INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

CELLULASES EFFECTIVENESS IN ANTIBACTERIAL ACTIVITY FOR OLIVE LEAVES AQUEOUS EXTRACT

ASWAN HAMDULLAH BAYAR[a], NIDHAL MOHAMMED SALIH[b] AND ALI AMEEN YASEEN[c]

[a] Department of Food Science, University of Baghdad, Baghdad, Iraq.
[b] Department of Food Science, University of Baghdad, Baghdad, Iraq.
[c] Department of Food Science, University of Al-Anbar, Al-Ramadi, Iraq.

Corresponding Author: aswanbayar@yahoo.com

Abstract

Aqueous extracts of olive leaves were achieved by three methods of extraction, first by boiled distilled water, second by using 2% NaOH and third by using fungal cellulases from local isolate of Aspergillus sp. to study the antimicrobial activity of these extracts against Bacillus cereus, E. coli, Pseudomonas aeruginosa, Staphyllococcus aureus, Salmonella typhimurium. In first method there was no effect of inhibitory mentions within the concentrations used in the current study. In the second method there were influential inhibition towards all bacteria used in this study with the amount of variation in sensitivity depending on the type of bacteria and the concentration, E. coli, Pseudomonas aeruginosa, Salmonella typhimurium had been effected at the lowest concentration of 2.5 mg/ml , with a diameter zone of inhibition of 5.5 , 1.2 and 4.5 mm, respectively. While Bacillus cereus and Staphyllococcus aureus weren't affected only by 250 mg/ml concentration and the inhibition zone diameter was 6 mm for each. In third method, results indicate clearly the role of cellulases in increasing the inhibitory effect of the extract, although there are differences according to the type of bacteria and concentrations, the inhibitory effect is clear for the bacteria Bacillus cereus and E. coli in less concentration of 2.5 mg/ml reaching 5.15 and 5.1 mm, respectively , while the effect on Staphyllococcus aureus and Salmonella typhimurium begins at the concentration 25 mg/ml as its diameter zone of inhibition was 4.75 and 4.5 mm, respectively, and went up 6.5 and 8.5 mm respectively in the concentration of 250 mg/ml. Phenolic compounds of aqueous and alcoholic extracts of olive leaves were detected, they were 10.504% and 13.94%, respectively, flavonoids of aqueous and alcoholic extracts of olive leaves were detected also, they were 3.11% and 8.33% respectively.

Keywords: Antibacterial activity, cellulases, olive leaves, aqueous extract, alcoholic extract.

Introduction

The olive trees class *Olea europaea L*. is the most important in the countries of the Mediterranean basin [15], and has leaves naturally resistant to many of the injuries insecticides and etiology of microbiology [13], extracts of leaves and olives contain many phenolic compounds multiple (polyphenols) that have high biological activity, which are usually extracted from fruit's oil during manufacturing due to their possession of a bitter tart taste [1]. Olive's leaves are containing materials holding the most important of tannin, which enters in the composition of cosmetic gurgling and anti-disease pathogens [4].Artificial preservatives such as industrial sorbic acid and benzoic and propionic are used in abundance in food to reduce the effectiveness of microorganisms and what are they caused of deterioration in taste as well as what are they produce of toxins , and because some of these preservatives is accused of being carcinogenic, researchers resorted to use natural alternatives to prevent molds and bacteria and yeasts provided that it will not cause a negative impact on taste, smell and color [6] , plant extracts are the most important in this regard, including specifically the extract of olive leaf , which contains a substance oleuropein, which decompose in the human body to

another material called enolinate which confirmed by recent studies ongoing outside the body of the organism (in vitro) its ability to kill pathogenic bacteria and viruses, fungi , as well as beneficial microorganisms, on the other hand some studies failed on the adverse effects of enolinate toward beneficial neighborhoods inside the body of a living organism (in vivo) to confirm that [9] , and in all conditions indicated all studies on olive leaf extract possesses positive action in reducing blood pressure in laboratory animals . This study aimed to identify the nature of the impact of the use cellulases act in an anti- aqueous extracts of the leaves of the olive tree class *Olea europaea L*. against certain types of bacteria.

Experimental

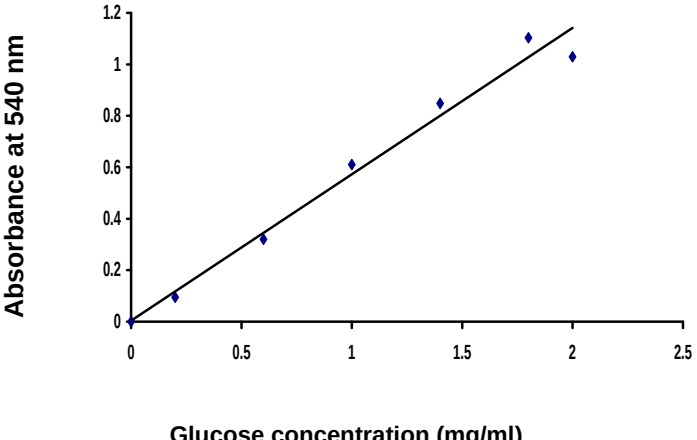
Microorganisms

The following microorganisms were used in this study: *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *Staphyllococcus aureus*, *Salmonella typhimurium*.

Olive leaves: Olive leaves have been collected from Gardens of Agriculture Faculty / University of Baghdad at December 2005 at a rate of temperature 22℃, washed and dried in an oven at 65℃ after putting them in punch paper bags until the weight is stable, milled and placed in glass bottles, closed carefully for the purpose of study.

Cellulases enzymes

crude enzyme solution was obtained from the mold Aspergillus sp. by using solid-state fermentation of wheat straw treated with (NaOH) and hydrogen peroxide [2].Enzymatic activity was calculated for enzymatic crude extract (units / ml) depending on the standard curve for glucose (Figure 1).



The unit of enzymatic activity is defined as the amount of enzyme required to liberate 1 micromole of reducing sugars per hour under the conditions of the experiment.

The aqueous extraction of olive leaves

The aqueous extraction of dried olive leaves was done by adding 300 ml of distilled boiled water to 10 grams of dried leaves in a glass beaker for 24 hours at 40℃ with stirring, and then the mixture was filtrated, then concentrated by rotary evaporator and then dried in an oven at 65℃.

The aqueous extraction of olive leaves with NaOH

The aqueous extraction of dried olive leaves was done by adding 300 ml of 2% of NaOH to 10 g of dried leaves in a glass beaker for 24 hours with stirring, adjusts the pH of mixture to 5.0 by using H_2SO_4, and then the mixture was filtrated, then concentrated by rotary evaporator and then dried in an oven at 65℃.

The aqueous extraction of olive leaves with NaOH and cellulases enzymes

The aqueous extraction of dried olive leaves was done by adding 300 ml of 2% of NaOH to 10 g of dried leaves in a glass beaker for 24 hours with stirring, adjusts the pH of mixture to 5.0 by using H_2SO_4, the cellulases crude enzyme was added to the mixture at the rate of 100:1(mixture:enzyme) at 40℃ for 4 hours, and then the mixture was filtrated, then concentrated by rotary evaporator and then dried in an oven at 65℃.

Inhibition activity of extracts against microorganisms

The inhibition activity of extracts prepared in the above has been estimated by using the method of Disc diffusion assay described by Faleiro et al [7] , by preparing steriled petri dishes containing 15 ml of sterilized nutrient agar and inoculated with bacteria by surface inoculation, and discs with 4 mm diameter saturated with extracts olive leaf treatment , according to the fore mentioned , were put on the surface of petri dish, which represent concentrations of 2.5, 25 and 250 mg / ml of each and with calculated distances to avoid any impact effect, then incubated for 24 hours at 37℃. After the end of this period the growth inhibition zones were measured (in mm) to express the results.

Total phenolic compounds assay*:*

The method we followed to determine total phenolic compounds was a method described by [5]. The

recorded results was at 760 nm by using a spectrophotometer Kind Pye unicum. We depend on the standard curve of gallic acid (Fig. 2).

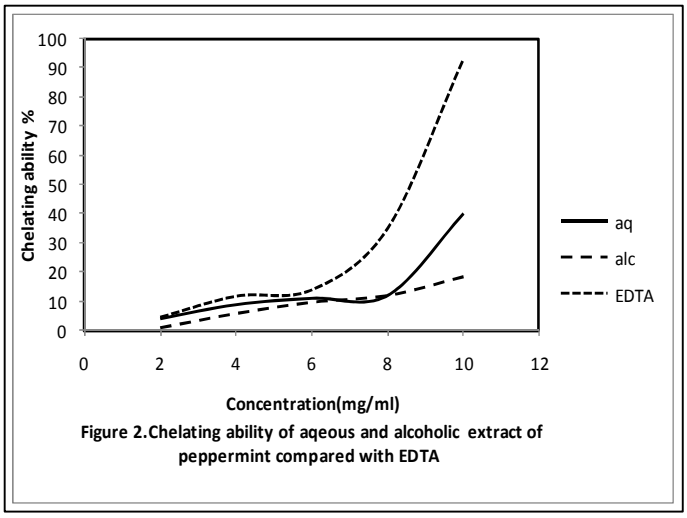
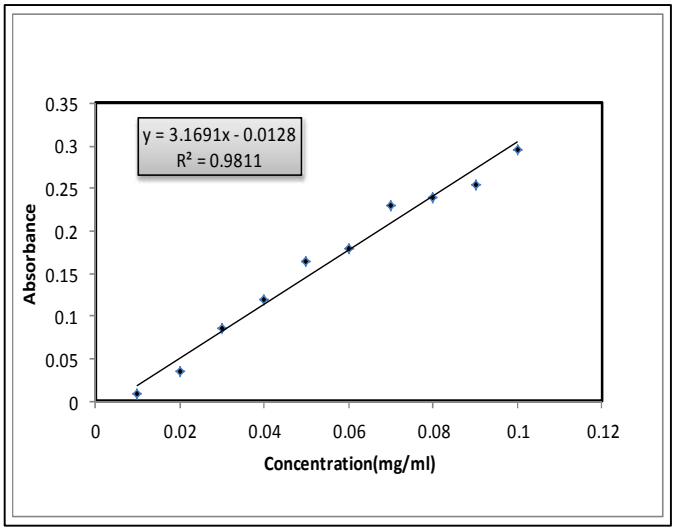


Figure 2.Chelating ability of aqeous and alcoholic extract of peppermint compared with EDTA

Determination of flavonoids

The total flavonoids for both extracts were determined according to (18). They were measured at 510 nm. The flavonoid compounds were determined according to catechin standard curve (Fig.3).



Results and Discussion

The results indicate (Table 1) that the inhibitory effect of aqueous extracts of the leaves of olive untreated with NaOH or the solution of the crude enzyme of cellulases microorganisms, has been limited only to bacteria *Ps. aeruginosa* with two concentrations 25 mg/ml and 0.250 mg / ml, the inhibition zone diameter for each was 4.5 and 0.5 mm, respectively , and there was no effect of inhibitory mentions within the concentrations used in the current study to other bacteria and possibly necessary to use higher concentrations , a study in this regard found that *E. coli* was sense at low concentration of olive leaf extract about 0.6% (weight / volume) [14] .Said Yang et. al. [17] that the aqueous extract of guava gave the highest effective inhibition against E. coli. While the addition of 2% of NaOH showed positive in the effectiveness of inhibitory, from table (2) we can find that there are influential inhibition towards all bacteria used in this study with the amount of variation in sensitivity depending on the type of bacteria and the concentration, where we find that *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* have been effected at the lowest concentration of 2.5 mg/ml , with a diameter zone of inhibition of 5.5 , 1.2 and 4.5 mm, respectively. While *Bacillus cereus* and *Staphyllococcus aureus* weren't affected only by 250 mg/ml concentration and the inhibition zone diameter was 6 mm for each. The increase in inhibitory effect of aqueous extracts by using the NaOH may due to the increase in the level of compounds which possess learned and influential inhibition towards microorganisms. One of the main compounds studied and found in olive leaf, which owns influential inhibition towards microorganisms are flavonoides and glycosides specifically querocitin, rutin and leutolin, it contains not bad quantities of these materials [8].

The table (3) shows the results of the use of aqueous extract of the olive leaves obtained by the presence of NaOH and the solution enzymatic of crude cellulases. The results indicate clearly the role of cellulases in increasing the inhibitory effect of the extract, although there are differences according to the type of bacteria and concentrations, the inhibitory effect is clear for the bacteria *Bacillus cereus* and *E. coli* in less concentration of 2.5 mg/ml reaching 5.15 and 5.1 mm, respectively , while the effect on *Staphyllococcus aureus* and *Salmonella typhimurium* begins at the concentration 25 mg/ml as its diameter zone of inhibition was 4.75 and 4.5 mm, respectively, and went up 6.5 and 8.5 mm respectively in the concentration of 250 mg/ml , one of the researchers have found [3] that the aqueous extract of olive leaves showed good inhibition towards microorganisms and was higher inhibition against *Salmonella typhimurium* PTCC 1639 where the inhibition zone diameter was 11.5mm. Other researcher found that hexenal extract of olive was found to possess activity against *Salmonella choleraesuis* with the minimum bacterial concentration (MBC) of 100µg/ml (0.98 mM). The bactericidal action of these aldehydes tested come in part from their ability to act as nonionic surfactants [12].From this it may be concluded that the use of cellulases has led to extract other compounds affected these bacteria in low concentrations, as cellulases are

responsible for the disintegration and decomposition of plant cell walls of cellulose in the leaves which increases the amount of extracted compounds. While we find that this extract did not affect *Pseudomonas aeruginosa*, the reason for that is may be for extraction compounds with inhibitory effect of the compounds resulting from the aqueous extract, which encouraged the growth of bacteria in all concentrations used in the research. That more phenolic compound with effective impact in microorganisms is Oleuropein, and as the more the extract contains this compound, the good results we obtained, as this compound is metabolized to elenolic acid during digestion, which observed its effect broad inhibition on microorganisms [1]. Most of the phenolic or polyphenolic compounds in nature have antioxidative activities, example include tocopherol, flavonoid and other organic acids [11].

Table 4 showed the content percentages of phenolic compounds of aqueous and alcoholic extracts of olive leaves which were 10.504% and 13.94%, respectively, flavonoids content percentages also have been detected, they were 3.11% and 8.33% respectively. It is clear that the contents were increased in alcoholic extracts

.

Table1. Effect of olive leaves aqueous extract in inhibition of microorganisms

Microorganism	Extract concentration(mg/ml)	Inhibition rate (mm)
E. coli	2.5	----
	25	----
	250	----
Salmonella typhimurium	2.5	----
	25	----
	250	----
Bacillus cereus	2.5	----
	25	----
	250	----
Staph. aureus	2.5	----
	25	----
	250	----
Pseudomonas aeruginosa	2.5	----
	25	4.5
	250	5

Table 2. Effect of olive leaves aqueous extract with NaOH in inhibition of microorganisms

Microorganism	Extract concentration(mg/ml)	Inhibition rate (mm)
E. coli	2.5	5.5
	25	7.0
	250	8.0
Salmonella typhimurium	2.5	4.5
	25	5.0
	250	6.0
Bacillus cereus	2.5	----
	25	----
	250	6.0
Staph. aureus	2.5	----
	25	----
	250	6.0
Pseudomonas aeruginosa	2.5	1.2
	25	7.2
	250	8.5

Table 3. Effect of olive leaves aqueous extract with NaOH and cellulases in inhibition of microorganisms

Microorganism	Extract concentration(mg/ml)	Inhibition rate (mm)
E. coli	2.5	5.1
	25	5.5
	250	7.5
Salmonella typhimurium	2.5	----
	25	4.5
	250	8.5
Bacillus cereus	2.5	5.15
	25	5.75
	250	7.1
Staph. aureus	2.5	----
	25	4.75
	250	6.5
Pseudomonas aeruginosa	2.5	----
	25	----
	250	----

Table 4 Phenolic and Flavonoids content in olive leaves extracts.

Extraction	Phenolic content%	Flavonoids content %
Aqueous	10.504	3.11
Alcoholic	13.94	8.33

Flavonoids have strong anti-inflammatory, antiviral, antioxidant, antiallergenic, anti-fungal, antibacterial, anticancer, cytotoxic and hepalo protective activities thus generated curiosity about flavonoid containing plants [10].

Phenol extract of high hydroxyfyrosol (OLPE) content was obtained from olive leaves (*Olea europaea L*.), was about 92% of the total phenols present in OLPE showed antioxidant effects on different food lipids and did not inhibit lactic acid bacteria growth [16].

Conclusion

The using of alkaline solutions and cellulases in extraction of olive leaves support the increasing of antibacterial activity. The information obtained in this paper about phenolic compounds and flavonoids might be useful for the appropriate additives with antioxidant properties of *Olea europaea* leaves.

Acknowledgments

This paper was done at the laboratories of food science department; we would like to thank the staff of these laboratories for their help. We (all authors) would clear that we have no conflict of interest.

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