

Study of the Antimicrobial effect of *Melia azedarach* L. plant

دراسة للفعالية ضد الجرثوميه لنبات السبج *Melia azedarach* L.

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Abstract

This study was aimed to evaluate the antimicrobial activity of the leaf extracts of *Melia azedarach* L. plant against different pathogenic microorganisms such as (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsilla sp.*, *Candida albicans*). Two plant extracts (Aqueous and ethanolic) under four different concentrations (25, 50, 75, 100) mg/ml were used by Agar-well diffusion method. Chemical detection of extract showed that the extracts contain tannins, flavonoids, terpins, steriods, alkaloids, and saponins. Aqueous leaf extract showed no effect against all tested microorganisms at (25, 50, 75, 100) mg/ml concentration except *Candida albicans* which was sensitive to 100mg/ml concentration. While Ethanolic (80%) leaf extract showed sensitivity on *Staphylococcus aureus* at 100mg/ml concentration.

المستخلص

الهدف من هذه الدراسة هو تقييم القدرة التثبيطية للمستخلص المائي والكحولي لاوراق نبات السبج *Melia azedarach* على نمو الاحياء المجهرية الممرضه مثل (*Staphylococcus aureus* ، *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsilla sp*) وبالتركيز (25،50،75،100) ملغم/مل وذلك باستعمال طريقة الحفر. اجري الكشف الكيميائي للنبات واطهرت النتائج احتواء النبات على المركبات الفعالة مثل التانينات والتربينات والستيرويدات في المستخلص المائي ، اما المستخلص الكحولي يحتوي على التانينات والفلانويدات والتربينات والستيرويدات وكذلك الصابونيات . لم يظهر اي تثبيط للمستخلص المائي على الكائنات المجهرية المستخدمة ما عدا الخميرة *Candida albicans* فقد ابدت تحسسها للتركيز 100 ملغم/مل ، بينما اظهرت البكتريا *Staphylococcus aureus* تحسسها للتركيز العالي 100 ملغم/مل من المستخلص الكحولي 80% ايثانول

Introduction

The demand for herbal medicinal plant is growing very fast in recent years, because of the patient's immunity to medicine and its side effects [1]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent potential source of antibiotic prototypes [1].

Herbal medicines are dietary supplements that contain herbs, either singly or in mixture. An herb also is a plant or plant part used for its scent, flavor, and/or therapeutic properties. Products made from botanical parts that are used to maintain or improve health are called herbal supplements, botanicals, or phytomedicines [2].

Melia azedarach Linn. belongs to (Meliaceae) family, commonly known as "Persian Lilac", is one of these important plants which contain a number of medicinally compounds [3]. *M. azedarach* is used orally and topically as an antiparasitic and antifungal agent [4,5]. Chemical composition reveals the presence of alkaloids,

Key words: *Melia azedarach*; Antimicrobial activity; aqueous extract; ethanolic extract

tannins, meliottannic acid, benzoic acid, vanillic acid, and others [6,7]. The aim of this current work was to evaluate the antimicrobial potential of *M. azedarach* extracts in different solvents against some pathogenic microorganisms.

Materials and Methods

Preparation of leaf extracts

Healthy plant leaves were collected from the University of Al-Nahrain garden, in April 2011 and 2012 and identified at the Department of Biology, University of Baghdad. The leaves washed thoroughly in running tap water, 100 grams of fresh leaves of *M. azedarach* plant were weighed and put in conical flask with 1000ml of distilled water to prepare the aqueous extract, then in water bath for 2hr at 40°C. The extract was filtered through whatman No.1 filter paper. The filtrate was concentrated in vacuum using rotary evaporator at 40°C then the extract was sterilized with millipore filter (0.22µm) [8].

100gm of fresh leaves were blended with 500ml of alcohol (80% ethanol) and stored at room temp. for 5 days. The extract was filtered through cheesecloth then through whatman No.1 filter paper. The filtrate was concentrated by using rotary evaporator at 45°C. Both extracts were left at 4°C until used in the assay. The extraction was repeated three times.

Test microorganisms

Four strains of Gram-positive and Gram negative bacteria were used to estimate the antimicrobial activity were obtained from Biotechnology Research Center at Al-Nahrain University. The microbial strains used in the study were (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsilla sp.*) and *Candida albicans*.

Antimicrobial assay

Muller Hinton Agar medium (MHA) was used for the antibacterial susceptibility study. The bacterial assay was performed by agar well diffusion method [9,10]. (3.1 g/100 ml) of (MHA) was weighed and dissolved in 100 ml of distilled water, then sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into sterile Petri plates. The culture medium was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Microorganisms' concentration was 10^2 . Wells (6) mm were punched into the agar and filled with the extracts. Four different concentrations of plant extracts (25,50,75,100) mg/ml filled the wells separately. The plates were incubated at 37°C for 18 h. and the antibacterial activity was determined by measuring the diameters of inhibition zone in millimeter. Controls were maintained where pure solvents were used as negative controls instead of the extracts. The experiment was repeated three times and the mean values were presented.

Results

Phytochemical analysis

The preliminary phytochemical screening of different extracts was done to ascertain the presence of bioactive components. The presence of alkaloids, flavonoids, tannins, terpenes, steroids, and saponins was determined [11,12], Table (1) showed the results

Table (1): Bioactive components of *M. azedarach* leaf extract (aqueous and ethanolic)

Phytochemicals	<i>Melia azedarach</i>
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Terpins	+

The results showed also that aqueous leaf extract of *M. azedarach* did not possess antibacterial activity against tested gram positive, and gram negative bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*) at all concentrations (25, 50, 75,100) mg/ml of aqueous and solvent leaf extract as shown in Table (2).

While the ethanolic leaf extract of *M. azedarach* showed that it possessed antibacterial activity against *Staphylococcus aureus* bacteria at 100 mg/ml. The *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* were resistant to ethanolic leaf extract. While the ethanolic leaf extracts showed a slight effect on *Candida albicans* in aqueous leaf extracts Table (2).

Table (2): Antimicrobial potential of *Melia azedarach* crude leaf extracts

Tested microorganisms	Extracts	concentrations (mg/ml)			
		Inhibition zone diameter (mm)			
		25	50	75	100
<i>Pseudomonas aeruginosa</i>	AQ	–	–	–	–
	EA	–	–	–	–
	C	–	–	–	–
<i>Staphylococcus aureus</i>	AQ	–	–	–	–
	EA	–	–	–	16
	C	–	–	–	–
<i>Escherichia coli</i>	AQ	–	–	–	–
	EA	–	–	–	–
	C	–	–	–	–
<i>klebsilla sp.</i>	AQ	–	–	–	–
	EA	–	–	–	–
	C	–	–	–	–
<i>Candida albicans</i>	AQ	–	–	–	14.5
	EA	–	–	–	–
	C	–	–	–	–

AQ=Aqueous, EA=Ethyl acetate, C= Control

Discussion

Aqueous extract did not show any antibacterial activity against all the bacteria studied. Similar results have been reported in the literature [13,14,15,16,17].

The difference in sensitivity might be ascribed to the difference in morphological constitutions between Gram-positive and Gram-negative organisms. Many plant species present inhibition zones of differing diameters; however, size difference of the inhibition zone depends primarily upon many factors for e.g. diffusion capacity of substances (present in the extracts) in the agar medium, antimicrobial activity of diffused substances, growth and metabolic activity of microorganisms in the medium. Inhibition zone diameter can further be associated with polarities of substances which make up the tested extracts, and also with cell wall composition of tested organisms since Gram-positive bacteria present cell walls with lower lipid levels than do Gram-negative bacteria [18].

Based on these results, it can be concluded that *M. azedarach* plant leaf extracts have potential as antimicrobial compounds against some microorganisms such as *S. aureus* and *Candida albicans*.

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