Anti-Microbial and Antioxidant Effect of Water Extract of Eucalyptus globulus And Quercus persica Plants on Gram Positive and Gram Negative Bacteria

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Abstract

Background: Since early times, plants as a source of medicinal compounds, has been playing a prominent role in the human health maintenance. They have been utilized in traditional medicine containing a vast number of substances that can be considered in the treatment of chronic diseases and a variety of infections.

Objective: To Antioxidant activity estimation of plant extraction, effect of the antimicrobial activity and estimation of total tannin.

Patients and Methods: The leaves of Eucalyptus and the seeds of Quercus were collected in Erbil and Shaqlawa during September 2014. The leaf water extracted from *Eucalyptus globulus* and *Quercus persica* plants were studied its antibacterial activity against two types of bacteria *Staphylococcus aureus* and *E. coli* each plant was used in three different concentrations (25%, 50% and 100%).

Results: It was recognized that both plant extracts have inhibitory effects against two tested bacteria with the observations that the Eucalyptus has more anti-bacterial activity than Oak.

Conclusion: A significant effect on the growth inhibition of gram positive and gram negative bacteria occur during use water extract of *Eucalyptus globulus* and *Quercus persica* Plants

Key words: Antioxidant, *Eucalyptus globulus*, *Quercus persica*, *Staphylococcus aureus* and *E. coli*.

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Introduction

Since early times, plants as a source of medicinal compounds have been playing a prominent role in the human health maintenance. According to reports from World Health Organization, plant extracts or their active ingredients constitute about 80% of folk medicine in the globale traditional therapy. Indeed, more than 50% of modern clinical drugs are originated from natural products [1].

Plants that have been utilized for traditional medicine contain a vast number of materials applicable for the treatment of chronic diseases and variety of infections. The substances capable of inhibiting microbial growth or killing them are considered as promising candidates for the treatment of various infectious diseases. As a traditional medicine, medicinal plants are well recognized in rural communities of many developing countries [2][3]. The immunomodulatory and characteristics of

antioxidant medicinal plants elucidate their antibacterial activities. Their many-sided immunomodulatory action is initiated by stimulating together non-specific and specific immunity [4].

Implementing plant extracts and phytochemicals combined with antimicrobial properties, can be of great implication in therapeutic actions. In latest years, many types research have been showed around the world to prove such effectiveness. Numerous plants have been applied in view of their antimicrobial behaviours which are because of compounds synthesized in the plant secondary metabolism [5].

Human defence mechanism with its enzymatic and non-enzymatic antioxidant systems can limit and protect the body against reactive oxygen species (ROS) [6]. Nonetheless, the innate defence may not be enough for continued or severe oxidative stress. Therefore, certain amounts of exogenous antioxidants are constantly wanted to preserve an adequate level of antioxidants to re-equilibrium the ROS in the human body. Numerous synthetic antioxidants, such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxyltoluene (BHT) are extremely effective. However, due to side impacts of synthetic antioxidants, the request for natural antioxidants has been increased [7].

Escherichia coli are facultative anaerobic, rod shaped, motile, spore making Gram-negative bacteria existing in the lower intestine [8]. The bacteria belong to the Entrobactreiaceae family. Though most strains of *E. coli* are non-pathogenic they may cause some diseases in the lower intestine such as gastroenteritis, urinary tract infection, mastitis, vaginosis, septicaemia, peritonitis. This enterotoxigenic bacterium commonly causes diarrhoea in children [9].

Staphylococcus aureus is a gram-positive, non-spore making globular bacterium from Staphylococcus genus. *S. aureus* has the ability to producing a kind of toxin known as staphylococcal enterotoxin (SE) which is responsible for nearly all staphylococcal food poisoning [10]. *Staphylococcus aureus* is regarded as facultative anaerobic that has the ability to grow under aerobic and anaerobic conditions [11].

It was recognized that the presence of the antioxidative and antimicrobial agents in *Eucalyptus globulus* and *Quercus persica* can be effective on the growth inhibition of gram positive and gram negative bacteria.

The aim of this study was to appraisal of antibacterial activities of *Eucalyptus* and Oak plants, also to determine antioxidant activity estimates of plant extraction and estimation of total tannin.

Patients and Methods

Collection of plant

The leaves of *Eucalyptus globulus* and fruit of *Quercus persica* were collected in Erbil and Shaqlawa during September 2014. The identification of plants was by senior taxonomist: Dr Yasin A. Rashid, Salahaddin University, College of Sciences, Biology Department, and Erbil, Iraq. The fresh samples of *Eucalyptus globulus* and *Quercus persica* were the first washed by tap water to remove the undesired particles and the most important thing to do with freshly collected material to dry it as fast as possible to prevent fungal infection and preserve colour. Therefore, plants had been dried in ventilated an oven at 40 °C for three days. The dried examples were differently crushed into fine powder by using an electric blender and stored at 2- 4 °C in dark containers [12].

Preparation of extracts

The sample of (*Eucalyptus and Oak*) were separated and dried in an oven at 40Co for 72 hrs. Then samples were powdered by a grinder. Then samples sieved through a mesh

2 mm, and then put ten grams (10 g) of each powder into mortar and pistil and added solvent (table 1) into it. After that collect mixture in a conical flask. Then put conical flask sample on a shaker for 24 hours. Next, it was filtered by vacuum Buchner funnel. Finally, collected sample into a cylinder to complete to 100 extracts. Extract sample was they stored in wide mouth dark glass bottles and kept in deep freeze to avoid degradation until use [13]. At this volume, the solution considered as 100% which was diluted to the concentration of (100%, 50% and 25%). and the control gets distilled water.

Table (1): Properties of solvent that used in this study.

Solvent	Formula	Boiling point (°C)	Melting point (°C)	M. weight (g/mol)	Density (g/mL)	Polarity Index	pH
Distil Water	H2O	100	0.00	18	0.998	9.0	7.00

Yield determination

The extraction yield is a measure of solvent efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of dry sample [14]. Powder roots (0.5 g) were extracted with 50 ml ethanol separately by using varies extraction methods described before. The yield percentage of the extract was determined by using the following formula:

Yield percentage (%)=X/Y*100

X is the oven dry weight of extract (g),

Y is the oven dry weight of the sample (g).

Determination of total condensed tannin

This assay was carried out by Shimadzu UV-visspectrophotometer.Extraction solution was prepared by mixing 0.05 g of Fe2SO4, 95 ml N-butanol and 5 ml Hydrochloric acid (35%). For determining the condensed tannin, 0.01 g of both Eucalyptus and Oak plants and mimosa tannin put separately in a test tube and 10 ml of extraction solution was added and placed in water bath for heating 1 h. The absorbance was measured at 580 nm wavelength [15].

DPPH radical scavenging activity

The plant extracts of free radical scavenging activity of *Eucalyptus* and *Oak* had been determined by means of the DPPH assay described by the moderate amendment [16]. In its radical form, 1,1-diphenyl-2-picrylhydrazyl (DPPH) absorption level decreases at 517 nm with reduction of an antioxidant or a radical specie. Briefly, 0.1 mM DPPH was once prepared for plant extraction. Then 0.1, 0.2 and 0.3 ml of pattern solutions combined with solvent up to 3 ml in a test tube, individually. Next, 1 ml of DPPH was added. The mixture was then shaken vigorously by Vortex and placed in a dusky room temperature for 30 min. Later, Shimadzu UV-vis 1240 spectrophotometer at 517 nm used for measuring the absorbance of samples. Butylated hydroxyltoluene (BHT) was used as a reference. Radical scavenging effectiveness was expressed as the inhibition percent of free radical through the pattern and calculated with the following equation [17].

Inhibition of DPPH radical scavenging activity (%)=A-B/*100 where, A is the absorbance of DPPH, B is the absorbance in the presence of sample and BHT.

Microorganisms

All the microorganisms had been obtained from microbiology laboratory at Salahaddin University, College of Sciences, Department of Biology, Iraq including: *Escherichia coli* G- bacteria and *Staphylococcus aureus* G+ bacteria.

Antimicrobial activity

Two pathogenic bacteria had been used for the antimicrobial activity. Eucalyptus and Oak extracts had been validated separately against all microorganisms using well diffusion method [18]. The nutrient agar (NA) media were disinfected by autoclaving for 15 min at 121 ºC. The medium had been transferred aseptically into each sterilized petri plate. The Petri plates of nutrient agar were inoculated by bacterial suspensions (*Escherichia coli* and *Staphylococcus aureus*) prepared from 24 hours' cultures, using sterile Swabs. The effect of extracts on bacteria had been studied utilize well diffusion method through making four holes on each plate then the holes were filled with extracts. The plates had been incubated at 37C for 24 hours for each test, respectively. After that, the outcomes were obtained by measuring the diameter of inhibition zones by millimetre. Total tests had been performed in triplicate. For more confirmation, ampicillin and gentamicin (10 µg/ml) were used as comparative for antibacterial activity (6 mm in diameter).

Statistical analysis

The data for antimicrobial activity were analysed by ANOVA using SPSS (version 18) statistical program. The mean differences were compared as significant with (LSD) Least Significant Difference.

Results

The various extract of the *Eucalyptus* and *Oak*. They were statically significant by the different diameters of inhibition zones of most the activities against the organisms tested were given in Table 2. The mean inhibition zones against all test bacteria ranged from 1.1-2.3 cm. The lowest inhibitory zone 1.1 mm of *Quercus* extract at concentrate 25 % was against *Escherichia coli*. Whereas, the highest inhibitory zone 2.3 cm of Eucalyptuses extract at concentrate 100 % was against *Staphylococcus aureus*.

Table (2): Inhibition zones (cm) of the root extracts against the microorganisms**.**

Organisms		**Inhibition zone (cm)**									
		Eucalyptus extract			LSD		Quercus extract			**LSD**	
		25 %	**50 %**	**100 %**	**0.1**	**0.5**	**25 %**	**50 %**	**100 %**	**0.1`**	**0.5**
B 1	Mean	1.85	2	2.25	0.26	0.3	1.1	1.525	1.45	0.19	0.23
	SD	±0.57	±0.57	±0.57			0.3	±0.57	±1.0		
B 2	Mean	1.675	1.825	2.3	0.3	0.4	1.15	1.325	1.625	0.34	0.39
	SD	0.33	±1.0	±0.57			±0.57	±0.57	±0.57		

Note: B1represents *Escherichia coli* and B2represents *Staphylococcus aureus*.
The values presented as mean ± SD of three replication.

Table (3): Synthetic antibiotic activities against the microorganisms.

Organisms	**Inhibition zone (mm)[1]**	
	Ampicillin 10µg/ml	**Gentamicin 10µg/ml**
Escherichia coli	–	34.3±1.52
Staphylococcus aureus	6.3±0.57	36.3±1.52

Figure (1) indicated that all treatments; t1 (25%), t2 (50%), t3 (100%) aqueous extract of leaf Eucalyptus caused a significant effect on the growth of *Escherichia coli* in

compared with control respectively. Figure (2) clearly manifested all treatments; treatment 1 (25%), treatment 2 (50%), and treatment 3 (100%) aqueous extract of leaf Eucalyptus showed the significant effect on the growth of pathogenic bacteria *Staphylococcus aureus* in compare to control respectively. It is shown in figure (3) the result of all treatments; t1 (25%), t2 (50%), t3 (100%) aqueous extract of Oak seed had a significant impact on the growth of *Escherichia coli* respectively. It is obvious from figure (4) the significant effect of all treatments; t1 (25%), t2 (50%), t3 (100%) aqueous extract of *Oak* seed caused a significant effect on the growth of Staphylococcus aureus respectively.

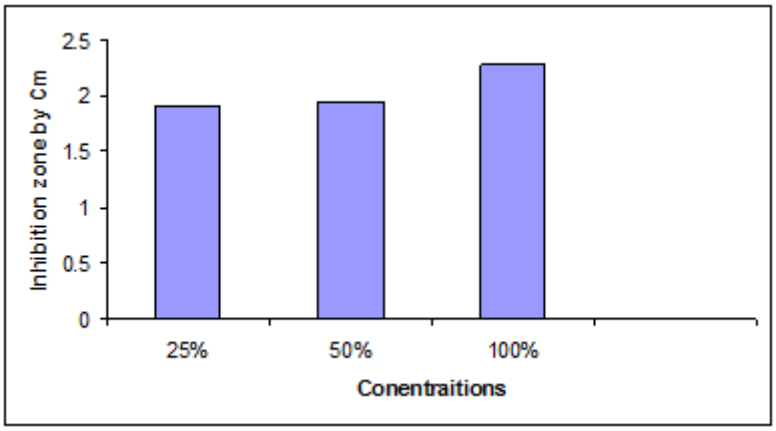

Figure (1): Effect of *Eucalyptus on E. coli*.

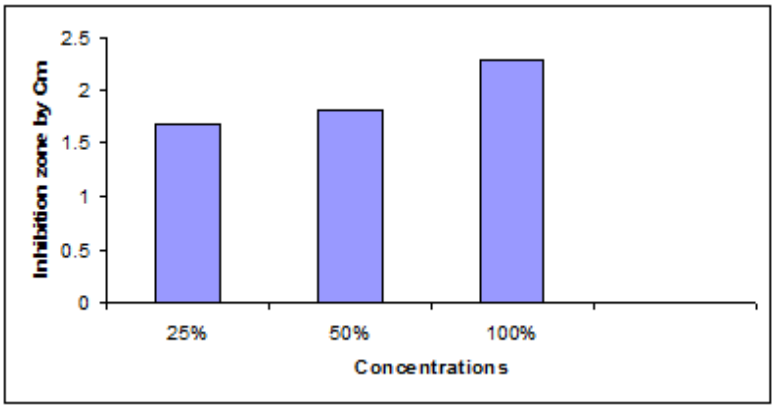

Figure (2): Effect of *Eucalyptus on Staphylococcus aureus*.

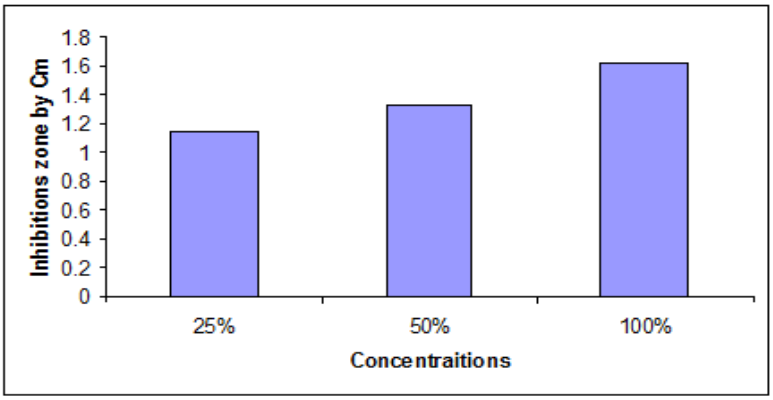

Figure (3): Effect of *Quercus on E. coli.*

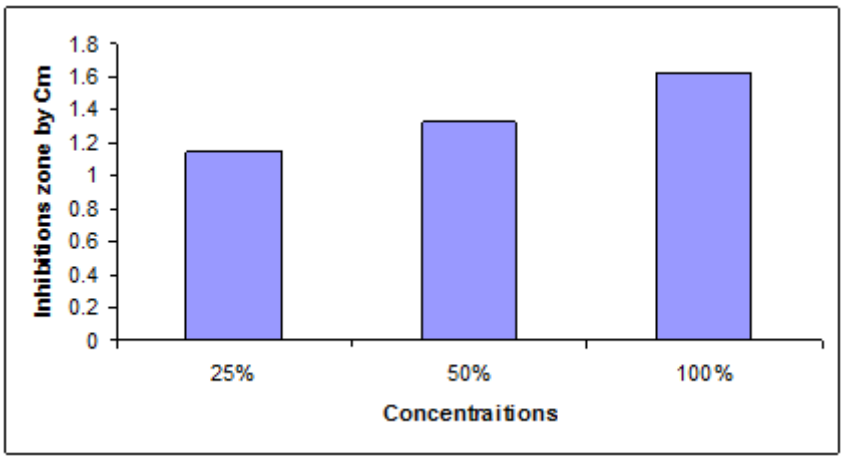

Figure (4): Effect of *Quercus* on *S. aureus*.

Yield Determination

The yield percentage of the Eucalyptus and Oak extracts prepared by using ethanol is summarized in Table 4.1.

Table (4): Yield percentage in the root extracts of Eucalyptus and Oak.

Solvent	Yield (%)	
	Mean[1]	SD[2]
Eucalyptus	19	± 0.1
Quercus	8	± 0.15

1Values presented as mean ± SD of three measurements.
2SD: Standard deviation

Total Condensed Tannin

Total condensed tannin concentration of the Eucalyptus and Oak is presented in Table 4.2. The tannin concentration was calculated by using standard calibration curve (R2= 0.999) with concentration ranged from 6.25 mg/L to 50 mg/L detailed in Table 4.3 and Figure 4.2.

Table (5): Total condensed tannin concentration in roots of Eucalyptus and Oak.

Plants	Condensed Tannin (mg/L)			Average	Standard deviation	Variation (V) mg/g
Eucalyptus	223.83	247.11	255.85	241.29	±16.33	6.77
Quercus	0.44	1.03	1.17	0.84	±0.37	44.35

Tannins are water-soluble antioxidant with molecular weight of 500-3000 g/mol. Tannins are natural polyphenols ubiquitously distributed in plants, such as vegetables, fruits and seeds. Tannins are widely used in wine industry for color stabilizer; balancing the complexity in wines, inhibit certain enzymes in infected fruits and act as wine fining agents [19].

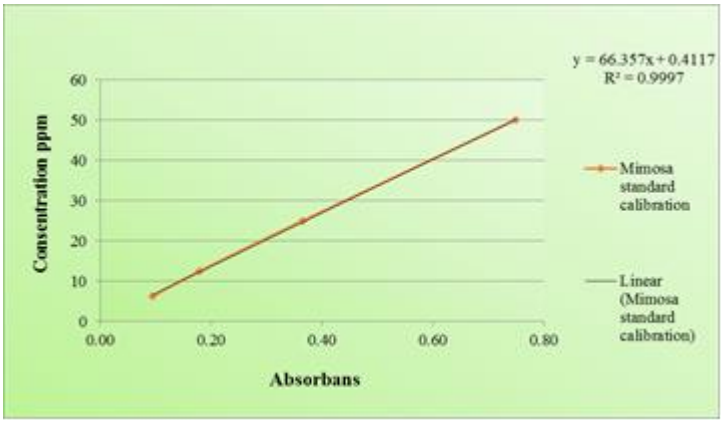

Figure (5): Mimosa tannin calibration curve.

Table (6): DPPH scavenging activities.

Plants	DPPH Radical Scavenging activity (%)					
	Extract volume (ml)			BHT volume (ml)		
	0.1	0.2	0.3	0.1 ml	0.2	0.3
Eucalyptus	97.63 %	93.77%	93.69%	67.4	65.6	78.3
Quercus	98.74%	98.45%	98.67	67.4	65.6	78.3

Discussion

The current medicine has successfully been used for years in the treatment of infectious diseases [20]. Many pathogens are becoming resistant to antibiotics increasing the alarm for a mounting threat. This, in turn, urges for placing strategies upon which new pharmaceutical improvements are envisaged [20]. Investigating the antibacterial activity of extracts has shown that a great number of these plants contain active compounds. The occurrence of antifungal, antibacterial and additional biological activities has been confirmed in various plant extracts from diverse traditional medicine practices [22].

The leaves essential extract added into culture media inoculated with S. aureus and *E. coli* inhibited the development of bacteria. The inhibition zone was greater, on gram negative bacteria (i.e. *E. coli*) than on gram positive bacterium (i.e. *S. aureus*) which might be due to their difference in cell wall.

These findings are incredibly similar to those demonstrated by other researchers working on the antimicrobial activity of essential extract of *E. globulus* leaves [23][24]. The growth of tested bacteria in the presence of high concentrations of essential leave's extract was extremely inhibited. Thus, it was assured that both bacterial species were sensitive to the extract. In fact, the chemical compound of extract leads to growth inhibition of bacteria. This characteristic of extract needs further investigations in terms of constituents and antimicrobial contents.

Some scientists have been observed that gram-negative microorganisms are extra sensitive to essential oils than gram-positive [25][26]. In view of this fact, gram-positive and gram-negative are different in several aspects particularly their cell walls mainly with regard to the occurrence of lipoproteins and lipopolysaccharides in gram-negative bacteria that form a fence to hydrophobic compounds [27][28].

Radical scavenging activity mechanism of herbal extracts could be related to the occurrence of polyphenolic compounds. It has previously been showed that the

polyphenolic compounds are in charge of radical scavenging activity because of their hydrogen atom endowment to active free radicals [29]. Phenols are high significant plant constituents as of their scavenging [30]. or antioxidative abilities [31]. Similarly, in this study, the antioxidative characteristic of *Eucalyptus globulus* and *Quercus persica* have demonstrated a significant effect on the growth inhibition of Gram positive and Gram negative bacteria.

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