacterial Activity of Ethanolic Extracts of Olea europaea and Ficus carica Leaves Against Methicillin-resistant Staphylococcus aureus

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ABSTRACT

This study was based on the evaluation of antibacterial activity of ethanolic extracts of the leaves of *Olea europaea* (Olive tree) and *Ficus carica* (Fig tree), separately and synergistically, against a standard Methicillin-resistant *Staphylococcus aureus* (SMRSA) strain using well diffusion method and minimum inhibitory concentration (MIC). The results revealed that the *Olea europaea* leaves extract was more potent than the *Ficus carica* leaves extract against MRSA, as the zone of inhibition was 12.6 mm and 6 mm respectively. By mixing both extracts, the results showed a slight synergistic effect, as the zones of inhibition were 10.6 mm,12 mm,11.3 mm and 12 mm for 1:1, 1:3, 1:6 and 1:9 ratios of the *Olea europaea* leaves and the *Ficus carica* leaves extracts respectively. The MIC for SMRSA growth was 200mg/ml for the *Olea europaea* leaves alone and mixed with *Ficus carica*. However, *Ficus carica* alone did not inhibit the growth of SMRSA. These results showing a potent anti-MRSA activity of the *Olea europaea* leaves and, therefore, their potential as a source of drug in the treatment of MRSA infections.

KEYWORDS: Antibacterial activity*, Ficus carica, Olea europaea, synergistic,* SMRSA

1. INTRODUCTION

Methicillin – resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens. MRSA strains are particularly serious and potentially lethal respiratory tract, and so threatens the public health and leading to high morbidity and mortility [2].

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 [3]. The *Ficus carica* is one of the oldest herbal trees that was used as a herbal medicine [4]**.** Galal (2012) studied the antimicrobial activity of aqueous and ethanolic fig leaves extracts from five different regions in Morocco against sixteen pathogenic bacterial strains including *Staphylococcus aureus* (*S. aureus*) and MRSA**.** The results of the study reported that the aqueous extracts had a better activity against gram-positive bacteria including *S. aureus* and MRSA than ethanolic extracts [5].

In a recent study**,** the in vitro antibacterial effect of ethanolic extracts of different *Ficus* species against clinical isolates of MRSA has been evaluated using well diffusion method. the results revealed an antibacterial activity with different zone of inhibition from 11 to 25 mm [6]. In contrast, the methanolic fig leaves extracts inhibited the growth of *Streptococcus pyogenes* (61%) and *Salmonella typhi* (55.5%), while it had no effect against *E. coli* and *S. aureus* [7].

In another study, the methanolic extract of fig leaves showed a strong antibacterial activity against oral bacteria pathogens that possess virulence factors including toxins, adhesins, enzymes and immunomodulators [1]. Moreover, it's also an important pathogen that causes a broad spectrum of infections including bones and joints, and exhibited less activity against the *S. aureus* reference strain ATCC 29213[8].

Olea europaea is cultivated from ancient time in the Mediterranean regions [9]. The phenolic compound (Oleuropein) represents the highest amount of olive leaves (up to 60-90mg/g dry leaves) and so reflect the antibacterial activity of different leaves extracts [10]. The antibacterial activity of olive leaves aqueous extracts against sixteen bacterial strains including *S. aureus*, Oxacillin – methicillin resistant *Staphylococcus aureus* (oxa-met RSA) ATCC43300 and methicillin- oxacillin resistant *Staphylococcus* MU-40 has been evaluated. The results showed an antibacterial activity against (oxa-met RSA) ATCC43300and methicillin- oxacillin resistant *Staphylococcus* MU-40 (15 mm) [11]**.** 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 *S. aureus* and MRSA, while ethanolic extract of olive leaves caused a high inhibition zone against both of them [12]. Additionally, the antimicrobial capacity order for several concentrations of olive leaves extracts (OLEs) was as follow; B. cereus~ C. albicans > E. coli > S. > C. neoformans ~ K. pneumonia ~ P. aeruginosa > B. subtiis [13].

Markin(2003) also reported that aqueous extract of olive leaf with a concentration of 0.6% w/v has killed *E. coli,*

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P. aeroginosa, S. aureus and *K. pneumoniae* in 3 hrs. exposure [14]. Moreover, the antibacterial activity of olive leaf extract with large variety of bacteria including *S. aureus* has been studied. The results indicated that OLE didn't present broad-spectrum antibacterial activity, but had an appreciable activity on *H. pylori* and *C. jejuni* [15].

The aim of this study was to evaluate the antibacterial activity of locally collected *Olea europaea* and *Ficus carica* leaves ethanolic extracts against MRSA and to evaluate their synergistic action against the same strain.

MATERIALS AND METHODS

2.1 MEDICINAL PLANTS MATERIALS AND PREPARATION

The leaves of two medicinal plants, namely *Olea europaea* and *Ficus carica*, were collected from Al'assabiea area in western Libya. The medicinal plants used in this study were identified and confirmed by Botany Department, 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.

2.2 PLANT ETHANOLIC EXTRACTION

Extraction procedure was carried out at the microbiology laboratory at Al'assabiea General Hospital as previously described [16]. Briefly, 50 grams of each finely powdered plant were separately dissolved in a flask containing 500ml ethanol 96% for 48 hours using hot plate magnetic stirrer. The samples were filtered using filter paper Whatman No. 1. The filtrates were collected and evaporated to dryness using hot air oven at 40ºC and the residue was kept in the refrigerator at 4 ºc until use.

2.3 MRSA STRAIN

Standard Methicillin-resistant *Staphylococcus aureus* strain (ATCC33591), was obtained from the Department of Microbiology at the Biotechnology Research Center in Tripoli. The bacterial isolate was grown aerobically on nutrient broth after incubation for 24hrs at 37 ºc using rotary instrument to enhance growth.

2.4 ANTIBACTERIAL ACTIVITY ASSAY

Antimicrobial activity of both leaves extracts were researched by well diffusion method on Mueller-Hilton agar (Oxoid CM337) [17]. The leaves extracts of both plants were dissolved in 2:4 Dimethyl Sulfoxide (DMSO) and water respectively. All assays were carried out under aseptic conditions. Suspension of the tested microorganisms (10^8 CFU/µL) was spread on the solid media plates [18]. Then the 6 mm diameter wells were punched into the Muller- Hinton Agar using sterile well cutter, 25µl of the desired extract(200mg/ml) from *Ficus carica* and *Olea europaea* 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.

2.5 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 [19]. Six different concentration were tested in the micro dilution 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. 100µl of nutrient broth only were dispensed in the first well to serve as a first negative control, then 100µl of DMSO and water (2:4) added to the second well as a second negative control, then 200µl of each 200mg/ml extract solution were added to the other well and serial dilution was performed by taking 100µl from the extract and transferred to other wells until reaching to last concentration 6.25mg/ml. Aliquot of 10µl MSAR bacterial broth 10^8 cfu/ml previously prepared was added to each well. Furthermore, the previously prepared 200 mg/ml extract of *Ficus carica* was mixed together with that of *Olea europaea* in a ratio of 1:1,1:3, 1:6 and 1:9 respectively and serial dilutions were done in the same manner. The plates were incubated for 24 hrs. at 37ºC. The MIC was detected by the lack of turbidity in the wells, for the confirmation of growth inhibition. Subcultures from no-growth wells were incubated for 24 hrs. at 37ºc.

2.6 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 analyzed by one-way analysis of variance (ANOVA) followed by Dunnett t-test (2-sided). $p < 0.05$ was considered significant.

RESULTS

There was a significant variation between *Ficus carica* and *Olea europaea* leaves ethanolic extracts at concentration 200mg/ml on the growth of SMRSA ($P<0.05$). *Olea europaea* leaves extract was 2 folds more effective for the inhibition of SMRSA than the *Ficus carica* leaves extract with zones of inhibition of 12.6mm and 6mm respectively, as shown in figure 1. In contrast, there were no statistically significant differences for the efficacy of synergistic activity of *Ficus* carica and *Olea europaea* leaves extracts on SMRSA growth ($P>0.05$) (Table1). The ratios 1:1,1:3,1:6 and 1:9 showed convergent zone of inhibition of 10.6mm,12mm,11.3mm and 12mm respectively (Fig1). The minimum inhibitory concentration exhibited by ethanolic extract of *Ficus carica* and *Olea europaea* leaves on growth of SMRSA showed in the (table 2).*Olea europaea* leaves extract inhibited SMRSA at concentration of 200mg/ml, while there was no effect of *Ficus carica* leaves extract against SMRSA alone but there was inhibition action against SMRSA with mixes of *Ficus carica* and *Olea europaea* leaves extracts in all ratios 1:1 (100/100 mg/ml), 1:3 (50:150 mg/ml), 1:6 (80:120 mg/ml) and 1:9 (20:180mg/ml)

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Table 1: The inhibitory effect of ethanolic extracts of *Ficus carica* and *Olea europaea* on the growth of SMRSA.

(I) plant extract (200mg/ml)	(J) plant extract (200mg/ml)	Mean Difference (I-J)	Std. Error	Sig.
Ficus carica	*Olea europaea*	-6.66667-*	2.10819	0.032
f/o 1:1	*Olea europaea*	-2.00000-	2.10819	0.805
f/o 1:3	*Olea europaea*	-0.66667-	2.10819	0.997
f/o 1:6	*Olea europaea*	-1.33333-	2.10819	0.950
f/o 1:9	*Olea europaea*	-.66667-	2.10819	0.997

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

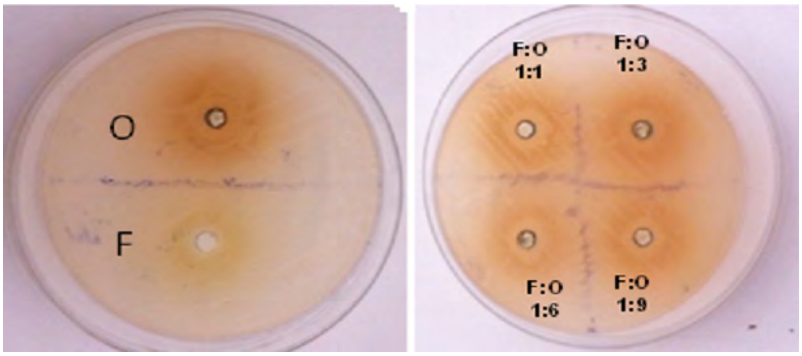

Fig 1: Zone of inhibition of *Ficus carica* (F) and *Olea europaea* (O) leaves extracts] on growth of SMRSA.

Table 2: MIC exhibited by ethanolic extract of tested plants on growth of SMRSA, non-growth (-) and growth (+)

Test plants extract	Tested concentrations (mg/ml) Serial dilution					
	200	100	50	25	12.5	6.25
Olea europaea	-	+	+	+	+	+
Ficus carica	+	+	+	+	+	+
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+					

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1- DISCUSSION

According to the World Health Organization, about 80% people of the world used traditional medicines for health care [20]. Several studies reported that there were many phenolic and flavonoid compounds produced by plant exerts and have antibacterial activity against MRSA [21]. Therefore, it is likely that the phenolic and flavonoid compounds in fig leaves may be related, in part, to the antibacterial effects observed in the present study. In addition, its reported by some researchers that the oleuropein in olive leaves has a lot of pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, anti-atherogenic anti-carcinogenic and antiviral activities [22]. Similarly, olive leaves in this study had an antibacterial activity against SMRSA. The increasing resistance of *S. aureus* to methicillin and other β-lactam antibiotics stresses the necessity to experiment the natural compounds for exploring their antibacterial activity and their potential as an alternative medicine. Two plants, *Ficus carica* and *Olea europaea,* were collected in Libya to be tested for their antibacterial synergistic activity against SMRSA. In this study, ethanoic extract was used because it is more active and safe in comparing with other organic solvents [19]. *Olea europaea* leaves extract exhibited stronger inhibitory action against (SMRSA) than *Ficus carica* leaves extract by 2 folds with zones of inhibition of 12.6 mm and 6 mm respectively, while both extracts mixed together showed relative differences in inhibitory zones indicating that there was slight synergism action between both extracts. In this study, the ethanol extraction was conducted because of that this extraction was more active than other organic solvents as mention in our previous study. The limitation of present study was the use of only one species of Gram-positive bacteria. furthermore, we used leaves of tested plants but not fruits. All these shortcomings would be considered in further studies. Our findings highlight that *Olea europaea* extraction had significant effect on SMRSA and this is the only study performed to investigate the synergistic action between the extraction of two mentioned plants. The high synergistic action of *Ficus carica* and *Olea europaea* leaves extraction (100:100 mg/ml) against SMRSA showed in this study maybe a useful observation for traditional medicine to decrease side effects of high concentration of *Olea europaea* extraction alone (200 mg/ml) on growth of SMRSA.

2- CONCLUSION

These results suggest that synergistic effect between ethanoic extract of *Ficus carica* and *Olea europaea* possess antibacterial activity, which can be used as antimicrobial agent in new drugs for eradication of MRSA. However, further research is needed to study the synergistic action of both mixed plant used with different organic solvents and different strains of bacteria. In addition, antibacterial effects of other plant component rather than leaves could be investigated.

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4- CONFLICT OF INTEREST

I declare that I contributed with my colleagues entitled in the design, execution and analysis of the paper and I have approved the final version. I here also declare that I have no conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

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