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The Antibacterial Effect of Olive Leaf in Sudan

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Abstract

In vitro study of the antimicrobial activity of Olive Leaf Extracts (OLE) was done against five different bacterial human pathogens. One of gram positive (*Staphylococcus aureus*) and four gram negative (*Proteus vulgaris*, *Escherichia coli*, *klebselia pneumonia* and *Pseudomonas aeruginosa*) bacteria. Extract obtained with methanol(80%),The antimicrobial activity evaluated with disc diffusion method using (0.5- 1.0- 1.5)g/ml from the extract study, showed sensitivy on(*S aureus* and *E.coli*) bacteria and resistant on The other(*Proteus vlugaris- klebselia pneumonia Pseudomonas aeruginosa*) bacteria.

The diameters zones of minimum inhibition concentration of the olive leaf extract observed (11-19) mm against *S aureus* in 0.5 g/ml and (9-11.)mm against *E coli*.in.5g/ml concentration .

Introduction:

Olive tree (*Olea europaea*) is one of the most important fruit trees in Mediterranean countries, where they cover 8 million ha, accounting for almost 98% of the world crop This demonstrates the great economic and social importance of this crop and the possible benefits to be derived from utilization of any of its byproducts^(1, 2). *Olea europaea* is widely studied for its alimentary use (the fruits and the oil are important components in the daily diet of a large part of the world's population), whereas the leaves are important for their secondary metabolites such as the secoiridoid compounds oleacein and oleuropein, the former responsible for hypotensive activity⁽³⁾ and the latter also for hypoglycemic activity⁽⁴⁾ Several reports have shown that olive leaf extract has the capacity to lower blood pressure in animals⁽⁵⁾ and increase blood flow in the coronary arteries⁽⁶⁾, relieve arrhythmia and prevent intestinal muscle spasms⁽⁷⁾. Also leaves may be used in infusions, allowing a considerable intake/uptake of bioactive component recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant. The non-availability and high cost of new generation antibiotics with limited effective span has led to the search for more effective antimicrobial agents among materials of Northern Iraq and Iran⁽⁸⁾.

The oleuropein is an important constituent in the leaf and fruit extracts and present in higher concentration in the leaves than in the fruits and other parts of the plant. The maximum antimicrobial activities were found for the methanolic extracts; this might be due to the good extraction efficacy of methanol compared to other solvents, the second and third effective solvents were ethanol and water⁽⁹⁾.

There for this study was done for detection of the activity of olive leave extract in Sudan and determined MIC value of the extract against some clinical isolated bacteria.

Materials and Methods:

Study design:

Experimental study

Study area:

The study conducted on Khartoum state.

Study duration:

The study conducted on March 2017 to October 2017.

Study population:

Clinical isolated bacteria

Sample size:

60 clinical isolated bacterial sample.

Data collection:

Primary data collection: Randomize collection of sample Secondary data collection: 1 plate of any aerobic bacteria isolated from clinical sample .

Preparation of plant extracts:

Sample of olive leave was collected from Botanic garden on Khartoum University faculty of agriculture. The leaf dried at room temperature then extracted by 80% methanol.

The methanol extract prepared by using 100 g of olive leaf powder with 800ml of 80% methanol.

Microorganisms Used *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

The organism isolated from different clinical samples, for every microbial strain two plates inoculated with the standardized broth culture using sterile cotton swab to be evenly spread on to Muller Hinton agar under aseptic condition, inoculums prepared by adjusting the turbidity of test microorganism suspension to 0.5 McFarland standards (1.5×10^8 CFU/ml) (Unnisa *et al.*,⁽¹⁰⁾

Determination of Antimicrobial Activity (Disc Diffusion Method)

Antibacterial activity of three dosage form of (0.5-1-1.5) g of extract Sterile filter paper discs (6 mm diameter soaked in the different concentration of extract under aseptic condition performed twice to check the results .

Sterile 6 mm diameter filter paper discs soaked in methanol 80 % using as control.

Aseptically placed the discs (0.5-1-1.5)g/ml and control disc on culture media Inoculated plates were incubating at 37c for 24 hour.

Results:

Three dosage form (0.5-1-1.5) g /ml of extract using Disc Diffusion method.

The zones of inhibition formed by the olive leaf extract very different in terms of effectiveness since some bacterial species are more resistant and some other more susceptible to the extract.

The diameters of inhibition zones of the olive leaf extract against *Staphylococcus aureus* on .5g/ml was observed (11-19mm) , 1g/ml was observed (12-20mm) on 1.5g/ml was observed (13-21mm) . *Escherichia coli* the diameters of inhibition zones on .5 mg/ml was observed (9-11) on 1g/ml was observed (10-12mm) on 1.5g/ml was observed (10-13mm).

On *Proteus. vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella Pneumonia* no zone of inhibition ,it was more resistant.

On control disc show no inhibition zone.

Minimal inhibitory concentration (MIC):

Staphylococcus aureus, (MIC) and antibacterial activity with a zone of inhibition ranging from (11-19) mm at .5g/ml It showed minimal inhibitory concentration of potent antibacterial activity.

Escherichia coli (MIC)

Antibacterial activity with a zone of inhibition ranging from (9-11) mm at 5g/ml It showed minimal inhibitory concentration of potent antibacterial activity.

Inhibition diameter zones (mm) on the tested bacteria of olive leaf extract

	<i>S. aureus</i>	<i>E.Coli</i>	<i>K.Pneumonia</i>	<i>P.Aeruginosa</i>	<i>Proteus vlugaris</i>
N	8	20	10	10	12
OLE .5g	11-19mm	9-11mm	R	R	R
OLE 1g	12-20mm	10-12mm	R	R	R
OLE 1.5g	13-21mm	10-13mm	R	R	R
NR	0	12	10	10	12
C	R	R	R	R	R

OIE olive leaf extract

N number of bacterial specie

NR number of resistant strain

R resistant strain (no zones of Inhibition)

C control disc (methanol without olive leave extract)

Discussion

Our results showed stronger antimicrobial activity of olive leaf on *Staphylococcus aureus*. *Escherichia coli* resistant strains with olive leaf showed, on *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Proteus. vulgaris* The MIC values reported 0.5g/ml for *Staphylococcus aureus*. And *Escherichia coli* , agree with

⁽¹¹⁾ Pereira *et al.* (2007) study who found that extracted olive leaves inhibited the growth of *E. coli* and *S. aureus* with MIC values of 50 µl/ml and 25 µl/ml respectively, at 37 °C.

In another study, Pereira *et al.* (2006) ⁽¹²⁾ ,reported that the MICs of a Portuguese table olive extract against *E coli*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, ranged from 10 to 100 µl/ml at 37 °C. *B. cereus* numbers were reduced by 2.7 log₁₀ CFU/ml in the presence of 5 µl/ml olive powder in BHI broth at 20 (Ferrer *et al.*) ⁽¹³⁾, 2009.

Sudjana⁽¹⁴⁾ and his coauthors in their study to investigate the activity of a commercial extract derived from the olive leaves of *Olea Europe* against a wide range of microorganisms, using agar dilution and micro dilution techniques, olive leaf extract was found to be most active against *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus*. They concluded that olive leaf extract was not broad spectrum in action, showing appreciable activity only against *H. pylori*, *C. jejuni*, *S. aureus* and. They reported given this specific activity. Olive leaf extract may have a role in regulating the composition of the gastric flora by selectively reducing levels of *H. pylori* and *C. jejuni*. Hussain *et al*⁽⁹⁾, in their study found that the antibacterial activity of olive leaf extract was higher for the gram negative strains as compared to gram positive strains disagree to our study.

Conclusion

The Methanolic extract of olive leaf had effect against (*S. aureus*, *-E coll*) and no effect on (*Proteus. vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella .Pneumona*)

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Limitations of the study:

Lack of the tree in Sudan, and difficulty of collection of Samples.

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Zone of inhibition

