

## Antimicrobial Effects of the Water Immiscible Solvent Extracts of Olive Tree Leaves

Abdulhakim Bawadekji<sup>1\*</sup> , Mohd. Imran<sup>2</sup>  and Mohammad Akram Randahawa<sup>3</sup> 

<sup>1</sup>Department of Biological Sciences, College of Science, Northern Border University, P.O. Box 1321, Arar 91431, Saudi Arabia. <sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, P.O. Box 840, Raffa 91911, Saudi Arabia. <sup>3</sup>Department of Pharmacology, Hazrat Bari Sarkar Medical & Dental College, Tarlai 45550, Islamabad, Pakistan.

### Abstract

The purpose of this work was to perform the phytochemical and antimicrobial activity evaluation of the water-immiscible solvent extracts of Olive tree leaves (*Olea europaea* L., Family: Oleaceae). Seven sample extracts of the leaves of *O. europaea* were obtained using dichloromethane (DCM), dichloroethane (DCE) along with their mixtures with chloroform (CH), and ethyl acetate (EA). The phytochemical studies were carried out using the standard procedures. Serial plate dilution technique was used to perform the antimicrobial activity of the extracts. The phytochemical tests revealed the presence of steroids, terpenoids, saponins and flavonoids in all extracts of olive leaves. The 1:1 mixture of ethyl acetate and dichloromethane (EA:DCM) exhibited equivalent MIC (20 µgml<sup>-1</sup>, 100%) values concerning *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with respect to ofloxacin (MIC = 20 µgml<sup>-1</sup>, 100%). Similarly, the 1:1 mixture of ethyl acetate and dichloroethane (EA:DCE) exhibited equivalent MIC (20 µgml<sup>-1</sup>, 100%) values concerning *E. coli* and *P. aeruginosa* with respect to ofloxacin (MIC = 20 µgml<sup>-1</sup>, 100%). The 1:1 mixture of EA:DCM and EA:DCE showed moderate antifungal activity (MIC = 20 µgml<sup>-1</sup>, 75%) with respect to fluconazole against *Aspergillus niger* and *Candida albicans*. The water immiscible solvent extracts showed more potent antimicrobial activity than the water extract of the olive leaves, which might be because of the presence of the lipophilic compounds in the water immiscible solvent extracts of the olive leaves. It is also expected that by increasing the concentration of ethyl acetate in EA:DCM & EA:DCE solvent system may further provide better MIC values.

**Keywords:** Olive leaves, Water immiscible solvent, Extract, Antimicrobial.

\*Correspondence: [hakimbawadekji@gmail.com](mailto:hakimbawadekji@gmail.com); +966557528348

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## INTRODUCTION

Antimicrobial resistance (AMR) has initiated the emergence of multidrug-resistant (MDR) pathogens and has also made management of many bacterial infections problematic<sup>1</sup>. It is informed that the infections triggered by antibiotic resistant bacteria execute about twenty thousand patients yearly in the USA and also primes to economic loss<sup>2</sup>. The circumstances of AMR infections are growing in Saudi Arabia due to inappropriate usage of antibiotics as well as owing to the socioeconomic and demographic physiognomies of different population residing in Saudi Arabia<sup>3,4</sup>. Additional factor causative to the expansion of AMR is letdown to ascertain newer antimicrobial agents<sup>5,6</sup>. At present, synthetic antimicrobials are extensively used for prophylactic or to cure many infections. The haphazard use of synthetic antimicrobials poses a severe threat to humankind<sup>5,6</sup>, because multidrug resistance is developing among the disease triggering microorganisms. Consequently, researchers are motivated to develop antimicrobial phytomedicines, which are non-toxic and can support to overcome the appearance of multidrug resistance concern<sup>5,6</sup>.

Olive tree (*Olea europaea* L.) relates to the Oleaceae family, which contains twenty-four genus and nine hundred species<sup>7</sup>. Many parts like leaves, fruits, and stems of this plant have been investigated for its biological effects<sup>7-12</sup>, for example, antioxidant activity, anti-inflammatory activity, antithrombotic activity, antihypertensive activity, cardioprotective activity, and hypoglycemic activity. The various extracts of the olive tree leaves have also been investigated for their antimicrobial activity<sup>13-20</sup>. According to the literature, mainly the extracts of the water-miscible solvents, have been investigated for the antimicrobial activity<sup>7-20</sup>. Because of the above facts, it has been decided to perform the phytochemical and antimicrobial activity evaluation of the water-immiscible solvent extracts of Olive tree leaves (*Olea europaea* L., Family: Oleaceae) that can lead to the identification of the possible new class of the phytoconstituents as antimicrobial agents.

## MATERIALS AND METHODS

### Collection of the Plant Material

The leaves of the Olive tree (*Olea*

*europaea* L., 2 kg, semi-dried) were acquired from irrigated plantation on 1<sup>st</sup> of April 2019 at Arar city, in northern border region, Saudi Arabia. The olive leaves were validated by Directorate of Environment, Water and Agriculture at Northern Border region at Arar city. Specimen were kept the facility of College of Pharmacy at Rafha city with the reference N. Oli 19. The leaves were cleaned and dried in air for seven days at room temperature (25°C-30°C). The completely dried material was ground into a powder using a grinder. The coarse powder was sifted and kept in polythene bags for the experiment/extraction purpose.

### Preparation of the Extracts

The powdered leaves (50 g) were taken in a 1000 ml flask. Dichloromethane (DCM, 500 ml) was added to the flask. The flask was plugged, and the combination was stirred at 25°C for about 30 minutes. The flask was kept for three days at (25°C-30°C) with infrequent shaking. The content was filtered with Whatman filter paper, and the obtained filtrate was concentrated in a rotary evaporator to get the semisolid residue. On the other hand, and in the similar manner, the extracts of dichloroethane (DCE), and the 1:1 mixtures of chloroform:dichloroethane (CH:DCE), chloroform:dichloromethane (CH:DCM), dichloroethane: dichloromethane (DCE:DCM), ethyl acetate:dichloroethane (EA:DCE), and ethyl acetate: dichloromethane (EA:DCM) were obtained.

### Phytochemical Studies

The Mayer's test & Wagner's test for alkaloids; FeCl<sub>3</sub> test and Lead acetate test for tannins; Baljet test and Kellar Killani test for cardenolides; Liebermann-Burchard test for steroids; Salkowski's test for terpenoids; Foam test for saponins; Borntrager's test for Anthraquinone; and Flavonoid test for Flavonoids were carried out using the prepared extracts of Olive leaves as per the standard procedures<sup>21,22</sup>.

### Antimicrobial Screening

Serial plate dilution procedure was employed to perform the antimicrobial activity of the extracts<sup>23-26</sup>. The minimum inhibitory concentrations (MICs) of the prepared extracts, fluconazole, and ofloxacin were defined against six microorganisms. Different dilutions (10-200 µgml<sup>-1</sup>) of fluconazole, ofloxacin, and the extracts were prepared in sterilised dimethylformamide

(DMF) solvent. The sterilised DMF was also used as control. The nutrient agar media was used for antibacterial activity assessment while Sabouraud dextrose media was used for the antifungal activity assessment. Three bacteria and two fungi; *Escherichia coli* Migula 1895 Castellani and Chalmers 1919, *Pseudomonas aeruginosa* (Schröter, 1872) Migula 1900, *Staphylococcus aureus* Rosenbach 1884, *Aspergillus niger* Van Tieghem and *Candida albicans* (C.-P. Robin) Berkhout (1923) were tested for antimicrobial activity. All strains were purchased from the College of Pharmacy, Rafha, Saudi Arabia.

#### Statistical Analysis

The data (N = 3, Mean±Standard Error Mean) was analysed by SPSS-software, in which  $p < 0.05$  specified the significant results.

## RESULTS AND DISCUSSION

Seven sample extracts of the leaves of *O. europaea* were obtained using dichloromethane

**Table 1.** Physical data of the extracts of *O. europaea* leaves

Extract	Percentage Yield	State	Colour
DCM	12	Semisolid	Green
DCE	14	Semisolid	Green
DCM:DCE (1:1)	12	Semisolid	Green
CH:DCM (1:1)	12	Semisolid	Green
CH:DCE (1:1)	15	Semisolid	Green
EA:DCM (1:1)	14	Semisolid	Light Green
EA:DCE (1:1)	15	Semisolid	Light Green

**Table 2.** Phytochemical assessment data of the extracts of *O. europaea* leaves

Phytochemical	DCM	DCE	DCM:DC E (1:1)	CH:DCM (1:1)	CH:DCE (1:1)	EA:DCM (1:1)	EA:DEC (1:1)
Alkaloids	-	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-
Cardenolides	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+

(+) = Present; (-) = Absent

(DCM), dichloroethane (DCE) along with their mixtures with chloroform (CH), and ethyl acetate (EA) (Table 1).

The greenish semisolid extracts were obtained in the yield of 12% to 15%, wherein the highest yield (15%) was obtained for the 1:1 mixture of EA and DCE, and the 1:1 mixture of CH and DCE. The lowest yield (12%) was obtained for the DCM, 1:1 mixture of DCM and DCE, and 1:1 mixture of CH and DCM. It is suggested that this yield variation was because of the solvent/solvent system polarity. Standard chemical tests<sup>21,22</sup> were used to determine the presence of alkaloids, tannins, cardenolides, steroids, terpenoids, saponins, anthraquinones, and flavonoids (Table 2).

These tests discovered the presence of steroids, terpenoids, saponins and flavonoids in all extracts of olive leaves, wherein these tests revealed the absence of alkaloids, tannins, cardenolides, and tannins, cardenolides, and anthraquinones. These findings were in concurrence with the previously published report on the phytochemistry of the leaves of *O. europaea*<sup>27</sup>, wherein it was shown that steroids, terpenoids, saponins and flavonoids are among the main chemical constituents of the leaves of *O. europaea*.

Serial plate dilution technique<sup>23-26</sup> was employed to perform the antimicrobial activity of the olive leaves extracts against two fungi and four bacteria fluconazole and ofloxacin as standard drugs, respectively. The MIC values of the extracts, ofloxacin, and fluconazole concerning the tested microorganisms are presented in Table 3.

**Table 3.** Antimicrobial assessment data of the extracts of *O. europaea* leaves

Solvent Extract	MIC ( $\mu\text{gml}^{-1}$ ) (% MIC concerning the standard)					
	<i>Escherichia coli</i> (Gram -ve)	<i>Klebsiella pneumoniae</i> (Gram -ve)	<i>Pseudomonas aeruginosa</i> (Gram -ve)	<i>Staphylococcus aureus</i> (Gram -ve)	<i>Aspergillus niger</i>	<i>Candida albicans</i>
DCM	25(80%)	30(66.66%)	30(66.66%)	40(50%)	40(37.5%)	40(37.5%)
DCE	25(80%)	30(66.66%)	30(66.66%)	25(80%)	30(50%)	30(50%)
DCM:DCE (1:1)	30(66.66%)	30(66.66%)	25(80%)	30(66.66%)	30(50%)	30(50%)
CH:DCM (1:1)	30(66.66%)	35(57.14%)	30(66.66%)	40(50%)	50(30%)	40(37.5%)
CH:DCE (1:1)	25(80%)	25(80%)	25(80%)	25(80%)	25(60.0%)	25(60.0%)
EA:DCM (1:1)	20(100.0%)	25(80.0%)	20(100.0%)	20(100.0%)	20(75.0%)	20(75.0%)
EA:DCE (1:1)	20(100%)	25(80%)	20(100%)	25(80%)	20(75%)	20(75%)
Fluconazole	-	-	-	-	15(100%)	15(100%)
Ofloxacin	20(100%)	20(100%)	20(100%)	20(100%)	-	-

\* $p < 0.05$  &  $N = 3$ .

For comparison, the MIC values of standard drugs (fluconazole & ofloxacin) are considered 100%. All the values exhibited statistically significant ( $p < 0.5$ ) results. It is obvious from Table 3 data that the 1:1 mixture of EA:DCM exhibited equivalent MIC (20  $\mu\text{gml}^{-1}$ , 100%) values concerning *S. aureus*, *E. Coli*, and *P. aeruginosa* with respect to ofloxacin (MIC = 20  $\mu\text{gml}^{-1}$ , 100%). Similarly, the 1:1 mixture of EA:DCE exhibited equivalent MIC (20  $\mu\text{gml}^{-1}$ , 100%) values concerning *E. coli* and *P. aeruginosa* with respect to ofloxacin (MIC = 20  $\mu\text{gml}^{-1}$ , 100%). The 1:1 mixture of EA:DCM and EA:DCE also showed most promising MIC values ranging from 75% to 80% with respect to standard drugs, ofloxacin and fluconazole, against *K. pneumoniae*, *C. albicans*, and *A. niger*. All other extracts revealed antimicrobial activity ranging from 50% to 80% with respect to standard drugs against the tested microorganisms. As per published reports<sup>28</sup>, the conventional antibiotics have MIC range from 15  $\mu\text{gml}^{-1}$  to 105  $\mu\text{gml}^{-1}$ . Accordingly, it can be assumed that our extracts are potent antimicrobials. However, this generalized assumption may not be applicable to our extracts because our extracts are not single compounds like conventional antibiotics, rather they may contain a mixture of many compounds. It has been suggested that the water-immiscible solvent systems (EA:DCM & EA:DCE) having more polarity had better antimicrobial activity in terms of the MIC values. On the other hand, the water-immiscible solvent systems having lesser polarity

had mild to moderate antimicrobial activity in terms of the MIC values. It is expected that the antimicrobial activity of the water-immiscible solvent extracts is attributed to the occurrence of steroids, terpenoids, saponins and flavonoids in the solvent extracts. Secondly, more amount of steroids, terpenoids, saponins and flavonoids might be existing in the higher polarity solvent system of the present study (EA:DCM & EA:DCE), which attributed to the equivalent MIC values against ofloxacin, and fluconazole<sup>27</sup>. Thirdly, the water-immiscible solvent systems are generally more lipophilic. The lipophilic constituents can easily interrupt with the lipophilic cell membrane of the microbes. This may be the reason for better antimicrobial activity of the EA:DCM & EA:DCE solvent system<sup>20,25</sup>. The results also suggested that our extracts have lesser MIC value than the water extract of the olive leaves<sup>13</sup>. The water extracts of the olive leaves<sup>13</sup> exhibited MIC values of 0.13  $\text{mgml}^{-1}$  (*P. aeruginosa*), 0.3  $\text{mgml}^{-1}$  (*K. pneumoniae* and *E. coli*), and 0.6  $\text{mgml}^{-1}$  (*S. aureus*), whereas our extracts showed their MIC values in the range of 20  $\mu\text{gml}^{-1}$  to 40  $\mu\text{gml}^{-1}$ . This shows that our extracts are more potent antimicrobials as compared to the water extract of the olive leaves. The possible reason for this phenomenon is that the bacterial cell wall mainly consists of lipid. Our water immiscible extracts contain lipid soluble compounds, which can easily pass through the lipid membrane of the microbes. The water extract mainly contains water soluble compounds, which

may not be able to cross the lipid membrane of the microbes<sup>20,25</sup>.

## CONCLUSION

The water immiscible solvent extracts showed more potent antimicrobial activity than the water extract of the olive leaves, which might be because of the presence of the lipophilic compounds in the water immiscible solvent extracts of the olive leaves. The 1:1 mixture of EA:DCM & EA:DCE exhibited the most promising and in some cases, equivalent MIC values with respect to ofloxacin and fluconazole. The promising MIC values of these two solvent system extracts might be due to the presence of more amount of steroids, terpenoids, saponins and flavonoids. It is also expected that by increasing the concentration of ethyl acetate in EA:DCM & EA:DCE solvent system may further provide better MIC values.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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