



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(1): 1493-1495

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Received: 26-11-2017

Accepted: 27-12-2017

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Antimicrobial activity and evaluation of genetic effects of olive leaves using molecular techniques

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Abstract

Effective control of pathogenic bacteria key to the prevention and treatment of disease, therefore many plants are used in traditional medicine as treatment for bacterial infections. In the present study (7) microbial pathogens as (*Staphylococcus aureus*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Streptococcus mutans*) isolated in the laboratory of veterinary medicine collage of Baghdad university. The present results show that aqueous olive leaves extract have potential antibacterial activities against some of bacterial strains, then select *Staphylococcus aureus* and *Streptococcus mutans* which are more sensitive to different concentrations (0.5, 1, 3, 6%) than other bacterial strain for the ability of hot aqueous extract of olive leaves. Plasmid profiling is among the methods used to determine and characterize aqueous olive leaves extract traits in bacteria. To induce the cleavage in cells, DNA fragmentation of microorganisms used. In addition to those treated with antibiotic (kanamycin and ampicillin) for comparing the results, the DNA fragments were observed using agarose gel electrophoresis. This phenomenon was tested for the ability of extract of olive leafs to induce the cleavage in cells (DNA fragmentation).

Pathogenic bacteria *Staphylococcus aureus* and *Streptococcus mutans* were exposed to at (0.5, 1, 3 and 6%) concentration and incubated for 24 hours. *Staphylococcus aureus* and *Streptococcus mutans* when applied with the extracts olive leaves showed a marked DNA fragmentation, and no fragmentation was observed in untreated cells. The results confirmed that the extracts of olive leaves can interact with DNA of the bacteria. This may explain the inhibitory action on DNA synthesis. Hence, the hot aqueous extract of olive leaves possessed antibacterial potential against these microorganisms. While kanamycin and ampicillin not posses such activity.

Keywords: Antibacterial, anti-plasmid, aqueous extracts, pathogenic bacteria

1. Introduction

Medicinal plants are from the most advantageous source of life saving drugs for humans, animals and plants. Bioactive compounds usually extracted from plants are used as medicines and fine chemicals. In some Asian and African countries, 80% of the population are reliant on medicinal plants to maintain their health and to cure their ailment [1]. Visioli *et al.*, 2002 were find demonstrate that olive oil phenolic are powerful antioxidants, both in vitro in vivo and possess other potent biological [2]. Olive leafs extract can be considered a plant antimicrobial with both antimicrobial and antioxidant activities [3]. It also has health benefits such as increasing energy levels, lowering blood pressure, and supporting the cardiovascular and immune systems [4]. The antimicrobial potential of eight phenolic compounds isolated from olive leafs was tested against the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, Oleuropein, p-hydroxy benzoic, vanillic and p-coumaric acids (0.4mg/ml) completely inhibited the growth of *E. coli*, *K. pneumoniae* and *Aspergillus parasiticus* [5]. Plasmid-mediated multidrug resistance is one of the most upcoming problems in the treatment of infectious diseases, as bacteria have reached the resistance to most of the antibiotics that are available for treatment. Plasmids are extra chromosomal pieces of double stranded DNA which multiply independently inside the bacteria [6]. Antibiotic resistance in bacteria may be an inherent trait of the organism that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance conferring DNA from another source [7], a major class of active compounds which can serve as potential plasmid eliminating agents. Various herbal extracts from plants like *Cinnamum verum*, *Zingiber officinale*, *Nigella sativa*, *Piper nigrum*, *Plumbago zeylenica*, etc. containing phenol (eugenol), tannins, flavonoids, terpenoids, naphthoquinones, alkaloid, saponin could be used for plasmid curing [8]. Aim of the

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present study was the use of plasmid – curing herbal hot aqueous extract of olive leaves which could be used to inhibit the development of resistant plasmid

2. Materials and Methods

2.1 Preparation of Olive Leaves Extract

Preparation of olive leaves aqueous extract was used in this study collected from farmer in Baghdad city. They were collected in winter (January). The leaves were sun dried, pulverized and then sieved. Twenty-five (25) grams of the powdered leaves was weighed out and dissolved in 250ml of distilled water to make extract (0.5%, 1%, 3% and 6%) solutions in distilled water were prepared.

2.2 Samples

Isolated in the laboratory of veterinary medicine collage of Baghdad University.

2.3 Antimicrobial activity

2.3.1 Disc diffusion assay

Antimicrobial activity of hot aqueous olive leaves extract was firstly tested using the inhibition zone width method. A clear inhibition zone of the studied bacteria were caused by the treatment with (0.5, 1, 3, 5%) of aqueous olive leaves extract, The diameter of all inhibition zones (DIZ) were measured, The results that aqueous olive leaves extract has potential antibacterial activities against some of bacterial strains. In the

present study results show that *Staphylococcus aureus* and *Streptococcus mutans* more sensitive to different concentration (0.5, 1, 3, 6%) than other bacterial strain.

2.4 Curing of plasmid mediated

Isolation of plasmid DNA content for plasmid profile (fermentas). Agarose electrophoresis technique. According for Maniatis, T. *et al.* (1982) [9]

3. Result

The results showed that the hot aqueous extract of olive oil (0.5, 1, 3, 6%) on growth and inhibition activity against the pathogenic *Staphylococcus aureus* bacteria and *Streptococcus mutans* as follow: Table (1) The results that aqueous olive leaves extract have potential antibacterial activities against some of bacterial strains. In the present study results show that *Staphylococcus aureus* and *Streptococcus mutans* more sensitive to different concentration of (0.5, 1, 3, 6%) than other bacterial strains. Pathogenic bacteria (*Staphylococcus aureus* and *Streptococcus mutans*) were exposed to different concentrations of olive leaves extract (0.5, 1, 3, 6%) and showed that Plasmid gradual disappearance of increasing concentrations, and no plasmid disappearance was observed in untreated cells Fig (1). Figure (2) showed agarose gel electrophoresis analysis of plasmid DNA isolated from kanamycin and ampicillin resistant bacterial strains

Table 1: Zone of inhibition (mm) of olive leaves extract against bacterial strains.

Bacterial strains	Inhibition Zone (mm) in 0.5% conc.	Inhibition Zone (mm) in 1% conc.	Inhibition Zone (mm) in 3% conc.	Inhibition Zone (mm) in 6% conc.
<i>Staphylococcus aureus</i>	5	5.5	8	10.3
<i>Listeria monocytogenes</i>	0	0	1	2
<i>Pseudomonas aeruginosa</i>	2.2	2.3	3	4
<i>Salmonella typhimurium</i>	0	0	0	1
<i>Enterococcus faecalis</i>	0	0	0	0
<i>Klebsiella pneumoniae</i>	0	2	3.2	4
<i>Streptococcus mutans</i>	5.5	6.3	7	10

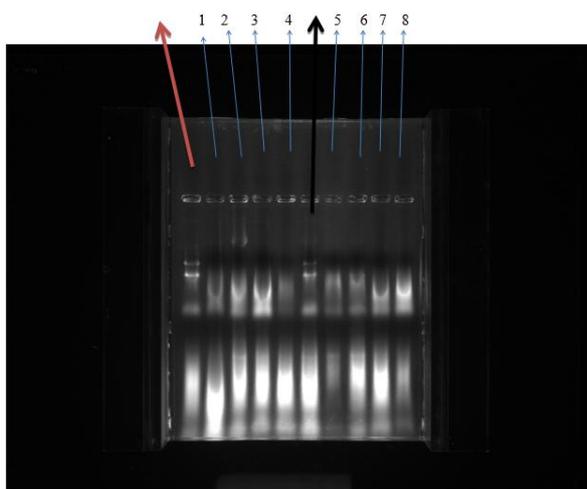


Fig 1: Agarose gel electrophoresis analysis of plasmid DNA from *Staphylococcus aureus* arrow red, *Streptococcus mutans* arrow black untreated. After treatment show DNA fragmentation:- *Staphylococcus aureus* 1- treated with 0.5%, 2- treated with 1%, 3- treated with 3% and 4- treated with 6% (Plasmid disappearance). *Streptococcus mutans* 5- treated with 0.5%, 6- treated with 1%, 7- treated with 3%, and 8- treated with 6%. (Plasmid disappearance).

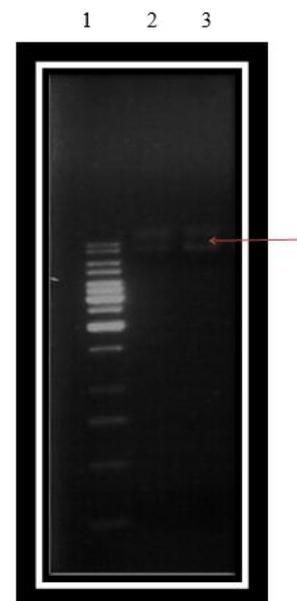


Fig 2: Agarose gel electrophoresis analysis of plasmid DNA isolated from kanamycin and ampicillin resistant bacterial strains. A: Lane 1: DNA marker; lanes 2-3: plasmids isolated from resistant bacterial strains.

4. Discussion

Olive leaves extracts is commonly used in folk medicine for the treatment of many diseases. In the present study, the antimicrobial activities of the aqueous extract of olive leaves against the microorganisms were examined and its potency was assessed by the presence of inhibition zone diameter, it was found when increased concentration ended with an increased inhibition zone diameter. The effect of this work reveals that the leaves of the plant, *Olea europaea*, contained bioactive agents which are connected with antimicrobial properties in plants. These agents are oleuropein (a phenolic compound), lobatannins, flavonoids, tannins and traces of alkaloids. Research work revealed that tannins from the barks, roots and leaves of many plants are used to treat cells that have gone neoplastic changes [10]. The physical loss of plasmid in the cured derivative when comparing between the untreated bacteria and those treated with different concentrations of the aqueous olive leaves extract Fig (1) was confirmed by agarose gel electrophoresis of the plasmid DNA preparation of respective cultures. These results suggest that there is an affection on gene expression and interruption in biochemical pathways of DNA and protein synthesis, consequently as alkaloids in olive leaves extract often do. The olive leaves contained significant levels of flavonoids based on the aglyco nesapigenin, quercetin, kaempferol, hesperitin [11, 12]. OLE is known to contain a mixture of polyphenolic compounds, among them oleuropein and hydroxytyrosol, both of which are readily absorbed and bioavailable. The biological activities of OLE are mainly derived from these compounds [13, 2].

In the present of study revealed presence of plasmids in untreated strain, but absence of plasmids in the treated which was the physical confirmation of plasmid curing of bacteria effected by the aqueous olive leaves extract, was confirmed with Sylvia [14]. And Fabiani [15] who showed that olive oil phenols have potent anti DNA damage effect.

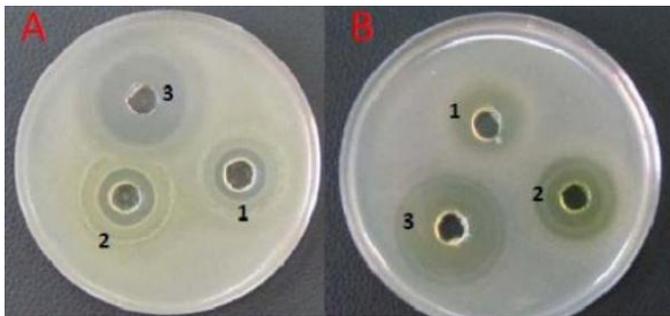


Fig 6: Biological application of the aqueous of Olive leaves extracts exhibited remarkable activity against some microorganisms. *Staphylococcus aureus*, *Micrococcus*, *pseudomonas aeruginosa* and *E. coli*. (Zone = mm)

5. Conclusion

It is concluded from this study that aqueous olives leaves extract have potential antibacterial activities against some of bacterial strains, like *Staphylococcus aureus* and *Streptococcus mutans*. As well as, after used Plasmid profiling to determine and characterize aqueous olive leaves extract straits in bacteria, observed the ability of extract of olive leafs to induce the cleavage in cells (DNA fragmentation)

6. Acknowledgment

At first, the prayerful thanks to our merciful ALLAH who help me ever thing I have. The completion of this under

taking could not have been possible without the participation and assistance of so many people whose names may not all be enumerated. I wish to express my deepest thank to the laboratory university of Baghdad.

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