The Study of Antibacterial Activity of *Juglans Regia* and *Thymus Vulgaris* Seeds

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الخلاصة

عرفت النباتات الطبية منذ القدم بفاعليتها ضد الأحياء المجهرية بسبب احتوائها على المركبات الفعالة مما جعلها مصدر مهم لأنتاج العقار . أن نباتي الجوز والزعتر يمتلكان المركبات الفعالة التي تجعلهما يستخدمان كمضاد لنمو الأحياء المجهرية . تم دراسة فعالية المستخلص الميثانولي والهكساني لبذور الجوز والزعتر بتراكيز تراوحت بين 3.1-50 mg/ml بأستخدام طريقة الأنتشار بالحفر ضد الأنواع البكتيرية:

Staphylococcus aureus, Listeria monocytogenes, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Salmonella typhi ,Klebsiella pneumoniae, Enterococcus sp .

اظهرت النتائج ان المستخلص الميثانولي لبذور الجوز كانت الأكثر فعالية يليه المستخلص الميثانولي لبذور الزعتر.حيث حددت فعالية المستخلص بقياس قطر منطقة التثبيط حيث أبدت كل من *Staphylococcus aureus, Streptococcus pyogenes Proteus vulgaris, Listeria monocytogenes* مناطق تثبيط عند التراكيز 3.1-50 mg/ml أما *Bacillus subtilis, Klebsiella pneumoniae* فأعطت مناطق تثبيط عند 12.5-50 mg /ml في حين *Pseudomonas aeruginosa* فابدت تحسسها عند 25−50mg/ml أما *Escherichia coli, Salmonella typhi,Enterococcus sp* لم تظهر أي منطقة تثبيط.

اظهر المستخلص الميثانولي لبذور الزعتر فعالية عند تركيز 6.25-50mg/ml ضد *Staphylococcus aureus, Listeria monocytogenes, Bacillus subtilis* اما *Escherichia coli, Klebsiella pneumoniae,Proteus vulgaris, Streptococcus pyogenes* فقد اظهرت تحسسا عند تركيز 12.5-50mg/ml بينما أبدت *Pseudomonas aeruginosa* تحسسا عند تركيز 25-50 mg/ml . لم تظهر مناطق تثبيط لنمو *Salmonellla typhi, Enterococcus sp* أما المستخلص الهكساني لبذور كلا النباتين كان أقل تأثيراً على نمو البكتريا المختبرة. نجد من نتائج البحث أن المستخلصات المستخدمة لها دور في مجال الصناعات الدوائية وكمواد حافظة للأغذية.

مفتاح الكلمات: الزعتر, الجوز, مستخلصات البذور, الفعالية المضادة للبكتريا.

Abstract

It has been well known since ancient times that medicinal plant have antimicrobial activity because of the presence of substances, therefore they become important sources of drugs production. *Thymus vulgaris* and *Juglans regia* have active compounds make them have antibacterial properties.

Antibacterial effects of different concentrations ranging from (3.1-50mg/ml) of hexane and methanol extracts of *Thymus vulgaris* and *Juglans regia* seeds was determined by using agar well diffusion method on clinical strains: *Staphylococcus aureus, Listeria monocytogenes, Streptococcus, pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Salmonella typhi Klebsiella pneumoniae and Enterococcus sp*.

Methanol extract of Juglans regia seed was the most active followed by methanol extract of *Thymus vulgsris* seeds. The activity of *Juglans regia* extract determined by measuring inhibition zone as following:

Staphylococcus aureus, Proteus vulgaris, Listeria monocytogenes and *Streptococcus pyogenes* showed inhibition zone at concentration of 3.1-50 mg/ml. *Bacillus subtilis* and *Klebsiella pneumoniae* showed inhibition zone of 12.5-50 mg/ml. *Pseudomonas aeruginosa* showed sensitivity at concentrations of 25-50mg/ml. There was no inhibition zone for *Escherichia coli*, *Salmonella.typhi* and *Enterococcus sp.*

Methanol extract of *Thymus vulgaris* showed activity at concentrations of 6.25-50mg/ml for *Listeria monocytogenes*, and *Bacillus subtilis.*

Escherichia coli, Staphylococcus. aureus, Proteus vulgaris, Klebsiella pneumoniae and *Streptococcus pyogenes* showed sensitivity at concentration of 12.5–50 mg/ml. *Pseudomonas aeruginosa* sensitive at concentration of 50-25mg/ml. There was no inhibition zone for *Salmonella.typhi* and *Enterococcus* sp. Hexane extracts of both plant seeds were less active then methanol extract against tested bacteria.

These results support the notion that plant extracts may have a role as pharmaceutical and preservatives.

Key words: *Thymus vulgaris, Juglans regia*, seeds extracts, antibacterial activity.

Introduction

One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic agents.

Medicinal plants might represent an alternative treatment in non sever cases of infectious disease. They can also be possible source for new potent antibiotics to which pathogenic strains are not resistant [1].

In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral. They contain numerous biologically active compounds, many of which have been shown to have antibacterial properties.

Juglans regia (walnut, Black walnut) has along history of folk use in the treatment of cancer [2]. The seeds are an tilithic, diuretic and stimulant. .

Internally use in treatment of back pain, chronic cough, asthma and frequent urination. Externally they are made into a paste and applied as poultice to areas of dermatitis and eczema [3].

The green parts of *Thymus vulgaris* (thyme, Za'ater) is almost popular herbal medicine used worldwide. Thyme photochemicals have been used as antioxidant [4], antibacterial [5, 6] antifungal [7] and wound healing [8].

Gram positive and Gram-negative bacteria were selected as the test microorganisms based on their clinical, pharmaceutical and bromatological importance in cases of infections and contamination of food.The aim of the present study was to evaluate the effect of methanol and hexane extracts of the seed against various pathogenic bacteria.

Materials and Methods

Thymus vulgaris and Juglans regia seeds:

The seeds were collected from herbal drugs shops.

Bacterial Strains:

All bacterial strains used in the study are clinical strains,and kindly provided by institute of genetic engineering and biotechnology, university of Baghdad at April – July 2008. They are *Staphylococcus aureus, Streptococcus pyogenes, Listeria monocytogenes, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, proteus vulgaris, Salmonella typhi, Klebsiella pneumoniae* and *Enterococcus sp*.

Preparation of extracts:

For extraction of *Thymus vulgaris* and *Juglans regia*, methanol and hexane were used as solvents, thirty grams of the seed powders were extracted with 300ml of methanol by using soxhlet apparatus for 10hr [9]. Then the extracts were filtered by using whatman No.1 filter paper and the solvent was evaporated using rotary distillation apparatus. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes and were left in 50°C oven for 24hrs. Then they were left at 4°C until assessments of their antibacterial activities.

For extraction of seed of *Thymus vulgaris* and *Juglans regia* with hexane, the same procedure was followed by using the same volume of hexane.

Antibacterial activity:

The hexane extracts dissolved in dimethylsulfoxide (DMSO) while methanol extracts were dissolved in distilled water in order to obtain the final concentrations: 50, 25, 12.5, 6.25 and 3.1mg/ml.

The agar well diffusion method was used to determine antibacterial activity of extracts [10]. The culture medium was inoculated with one of tested bacteria suspended in nutrient broth.

Six millimeter diameter wells were punched in to the agar and filled with 0.1ml of each extract. Solvents were used as negative control while antibiotic of streptomycin 10ug /disc (Oxoid) were used as positive control. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed.

Results

The results showed that the methanol and hexane extracts of *Thymus vulgaris* and *Juglans regia* seeds had the antibacterial activity. The different concentrations of hexane extracts of *Thymus vulgaris* seeds (table-1) produced inhibition zones against tested bacteria; *Listeria monocytogens* was sensitive to concentration ranging from 50-6.25mg/ml, it Produce the largest inhibition zone. *Bacillus subtilis, Streptococcus pyogenes* showed sensitivity at concentrations of 50-12.5mg/ml. *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoniae* produce inhibition zone at concentration of 50-25mg/ml.

There was no inhibition zone for *Enterococcus sp., Salmonella typhi* and *Pseudomonas aeruginosa*.

Bacillus subtilis and *Listeria monocytogenes* showed the highest sensitivity to methanol extract of Thymus vulgaris (table-2), they produced inhibition zones at concentrations ranging from 50-6.25mg/ml, followed by *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae* and *Streptococcus pyogenes*,they were sensitive to concentrations ranging from 50-12.5mg/ml. *Pseudomonas aeruginosa* sensitive at concentration 50-25mg/ml. *Salmonella typhi* and *Enterococcus sp* were insensitive to this extract.

Staphylococcus aureus, Proteus vulgaris, Listeria monocytogenes and *Streptococcus pyogenes* showed the highest sensitivity to methanol extract of *Juglans regia* seed (table-3), they were sensitive to concentrations ranging from 50-3.1mg/ml while *Bacillus subtilis* and *Klebsiella pneumoniae* were sensitive to concentration of 12.5mg/ml, *Pseudomonas aeruginosa* was sensitive at concentration of 50- 25mg/ml. *Escherichia coli, Salmonella typhi* and *Enterococcus sp* did not showed any inhibition zone to all concentrations of this extract. Hexane extract of *Juglans regia* was less effective against tested bacteria (table-4). *Proteus vulgaris* showed the highest inhibition zone at concentration raging from 50-12.5mg/ml. *Staphylococcus aureus, Listeria*

monocytogenes and *Streptococcus pyogenes* showed sensitivity at concentrations 50-25 mg/ml. while *Bacillus subtilis* and *Klebsiella pneumoniae* was sensitive to concentration of 50mg/ml. *Pseudomonas aeruginosa*, *Salmonella typhi*,*Escherichia coli* and *Enterococcus sp* did not showed any sensitivity to all concentrations were used. Solvents (negative controls) used for preparation different concentrations showed no activity against any tested bacteria. Streptomycin (Positive controls) at concentration of 10µg/disc showed inhibition zone ranging from 20-24mm against all tested bacteria except *Enterococcus sp* it was resistant.

Tested bacteria	**Inhibition zone diameter (mm) concentrations (mg/ml)**				
	50	**25**	**12.5**	**6.25**	**3.1**
Staphylococcus aureus	**8**	**7**	**-**	**-**	**-**
Listeria monocytogenes	**17**	**16**	**14**	**13**	**-**
Streptococcus pyogenes	**14**	**13**	**7**	**-**	**-**
Bacillus subtilis	**10**	**9**	**8**	**-**	**-**
Pseudomonas aeruginosa	**-**	**-**	**-**	**-**	**-**
Escherichia coli	**10**	**8**	**-**	**-**	**-**
Proteus vulgaris	**8**	**7**	**-**	**-**	**-**
Salmonella typhi	**-**	**-**	**-**	**-**	**-**
Klebsiella pneumoniae	**8**	**7**	**-**	**-**	**-**
Enterococcus sp.	**-**	**-**	**-**	**-**	**-**

Table 1: Antibacterial activities of hexane extract of *Thymus vulgaris* seeds.

Tested bacteria	Inhibition zone diameter (mm) concentrations (mg/ml)				
	50	25	12.5	6.25	3.1
Staphylococcus aureus	8	7	7	-	-
Listeria monocytogenes	11	10	8	7	-
Streptococcus pyogenes	13	11	10	-	-
Bacillus subtilis	15	13	11	8	-
Pseudomonas aeruginosa	15	13	-	-	-
Escherichia coli	12	10	8	-	-
Proteus vulgaris	15	12	7	-	-
Salmonella typhi	-	-	-	-	-
Klebsiella pneumoniae	15	14	13	-	-
Enterococcus sp.	-	-	-	-	-

Table 2: Antibacterial activity of methanol extract of *Thymus vulgaris* seeds.

Tested bacteria	Inhibition zone diameter (mm) concentrations (mg/ml)				
	50	25	12.5	6.25	3.1
Staphylococcus aureus	18	17	16	14	8
Listeria monocytogenes	19	18	17	15	10
Streptococcus pyogenes	20	18	17	17	15
Bacillus subtilis	12	10	8		-
Pseudomonas aeruginosa	8	7	-	-	-
Escherichia coli	-	-	-	-	-
Proteus vulgaris	23	21	20	18	15
Salmonella typhi	-	-	-	-	-
Klebsiella pneumoniae	15	10	8	-	-
Enterococcus sp.	-	-	-	-	-

Table 3: Antibacterial activity of methanol extract of *Juglans regia* seeds.

Tested bacteria	Inhibition zone diameter (mm) concentrations (mg/ml)				
	50	25	12.5	6.25	3.1
Staphylococcus aureus	**8**	**7**	**-**	**-**	**-**
Listeria monocytogenes	**8**	**7**	**-**	**-**	**-**
Streptococcus pyogenes	**7**	**7**	**-**	**-**	**-**
Bacillus subtilis	**7**	**-**	**-**	**-**	**-**
Pseudomonas aeruginosa	**-**	**-**	**-**	**-**	**-**
Escherichia coli	**-**	**-**	**-**	**-**	**-**
Proteus vulgaris	**20**	**18**	**17**	**-**	**-**
Salmonella typhi	**-**	**-**	**-**	**-**	**-**
Klebsiella pneumoniae	**8**	**-**	**-**	**-**	**-**
Enterococcus sp.	**-**	**-**	**-**	**-**	**-**

Table 4: Antibacterial activity of hexane extract of *Juglans regia* seeds.

Discussion

The present study was designed to obtain preliminary information on the antibacterial activity of *Thymus vulgaris* and *Juglans regia* seeds on pathogenic bacteria. The agar well diffusion method was preferred to be used in this study. The results showed aremarkable antibacterial activity of the methanol and hexane extracts of both plants. The methanolic extracts had the best antibacterial activity than hexane extracts, the relatively high potency of the methanolic extracts may be attributed to the dissolving power of alcohol over water. [11]
In literature it has been indicated that the anti bacterial activity is due to different chemical agents in the extract, including thymol, carvacrole, flavoniods, trepens, glycosides and phenolic compounds [12, 13].

The antibacterial activity of thymus vulgaris due to Thymol and carvacrol, while *Juglans regia* contain naphthaquinine juglone, flavonoids, phenolic acids and glycosides [14]. The results showed Gram-negative bacter ia were shown to be more resistant than Gram positive bacteria. The resistance of Gram negative bacteria towards antibacterial substances is related to lipopoly saccharides in their outer membrane [15].

The activity is refered to the presence of glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens [16], or

may be due to impairment of variety of enzyme systems including those involved in energy production and structural component synthesis [17].

Finally,the results of this study revealed that the seeds of *Thymus vulgaris* and *Juglans regia* possess some antibacterial properties as antibiotics principles ,the diameters of inhibition zone of the antibacterial agents i.e. *Thymus vulgaris*, *Juglans regia* and streptomycin were different according to the kinds, concentrations and purity, and this results obtained support the fact that more needs to be done on the purification, identification and quantification of the active of extracts components with the view of their use for invivo studies.

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References

1. Punopas, K.E.; Griangsak, C.; Benjamart and N. Pichaya (2003). The study of antibacterial activity of some nedicinal plants in lamiacea family. Suranaree J.sci technol.
2. Duke, J.A. and Ayensu. E.S. (1985). Medicinal plants of china References publications. Inc.
3. Yeung and Him-che. (1985). Handbook of Chinese Herbs and formula. Institute of cheese medicine, Los Angeles.
4. Remigins, C.; Hanneliese, M. and chlodwig, F. (2008). Antioxidant properties of *Thymus vulgaris* leaves: comparison of different oil chemotyypes. J. Agric. Foodchem. Abstract.
5. Al-Saimary, I.E.; Sundus, S.; Bassam, Y. and yass, K. (2007). Efficiency of antibacterial agents extracted from (lamiaceae) J. nutrition and wellness. 4(1-7).
6. Mohammed, M. (2007). Evalution of antibacterial Activity of selected Iranian Essential oil against *Staphylococcus aureus* and *Escherichia coli* in Nutrient Broth Medium. Pakstan Journal of Biology Sciences
7. Giordani, R.; Rogli, P. and Kalonstian, J. et al (2004). Antifungal effect of various essential oils against *Candida albicans*. Polentiation of antifungle action of amphotericin by essential oil from *Thymus vulgaris*. Phytother. Res.
8. Dursun, N.; Liman, N. and Ozyazan, L. et al (2003). Role of Thymus oil inborn wound healing. J. Burn care Rehabil.
9. Lin, J.; Opoku, A.R.; Geheeb-keller, M.; Hutchings, A.D.; Terblanche Jager, A.k. and vanstaden, J. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activity. J. Ethnopharmacol.

10. Anesini, C. and Perez, C. (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol.
11. Majorie, M.C. (1999). Plant products as antimicrobial agents Cli. Microbial. Rev.
12. Essawi, T. and M. Srour (2000). Screening of some Palestinian medicinal plants for antibacterial activity J. Ethnopharmacol.
13. Rojas, A.; L. Hernandez; R. pereda and R. Mata (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants J. Ethnopharmacol.
14. Evans, W.C. (2000). Pharmacognosy. 15th.ed. Saunders.
15. Gao, Y.; M.J. Belkun and M. Stiles (1999). The outer membrane of Gram-negative bacteria inhibits antibacterial activity of Brochacin C. Applied Environ. Microbial. 65(4329-4333).
16. Aboaba, O. and B.M. Efuwape (2001). Antibacterial properties of some Nigerian spices. Bio. Res. Comm.
17. Conner, D.E and L. Beuchat (1984). Sensitivity of heat stressed yeasts to essential oils of plants. Applied Environ. Microbial.