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Antibacterial Activity and Phytochemical Screening of Olea *Europaea* Leaves from Algeria

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Abstract: Because of growing emergence of the global phenomenon of bacterial resistance to antibiotics, the discoveries of new antimicrobial compounds become a primary objective in the fight against infections caused by resistant bacterial strains.

The main purpose of this study firstly, it is to find the chemical compounds in the *Olea europaea* leaves, and secondly, to evaluate the antibacterial activity of aqueous extract of olive leaves in Adrar.

Phytochemical screening revealed the presence of some active substances flavonoids, saponins and steroid, to express the desired activities.

Evaluating the antibacterial activity of aqueous extract of olive leaves, we use the diffusion method on solid medium and the direct contact method.

The aqueous extract reacted positively on all bacterial strains tested *Escherichia coli* ATCC25922, *Escherichia coli 2*, *Staphylococcus aureus* ATCC6538, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae, Enterobacter cloacae* ATCC13047, *Pseudomonas aeruginosa* ATCC10145 and *Bacillus stearothermophilus* ATCC 11778. Aqueous extract of *Olea europaea* leaves demonstrated the best inhibition against *Escherichia coli 2* with MIC of 150 µl / ml and inhibition zone of 15.3 mm in diameter.

Keywords: Active substances, antibacterial activity - antibacterial resistance, aqueous extract, *Olea europaea,* Phytochemical screening.

INTRODUCTION

The Mediterranean region is rich in plant species; there are about 2,600 species of which many are considered to have medicinal effects [1]. The *Olea europaea* which is one of the first domesticated agricultural tree crops in the family *Oleaceae*, is cultivated mainly for both edible oil and table olives. The domestication of *Olea europaea* is supposed to be realized some 5700–5500 years ago in the Near-East [2]; it is known that olive is native to coastal areas of the Mediterranean region such as Palestine, Syria Spain, Italy, Greece, France, Turkey, Algeria, and Morocco.

Pathogenic bacteria constitute a major cause of morbidity and mortality in humans. The emergence and spread of bacterial resistance made the treatment of infectious diseases more problematic [3].

The antimicrobial activity of a plant is highly related to secondary substances that are synthesized and produced by these plants [4]. Secondary metabolites are substances of low molecular weight, which were not the products of the primary metabolic pathway of the producing organism and at first thought to be with no advantage to the plant. Nowadays it is believed that they have vital functions [5]. The aim of this study was to determine the antimicrobial activity of the aqueous extract of *Olea europaea* leaves against contaminating or pathogenic microorganisms.

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MATERIAL AND METHODS

Plant Material

Olea europaea leaves specimens are collected every atumm between September and October why we used our leaves of the experience in september 2010 from Adrar located south west of Algeria. These leaves were dried for fifteen day at the ambient laboratory temperature (20-28°C). They was milled to a fine powder in an electrical mill and stored in the dark at laboratory temperature in closed containers until required.

Phytochemical Screening of the *Olea Europaea* Leaves

Ethanol; chloroform; hydrochloric and aqueous extracts were prepared for phytochemical screening of *Olea europaea* leaves. The extracts were subjected to phytochemical tests for leaves secondary metabolites, tannins, saponins, steroid, alkaloids, flavonoid, unsaturated sterol and terpen in accordance with [6, 7].

Preparation of *Olea Europaea* Leaves Extract

Decoction: 5 g of powdered leaves were boiled with 50 ml of distilled water for 30 min. After cooling the mixture

Table 1. Phytochemical Screening of *Olea Europea* Leaves

Phytochemicals	Leaves Extracts
Alkaloids	-
Flavonoids	+
Tanin	-
Saponins	+
Unsaturated sterols and terpens	+
Sterol and steroid	+

Keys: +: Present; -: Absent

was filtered with sterile filter paper and stored at 4 °C for further use.

Antibacterial Screening

The extract obtained from leaves was studied for antibacterial activity. The antibacterial activity of aqueous leaves extract against various Gram positive and Gram negative bacteria were observed. The organisms given below were obtained from the Pasteur Institute; Algiers; Algeria.

Gram negative: *Escherichia coli* ATCC25922; *Escherichia coli2*; *Pseudomonas aeruginosa* ATCC10145; *klebsiella pneumonia; Enterobacter Cloacae* ATCC13047.

Gram positive: *Staphylococcus aureus* ATCC6538; *Staphylococcus aureus* ATCC25923; *Bacillus stearothermophilus* ATCC 11778.

The strains were identificated by the use of Biochemical profiles according to the recommendation of the manual of clinical microbiology [8]. The bacterial strains were first subcultured in a nutrient broth and incubated at 37°C for 18 h.

Preparation of Standardized Bacterial Inoculums

Standardized bacterial inoculums was prepared and adjusted to 0.5 McFarland and then diluted to 10^6 CFU/ml.

Disk Diffusion Method

Petri dishes were prepared with 10ml of a base layer of Muller Hinton gelose medium and inoculated with 100µl of each bacterial suspension [9]. After drying in a sterile hood, 6mm diameter disks soaked with aqueous decoctate 0.1*u*l (Equivalent to 9 µg/disk). The dishes were incubated at 37°C for 24 hours. Activity was evaluated by measuring the diameter of the inhibition zone and presented in millimeter.

Minimum Inhibitory Concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of [10]. Different concentrations of the aqueous extract were added to 20 ml of Muller Hinton gelose for the following concentrations: 4.73µg/ml; 10µg/ml; 15.88µg/ml; 22.5µg/ml; 30µg/ml; 36µg/ml; 48.46µg/ml and 60µg/ml. After drying in a sterile hood the strains were ensemnced by streaking from bacterial inoculums diluted to 10^6 CFU/ml. The plates were later incubated at 37°C for 24. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

All tests were performed in duplicate.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical analysis conducted on *Olea europaea* leaves revealed the presence of flavonoids, steroids and saponins (Table **1**).

Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals. Result of phytochemical screening of *Olea europea leaves* us of the various extracts showed the presence of saponins, stérols, steroid, terpen and flavonoids. The HPLC-DAD analysis of olive leaf aqueous extract allowed the identification of seven phenolic compounds: caffeic acid, verbascoside, oleuropein, luteolin 7-*O*-glucoside, rutin, apigenin, 7-*O*-glucoside and luteolin 4'-*O*-glucoside. All these compounds were previously reported to occur in olive leaf [11, 12].

Disk Diffusion Method

In the present study, effectiveness of aqueous leaves extract of *Olea europaea* was also confirmed by filter paper disc diffusion assay and growth inhibitions zone diameters were measured in presence of each extract. Results are presented in Table **2**.

It is evident that the Gram-negative organisms *Escherichia coli* ATCC25922, *Escherichia coli*, *Pseudomonas aeruginosa* ATCC10145, *Klebsiella pneumonia* and *Enterobacter cloacae* ATCC13047, showed a slightly higher sensitivity to aqueous leaves extract of O*lea europaea* compared to the Gram-positive organisms used namely *Staphylococcus aureus* ATCC6538, *Staphylococcus aureus* ATCC25923 and *Bacillus stearothermophilus ATCC* 11778.

The Raison for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological difference between these microorganisms' Gram-negative bacteria has an outer phospholipidic membrane carrying. The structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes; while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600Da [13]. The Gram-positive bacteria should be more

Table 2. Antibacterial Activity of the Aqueous Extract of Olea *europaea leaves* by Disc Diffusion Method

Microorganismes	Inhibition Zone (mm)
Gram-	
Escherichia coli ATCC25922	13.5
Escherichia coli	15.3
Pseudomonas aeruginosa ATCC10145	13.3
Klebsiella pneumonia	11.7
Enterobacter cloacae ATCC13047	12.5
Gram+	
Staphylococcus aureus ATCC6538	09
Staphylococcus aureus ATCC25923	07
Bacillus stearothermophilus ATCC 11778	06.9

Table 3. MICS of Aqueous Extract of *Olea Europaea Leaves* on Bacteria Gram-Positive and Bacteria Gram-Negative

Microorganismes	CMI (µg/ml)
Gram-	
Escherichia coli ATCC25922	09.8
Escherichia coli	06.97
Pseudomonas aeruginosa ATCC10145	25.01
Klebsiella pneumonia	19.03
Enterobacter cloacae ATCC13047	21.29
Gram+	
Staphylococcus aureus ATCC6538	09.88
Staphylococcus aureus ATCC25923	09.12
Bacillus stearothermophilus ATCC 11778	26.36

MIC: Minimum Inhibitory Concentration

susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [14]. So this aqueous extract has hydrophilic properties and it can penetrate inside the bacterial cells Gram-negative.

There might be another possibility that aqueous leaves extract may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive contact with extract .It may also happen due to hydrophilic nature of bacterial cell wall [15].

Minimum Inhibitory Concentration (MIC)

As Table **3** shows, *Olea europaea* leaves extract exhibited inhibitory effects on *Escherichia coli2*, *Pseudomonas aeruginosa, Enterobacter cloacae Staphylococcus aureus, Bacillus stearothermophilus* and *Klebsiella pneumoniae*. The MICs are more or less important depending on the type of bacteria studied.

The aqueous extract fraction that shows good antibacterial activity with *Staphylococcus aureus* and *Escherichia coli* exhibited very low activity on *Pseudomonas aeruginosa* and *Bacillus stearothermophilus*.

The antibacterial action obtained in this study for aqueous *Olea europaea* leaves extracts is in agreement with that reported by [16].

Olea europaea leaves may be useful in cases where prolonged use of antibiotics encourage development of opportunistic infections [17] being especially effective against *Klebsiella* and *Pseudomonas*, two bacterial genera which pose a major resistance problem [18].

In conclusion, the data obtained in this study demonstrate that the use of olive leaves as nutraceuticals may lower the risk of microbial infections, particularly in the intestinal and respiratory tract, mainly due to the protective action provided by its phenolic compounds. The use of extracts is recommended to achieve health benefits due to the additive and synergistic effects of phytochemicals present in whole extract [19].

CONCLUSION

This study emphasizes antimicrobial properties of aqueous extract of *Olea europaea* leaves against human pathogenic bacteria. The strains studied are a sensible to the aqueous extract of *Olea europaea* leaves in different concentrations towards the characteristics of strains tested *in vitro*.

These aqueous extract may be effective on other Gram-positive and Gram-negative bacteria. More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Hence, this extract and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

CONFLICT OF INTEREST

This academic work is realized in Laboratory of Plant Resource Development and food Security in Semi-Arid Areas, South West of Algeria, Department of Biology, university of Béchar which all contributions financial have covered by the latter.

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