Biological Effect of Aqueous Extract and Ethanolic Extract of Malva Leaves and some Antibiotics against some Local Pathogenic Bacteria

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ABSTRACT

Malava is an important medicinal plant used as a remedy for cut wound, eczema, dermal infected wounds, bronchitis, and digestive problems. To evaluate the scientific basis for the use of the plant, the antimicrobial activity of different concentrations of aqueous and ethanolic malva leaves extract were investigated in vitro against five pathogenic bacteria (Proteus sp, Salmonell sp, Kliebsiella pneumoniae,Pseudomonas aeruginosa and Staphylococcus aereus) The results indicated that all concentrations showed an antimicrobial activity against all the tested bacteria, the highest activities were against S.aureus,with the inhibition zone was (25)mm ,(20)mm at (0.1)% ethanolic and aqueous con respectively .The biological activity of ethanolic and aqueous extract of malva leaves when compared with some antibiotic include: Tetracycline, Gentamycin, Ampicillin, Carbinicilin and Erythromycin. Antimicrobial of the lowest conc(0.01%) of malva leaves was better than all antibiotics against all tested pathogenic bacteria.

Keywords: Malava, Antimicrobial Activity.

التأثير البايولوجي للخلاصة المائية والخلاصة الكحولية لاوراق نبات الخبيزة وبعض المضادات الحياة على انواع من البكتريا المرضية المعزولة محليا

الخلاصة

الخبيزة نبات طبي مهم ,يستخدم كعلاج للجروح والاكزماوالاصابات الجلدية الملتهبة والمشاكل الهضمية . ولمعرفة الاساس العلمي الطبي الذي استخدم من اجله النبات درست داخل المختبر الفعالية المكروبية لتراكيز مختلفة من المستخلص المائي والايثانولي لأوراق النبات على خمسة انواع من البكتريا المرضية Proteus sp ,Salmonella sp, Kliebsiella pneumoniae, Peudomonasa eruginosa and Staphylococcus aereusباتباع طريقة الانتشار بالحفر .اشارت النتائج الى ان كل التراكيز لها فعاليةمايكروبية ضد كل الانواع المرضية.وان اعلى فعالية كانت ضد بكتريا Staphylococcus aereusاذ كان التثبيط (20و25)ملم عند تركيز 0,1% للمستخلص الايثانولي والمائي بالتتابع. ثم قورنت الفعالية البايولوجية للمستخلص الايثانولي والمائي للنبات مع بعض المضادات الحيوية فلوحظ ان اقل تركيز وهو0.01% لمستخلصات الاوراق كانت افضل من فعالية جميع المضادات الحيوية على جميع انواع البكتريا المرضية المدروسة.

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INTRODUCTION

For a long period of time, plants had been a valuable source of natural products for maintaining human health, especially in the last decade. The use of plant compounds for pharmaceutical purposes has gradually increased, and all according to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [12]. These products are Phenols compounds, flavonoids, steroids, resiens fatty acid gums and alkaloids which are the most common classes of phytochemicals that have exhibited promising activity against a wide range of bacterial species [18] [7], Compounds extracted from different part of plants can be used to cure diarrhea, dysentery, cough, cold and bronchitis [13]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

Malva is species of mallow belonging to family of Malvaceae known as common mallow Malva parviflora L. and Malva strumcoromandelianum L. belong to the family Malvaceae which has been famous for medicinal properties for many years [15]. The plants of this family are well known for their antibacterial and antifungal activities due to the presence of alkaloids, essential oils and phenolic quleoside [19]. It is an annual or perennial herb, growing to a height of four feet, and it has been used for the treatment of colitis and stomatitis, in cases of chronic bronchitis, against furuncle and abscess [14], contusions and haemorrhoids as well as other dolorous and inflammatory processes [8] .Because of their active constituents such as essential oils, terpenes, aromatic compounds, anthocyanins, mucilage, tannins and vitamins A, B, C [6]. The high mucilage content of Malva sylvestris makes it an excellent demulcent that can be used for many applications. In the digestive tract the fruit mucilage can be used to heal and soothe inflammations such as gastritis, peptic ulcers, enteritis, and colitis. [20].

Now days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effect on the host including hypersensitivity, immune-suppression and allergic reactions [1]. This situation forced scientists to search for new antimicrobial substances [4]. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, and there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious disease from medicinal plants [11].

In this study, ethanolic extract obtained from Malva leaves was tested *in vitro* against five bacterial strains by the agar diffusion method. Staphylococcus aureus, Klebsiella pneumonia, Salmonella spp, Proteus sp, and Pseudomonas aerugenosa were used in this investigation.

MATERIALS AND METHODS

MICROORGANISMS

The one Gram-positive (Staphylococcus aureus) and four Gram-negative (Salmonella typhi, Pseudomonas aureuginosa, Kliebsiella pneumoniae, Proteus sp)

bacterial strains obtained from general microorganisms Laboratory of biotechnology branch in school of applied sciences in university of technology, Baghdad.

Gram positive	Gram negative
Staphylococcus aureus	Salmonella typhi
	Pseudomonas aureuginosa
	Kliebsiella pneumoniae
	Proteus sp

PLANT EXTRACT

The Malva leaves were collected from a Botanical Garden, washed with clean sterile water and oven-dried for one hour at 40 ^{o}C. Dry plant material was blended into fine powder, and 50 g of dried powder was filled in the thimble and extracted successively with 500 ml(70% ethanol) in Soxhlet extractor for24 h. The extracts were filtered (through 0.45 mm filters under sterile conditions). The extracts solutions were concentrated at 40ºC and evaporated in oven and weighed. (0.1, 0.01, 0.02, 0.03, 0.04) % plant extract concentrations prepared by dissolving in [10] ml of sterile dimethyl sulfoxide (DMSO) [10]. Dry plant material was boiled with 500ml of distilled water in a conical flask, till the water reduced to 200 ml. The extract was then filtered through filter paper in a sterile bottle. This made a 50% extract of the herb [3], then prepared the same concentrations in above.

ANTIBACTERIAL ACTIVITY

Antibacterial activity was determined by the well diffusion method according to [2] Bacteria were sub cultured on nutrient agar at 37°C prior to be grown in nutrient broth over night. The antibacterial activity of the plant extracts was carried out by well diffusion assay, Muller Hinton agar (MHA) plates were swabbed with the freshly broth cultures of the organisms, five Wells were bored into the agar by sterile tip of micropipette, and 50 µl of prepared plant extract concentrations were tested Disc (6mmdiameter) of Gentamycin (CN10), Ampicillin (AM10), Carbnicilin (PY100), Tetracycline (TE30), Erythromycin (E15) was used as positive control [16]. Antibacterial assay plates were incubated at 37°C for 24h, the diameters of the inhibition zones were measured in cm.

RESULTS AND DISCUSSION

Results were showed that gram positive strain(*S.aureus*) was the most susceptible bacteria to all extract concentrations (2.0-2.5)cm which agrees with (15),They found the highest inhibitory zone by ethanolic extract of malva was against *Staphylococcus* and with [17] they found the highest inhibitory zone (2.2 cm) by ethanolic extract of malva was against Staphylococcus. Results showed that Gram-negative microorganisms are typically more resistant to antimicrobial agents than Gram-positive bacteria, This has long been explained by the presence of an outer-membrane permeability barrier in Gram-negative bacteria, which limits access of the antimicrobial agents to their targets in the bacterial cells [9], Flavonoids that appear to have greater activity against Gram-positive than Gram-negative bacteria.

Biological activity of ethanoloic extract of malva leaves was compared with number of antibiotics that known for their ability shown in Table (3).

In Table (2), the aqueous extract showed less effect than ethanolic extract on both gram$^+$ and gram$^-$ pathogenic bacteria tested in this study,but the stock concentration was better than the effect of antibiotics tetracyctin and gentamycin . The effect of aqueous extract was mentioned by [5] and valuable gains have been documented on the bacterial properties of malva extracts

Antibacterial activity of 0.01% concentration of ethanolic extract of malva leave was better than tetracycline and gentamycin against all tested bacteria. The some results were shown with the Carbinicilin, Ampicillin and Erythromycin Table (3).

Finally we can conclude from this study that the active material of leaves extract is a strong antibacterial agent from natural sources.

Figure (1) shows that the ethanolic extract was the highest effect , the second was aquous extract , were as the antibiotics were the lowest . The antibiotics (carbencillin and erythromycin) were no affected totally on all bacteria that were resistant against them. In this figure the lowest concentration (0.01%) was taken in the comparation of effect to show that malva extracts are good antibacterial within little concentrations.

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Table (1) Effect of malva leaves extract concentration on bacterial strains tested by well diffusion assay (cm.diameter)of inhibition zone(ethanolic extract).

Con%	S.aureus	K.pneumoniae	Proteus.sp	S.typhi	P.aerugenosa
0.1 Stock	2.5	1.7	1.9	1.2	2.2
0.01	1.7	1.0	1.6	0.9	1.3
0.02	1.8	1.1	1.7	1.0	1.9
0.03	1.8	1.2	1.7	1.0	2.0
0.04	2.0	1.3	1.7	1.1	2.0

Table (2) Effect of malva leaves extract concentration on bacterial strains tested by well diffusion assay (cm.diameter) of inhibition zone (aqueous extract).

Con%	*S.aureus*	*K.pneumoniae*	*Proteus.sp*	*S.typhi*	*P.aerugenosa*
0.1 Stock	2.0	1.4	1.8	1.0	1.8
0.01	1.3	0.9	1.2	0.7	1.0
0.02	1.6	1.0	1.4	0.8	1.2
0.03	1.7	1.1	1.5	0.8	1.3
0.04	1.9	1.2	1.7	0.9	1.5

Table (3) Inhibitions zone (cm.) of 5 antibiotics against 5 pathogenic bacteria.

Antibiotics	S.aureus	K.pneumoniae	Proteus sp.	S.typhi	P.aeurogenosa
Tetracyclin	0.5	R	0.9	0.6	R
Gentamycin	1.1	0.7	1.0	0.5	0.9
Ampicillin	0.4	R	R	R	R
Carbnicilin	R	R	R	R	R
Erythromycin	R	R	R	R	R

R= resistant.

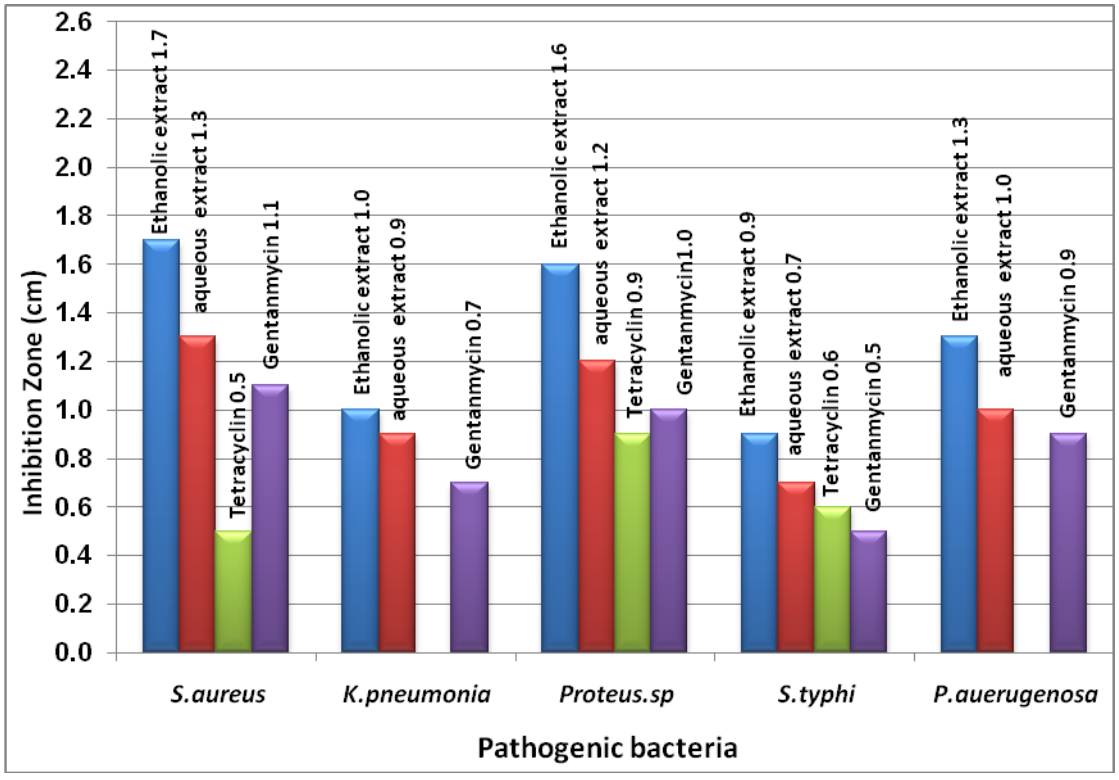

Figure (1) Effect of malva extract (0.01%) and antibiotics (Tetracyclin) &Gentamycin) on growth of some pathogenic bacteria.