Evaluation of Antibacterial Activity of Ethanolic Extracts for Three Local Plants

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Abstract

There is an increasing interest in the use of plant extracts as therapeutic agents, particularly their capacity to inhibit the growth of pathogenic microorganisms. In this study antibacterial effect of *Malva sylvestris*, *Anastatica hierochuntica* and *Vitis vinifera* leaves extracts were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus mirabilis*. The *in vitro* antibacterial activity was performed using agar well diffusion method and the minimum inhibitory concentration (MIC) was determined by microtitration technique. The result indicated that the extract of *V. vinifera* leaves inhibited with the growth of gram-positive bacteria, as well as gram-negative bacteria while the extract of *A. hierochuntica* showed inhibitory activity against *B. subtilis* and *S. aureus*. Finally the extract of *M. sylvestris* showed inhibitory activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *P. mirabilis*. Phytochemical studies showed that the active component in ethanolic extract of these plants have amino acid, glycoside, phenol, tannins and alkaloids.

Key words: *Malva sylvestris*, *Anastatica hierochuntica*, *Vitis vinifera* leaves, antibacterial activity.

Introduction

Over the past decade, there has been an explosion of interest in antibacterial and antifungal activity of natural products. Recommendation for the use of various natural products for infectious diseases is widespread and appears in a number of popular and other easily obtainable texts [1].

Today in most countries of the world, the knowledge of plant therapy has become an agenda in scientific researches and the use of herbal medicine is constantly growing. Medicinal plant have many healing properties without showing any significant side effect. Antibacterial activity is one of those properties among many others [2].

Malva sylvestris Linn. (Malvaceae) is an important medical plant whose flowers are used as a remedy for cut wound, eczema, dermal, infected wounds, bronchitis, digestive problems and urinary tract infections [3, 4].

Anastatica hierochuntica is a small gray annual herb that rarely grows above 15 centimeters, bears minute white flowers [5, 6]. *Anastatica* is found in arid areas in the middle east, including parts of North Africa, regions of Iran, Egypt, Palestine, Jorden, Iraq and Pakistan [7, 8]. It is used to treat gastrointestinal disorders, depression, high blood pressure, indigestion, cold, fever and diabetes [9, 10].

Grape (*Vitis vinifera*) belongs to family vitaceae [11]. *Vitis vinifera* is a deciduous woody climber with coiled climbing tendrils and large leaves. It has small, pal, green flowers in the summer followed by bunches of berry fruits that range from green to purple-black [12]. In Iraq, grape leaves are used in traditional food (dolma). Grape leaves with antioxidant activity have been reported to treat chronic venous insufficiency in human and nephrotoxicosis induced by citrine [13].

Theoretical

Selected bacteria are common bacteria in hospital infectious disease. *P. aeruginosa* is one of the important opportunistic pathogens in hospital which is clearly seen in affected to out immune system deficiency and in scaled, respiratory diseases, cancerous patients under chemotherapy, heredity cysticfibrosis bacteremia, septicemia and many other hospitals infections [14]. *S. aureus* is the main cause of bacteremia, surgical wound infections and the most common cause of skin and soft tissue infections [15]. *E. coli* is the agent of major urinary tract hospital infection [16].

B. subtilis is only known to cause disease in severely immunocompromised patients [17] *Proteus* include pathogens responsible for many urinary tract infections [18]. The aim of this study is to evaluate the antibacterial activity of *M. sylvestris*, *A. hierochuntica* and *V. vinifera* leaves against pathogenic Gram positive and Gram negative bacteria.

Material and Methods

Plant samples: *M. sylvestris*, *A. hierochuntica* and *V. vinifera* leaves were purchased from Baghdad markets, Iraq. The plants were dried at room temperature for fifteen days.

Preparation of plant extracts: 25 gm of each plant was extracted in 250 ml of solvent (80% ethanol) by soxhlet extraction techniques for 4 h. The extract kept for the next day at room temperature, then filtered by filter paper No. 42 extracts were concentrated by rotary evaporator [19].

Different concentrations of (25, 50, 75 and 100) mg/ ml of each plant extracts were prepared using dimethyl sulfoxide (DMSO).

Bacterial strains: Five bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *P. mirabilis*) were used to test the antibacterial activity in the plant extract. These strains were collected from center for market research and consumers protection-Baghdad University.

Culture media and antibacterial assay: Bacterial strains were grown on Muller Hinton agar (MH) and Nutrient agar (NA) at 37° C for 24 h were appropriately diluted using sterile normal saline solution to obtain cell suspension at 1.5 $\times 10^8$ colony forming units (CFU)/ml. To evaluate inhibiterial assay, an agar well diffusion method was used described by [20]. The organisms were spread on MH :NA agar plates by cotton swab. Well were punched into the agar medium and filled with 50 µL of plants extracts. The plates were incubated for 24 h at 37° C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.

Minimum inhibitory concentration (MIC): Determination of MIC of the plant extracts against bacterial strains was preformed according the microtitration technique described by [21].

Determination of phytochemical constituents: The freshly prepared extract was subjected to standard photochemichal analyses for different constituents such as alkaloids, glycosides, phenols, tanines and amino acid as earlier described by [22].

Results

Table (1) and Fig. (1) showed the effect of ethanolic extract of *A. hierochuntica* against the growth of bacteria. *A. hierochuntica* exhibited the strongest antibacterial activity against *S. aureus* (19 mm) at (100 mg/ ml). The ranking of antibacterial activity of *A. hierochuntica* against the five bacterial strains was *S. aureus* > *B. subtilis* > *P. aeruginosa* but it wasn't active against *E. coli* and *P. mirabilis*.

Table (2) and Fig. (2) showed the effect of ethanolic extract of *V. vinifera* leaves. *V. vinifera* leaves exhibited maximum antibacterial activity against *E. coli* (15 mm) at (100 mg/ ml). The ranking of antibacterial activity of *V. vinifera* leaves against the five bacterial strains was *E. coli* > *S. aureus* > *P. mirabilis* > *P. aeruginosa* > *B. subtilis*.

Table (3) and Fig. (3) showed the effect of ethanolic extract of *M. sylvestris*. *M. sylvestris* exhibited the strongest antibacterial activity against *S. aureus* (22 mm) at 100 mg/ ml. The ranking of antibacterial activity of *M. sylvestris* against the five bacterial strains was *S. aureus* > *P. aeruginosa* > *P. mirabilis* > *E. coli* and it had negative activity against *B. subtilis*.

The results showed that the ethanolic extract of *V. vinifera* leaves has the highest effective, the second was the extract of *M. sylvestris* while the extract of *A. hierochuntica* was the lowest.

Table (4) represented the chemical test for the active compounds like amino acid, glycoside, phenol compounds, tannins and alkaloids which was the active material of the plants extracts.

Table (5) showed the minimum inhibitory concentration of those studied local plants.

Discussion

The reason for the effectiveness of antibacterial to contain plant extracts like alkaloids, tannins, phenol and glycosides. Activity of the studied plant extract alkaloids characterized by their ability to break into the bacterial cell and interfering with DNA. Tannins have the ability to inhibit enzymes and transportation of proteins in the cell membrane. Phenolic in usability the formation of a complex with a soluble extracellular protein and activity was in cell wall lead to the treating of the cell[23, 24].

The common name for *M. sylvestris* is mallow and its local name is Khabaz. The active ingredients are found in the flowers and leaves, which are rich in mucilage; it is used in treatment for their expectorant properties [26]. This plant was largely used to soothe mucous and membrane in inflammations. *M. sylvestris* exhibited maximum antibacterial activity against *S. aureus*. The study was conducted by [27]. *M. sylvestris* is good for skin disorders,

as well as having good antibacterial and anti-inflammatory activity [28]. *V. vinifera* leaves are rich in phenolic compound such as myricetin, ellagic acid, kaempfrol, quercetin and gallic acid all of these compound have antibacterial activity [28, 29]. Also this antibacterial properties could be used to increase the shelf-life of food [30]. All part of *A. hierochuntica* are rich in minerals, these phenolic, have high antioxidant and free radical scavenging activities. These properties explain the therapeutic activities of these plants [31].

Conclusion

This study will help to identify active ingredients for the treatment of bacterial diseases. Additional studies are needed to assess the effect of the selected plants on other pathogenic organisms. Finally we can conclude from this study that *V. vinifera* leaves extracts is good antibacterial with in little concentration. This study could be done on each active compound (alkaloids, amino acid, phenolic compounds, glycosides and tannins) in the ethanolic extract.

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Table No. (1): The effect of ethanolic extract of *A. hierochuntica* against the growth of bacteria strains.

Concentration mg/ml	Inhibitory diameters (mm) mean				
	E. coli	*P. aeruginosa*	*P. mirabilis*	*B. subtilis*	*S. aureus*
25	-	-	-	4	-
50	-	-	-	8	8
75	-	-	-	12	18
100	-	15	-	16	19
DMSO	-	-	-	-	-

Note: '-', no inhibitory diameter

Table No. (2): The effect of ethanolic extract of *V. vinifera* against the growth of bacteria strains.

Concentration mg/ml	Inhibitory diameters (mm) mean				
	E. coli	*P. aeruginosa*	*P. mirabilis*	*B. subtilis*	*S. aureus*
25	4	2	3	2	4
50	8	5	6	3	7
75	11	7	9	4	10
100	15	10	12	5	13
DMSO	-	-	-	-	-

Note: '-', no inhibitory diameter

Table No. (3): The effect of ethanolic extract of *M. sylvestris* against the growth of bacteria strains.

Concentration mg/ml	Inhibitory diameters (mm) mean				
	E. coli	*P. aeruginosa*	*P. mirabilis*	*B. subtilis*	*S. aureus*
25	3	4	5	-	5
50	6	8	9	-	11
75	9	12	14	-	16
100	12	16	14	-	22
DMSO	-	-	-	-	-

Note: '-', no inhibitory diameter

Table No. (4): Result of photochemical screening of ethanolic extract of plant

Photochemical class	*A. hierochuntica*	*V. vinifera*	*M. sylvestris*
Alkaloids	+	+	+
Amino acid	+	+	+
Phenolic content	+	+	+
Glycosides	+	+	+
Tannins	+	+	+

Table No. (5): Minimum Inhibitory Concentration (MIC) of the ethanolic extracts of the plants against the test bacterium

Bacterial strain test	*A. hierochuntica*	*V. vinfera*	*M. sylvestris*
E. coli	-	**25**	**25**
P. aeruginosa	**100**	**25**	**25**
B. subtilis	**25**	**25**	-
S. aureus	**50**	**25**	**25**
P. mirabilis	-	**25**	**25**

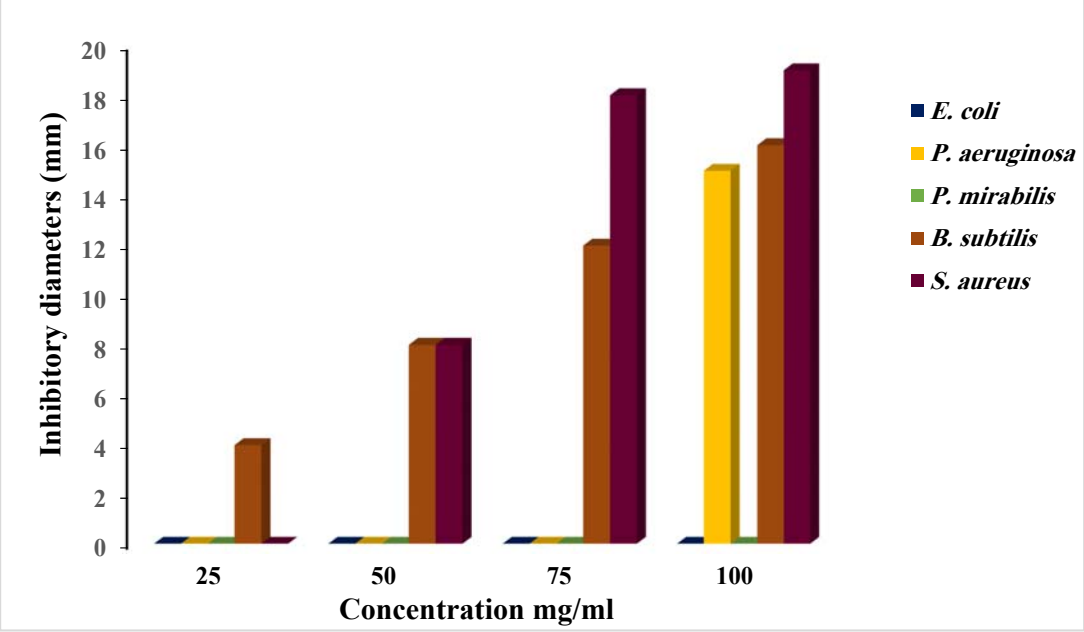


Figure No. (1): The effect of ethanolic extract of *A. hierochuntica* against the growth of bacteria strains.

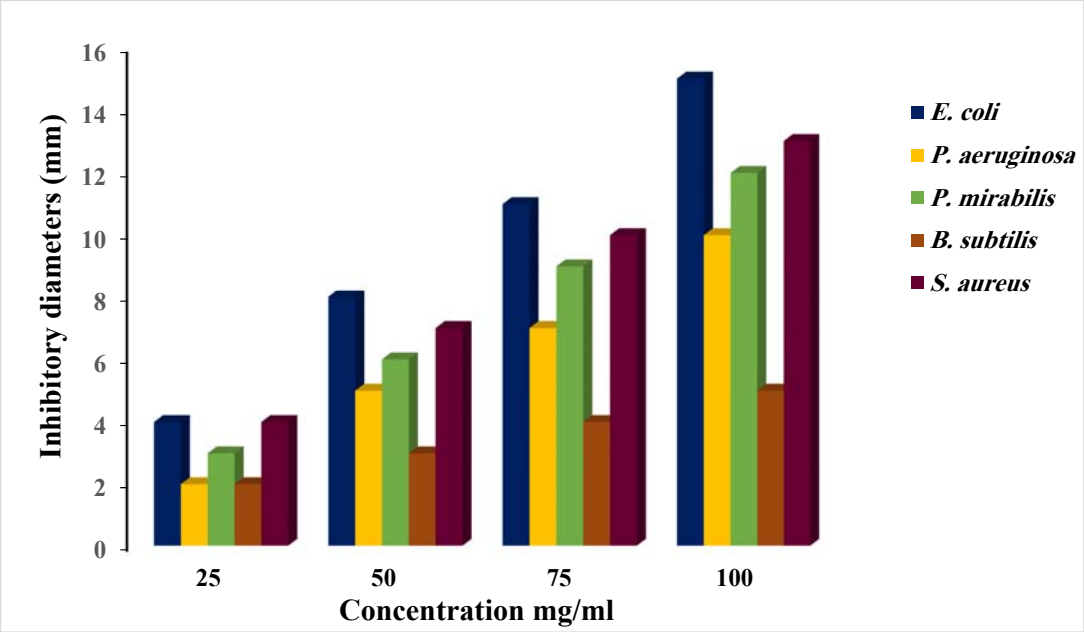


Figure No. (2): The effect of ethanolic extract of *V. vinifera* against the growth of bacteria strains.

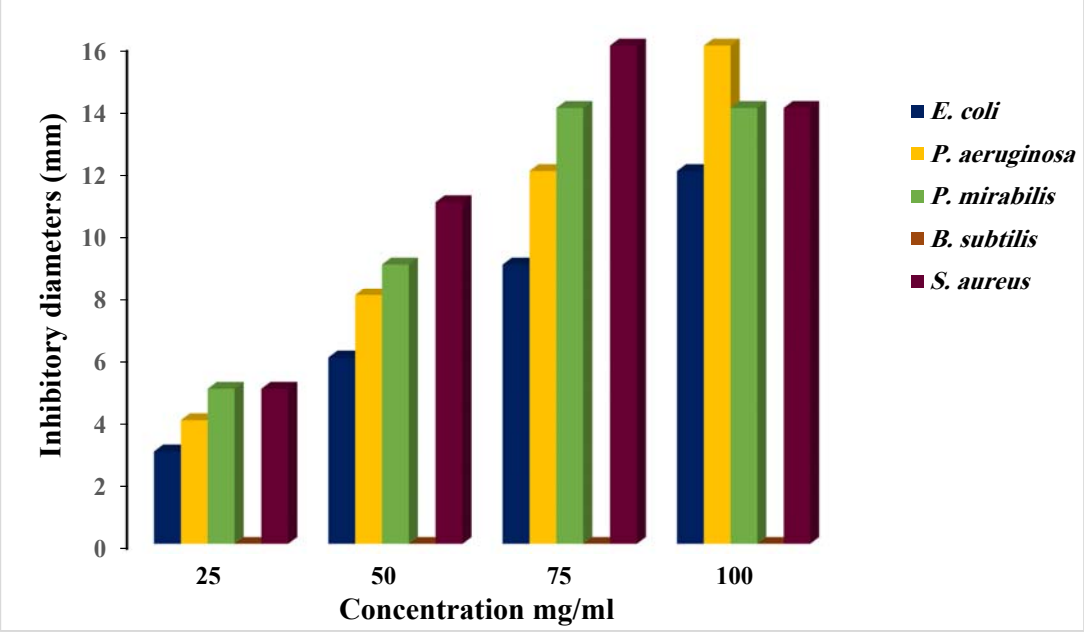
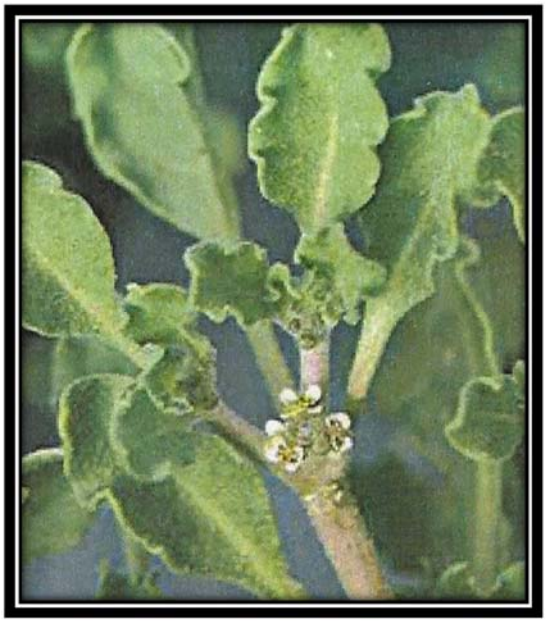
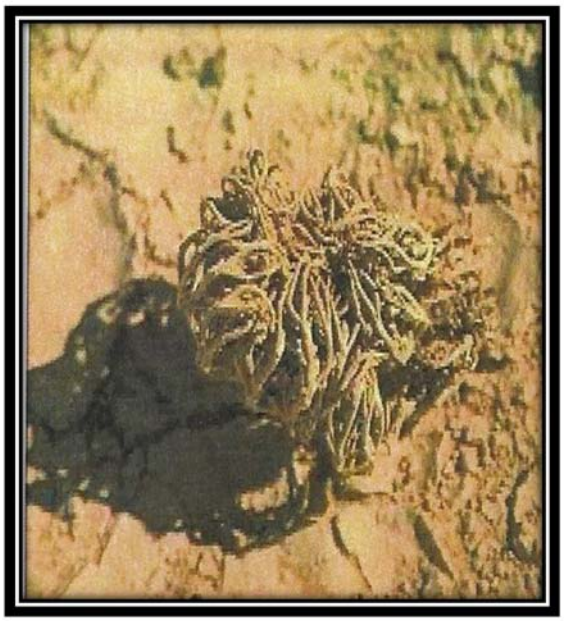


Figure No. (3): The effect of ethanolic extract of *M. sylvestris* against the growth of bacteria strains.

Malva sylvestris





Green plant Dry plant

Anastatica hierochuntica

تقييم التأثير التثبيطي لمستخلصات الإيثانول في ثلاثة نباتات محلية

خضير عباس حسن العامري

قسم علوم الحياة/ كلية التربية للعلوم الصرفة -(ابن الهيثم)/ جامعة بغداد

استلم البحث في :7/ايلول /2014 ،قبل البحث في 24/تشرين الثاني /2014

الخلاصة

هناك اهتمام واسع باستعمال المستخلصات النباتية كعلاج بسبب قدرتها الواسعة على تثبيط اكبر عدد من الاحياء المجهرية. أجريت هذه الدراسة لمعرفة التأثير التثبيطي ضد البكتريا لمستخلصات نباتات الخبّاز، كف مريم، وورق العنب، وتم تقويم التأثير التثبيطي للمستخلصات ضد بكتريا *Escherichia coli* و *Pseudomonas aeruginosa* و *Bacillus subtilis* و *Staphylococcus aureus* و*Proteus mirabilis* . اتبعت طريقة الانتشار بالحفر. وتقدير اوطأ تركيز مؤثر. أظهرت النتائج بان مستخلص ورق العنب ثبط كل البكتريا الموجبة والسالبة لملون كرام في حين أظهر مستخلص كف مريم قدره تثبيطية تجاه *S. aureus* و *B. subtilis*. واخيراً اظهر مستخلص نبات الخبّاز قدرة تثبيطية تجاه *P. mirabilis*، *P. aeruginosa*، *S. aureus* و*E. coli* .

أجريت فحوصات كيميائية للمستخلصات لمعرفة مكوناتها الكيميائية. أعطت المستخلصات نتائج إيجابية لكشوفات الاحماض الامينية، والكلايكوسيدات، والفينولات، والقلويدات والتانينات.

الكلمات المفتاحية: ورق العنب، وكف مريم، ونبات الخبّاز، والفعالية التثبيطية للبكتريا.