ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *Ammi visnaga* EXTRACTS AGAINST PATHOGENIC MICROORGANISMS

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Abstract

Antimicrobial activity of ethanolic and aqueous extracts from fruits of commonly used medicinal plant in Iraq *Ammi visnaga* was evaluated against eight pathogenic microorganisms *Staphylococcus aureus*, *Leuconostic mesontroide*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida tropicans* and *C. albicans* over a wide range of concentrations (0.05-50 mg/ml). Minimum inhibition concentration (MIC) and the diameter of inhibition zone (DIZ) were determined by In vitro bioassays using hole-plate diffusion method.

The ethanolic and aqueous extract from the fruit part of the plant showed antimicrobial activity against most tested microorganisms. The most active extract against Gram-positive bacteria was ethanol extract with a minimal inhibitory concentration (MIC) value of (5mg/ml) against *Enterococcus faecalis*. In addition, the same extract from the plant part demonstrated in antimicrobial activity against the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* with an MIC value of 12.5mg/ml.In yeast it need high concentration of extract to cause inhibition.

This study shed the light on the ability of extracts from *Ammi visnaga* to combat pathogens which will help as natural antimicrobial agents as well as can be used in pharmaceutical and food preservation systems.

الفعالية المضادة للبكتريا والفطريات لمستخلصات *Ammi visnaga* ضد بعض الأحياء الممرضة

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الخلاصة

تم دراسة تأثيرالمستخلصات الكحولية والمائية لثمار النبات الطبي *Ammi visnaga* الشائع الاستخدام في العراق على نموالاحياء المجهرية *(Staphylococcus aureus, Leuconostic mesontroid, Candida Enterococcus faecalis, Klebsiella pneumoniae, Candida tropicans). Escherichia coli Pseudomonas aeruginosa، albicans* لمعرفة الفعالية المضادة للمايكروبات

بطيف واسع من التراكيز تراوحت بين (50-0.05mg/ml) وتم تحديد التركيز التثبيطي الادنى وقطرهالة التثبيط بالاختبارات الحيوية في المختبرباستخدام طريقة الانتشار بالحفر.

المستخلص الكحولي والمائي لثمار النبات يبين الفعالية التثبيطية لهذه الثمار ضد اغلب الاحياء المجهرية المستخدمة، المستخلص الكحولي هو الاكثر فاعلية ضد البكتريا الموجبة لصبغة كرام *Enterococcus faecalis* وبقيمة التركيز التثبيطي الادنى (5mg/ml) فضلا عن المستخلص النباتي نفسه في البكتريا السالبة لصبغة كرام *(Escherichia coli, Klebsiella pneumoniae)* بتركيزتثبيطي ادنى (12.5mg/ml) وفي الخمائر تحتاج الى تراكيز عالية من المستخلص لحصول التثبيط.

هذه الدراسة سلطت الضوءعلى امكانية استخدام مستخلصات نبات ال *Ammi visnaga* المثبطة للممرضات كمواد طبيعية مضادة للمايكروبات فضلا" عن الى امكانية استخدامها في المجالات الصيدلانية وانظمة حفظ الاغذية.

Introduction

Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [1, 2], insecticidal, acaricidal and cytotoxic activity [3]. Medicinal plants have been prescribed and used with a strong belief in their ability to cure diseases for centuries [4]. Plants used in traditional medicine contain wide range of substances to treat chronic as well as acute diseases. The substances of plants can either inhibit the growth of microorganisms or kill them are commonly considered for developing new drugs for treatment of various infectious diseases [5]. Herbal medicine in the developing countries has evolved as an alternative solution to health problems as a cheap source [6]. Therefore, medicinal plants are intensely screened and tested for a wide range of applications including pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology food and preservation [7]. Research on plants used as remedies in traditional folk medicine can lead to identification of several biologically active molecules from the 250,000 documented higher plant species [8]. The success achieved using medicinal plants and herbal formulations therapeutically based on ethnomedicinal and traditional use against a number of bacterial infections, raises optimism about the future of phytoantibiotics [9]. Based on the indigenous and local knowledge, plants represent a rewarding untapped source with a significant potential for developing antimicrobial agents [10]. Iraq is very rich in botanical diversity with more than 2000 wild plant species belong to about 700 genera [11]. As many as 485 species from approximately 99 plant families are categorized as medicinal plants [12]. Biologically active compounds and extract isolated from plant used in traditional herbal medicine in Iraq have been the center of interest [13]. However, few studies on the antimicrobial activities of the Iraqian medicinal plants were carried out. Therefore, there is still a potential need to screen their effects on various pathogenic microorganisms. The aim of this study was to investigate the antimicrobial inhibitory effect of ethanolic and aqueous crude extract obtained from medicinal plant commonly used in traditional medicine in Iraq.Fruit Extracts of *Ammi visnaga* was used against eight microorganisms (*Staphylococcus aureus*, *Leuconostic mesontroide*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida tropicans* and *C. albicans*).

Materials and Methods

Material of Plant: One medicinal plant namely *Ammi visnaga* was collected from herbal drugs markets. Scientific name, English common name, part used regarding plant, the common medicinal uses and active constituents of the plant under this study are reviewed in Table (1).

Table1: Botanical data and active constituents of studied plant [14, 15]

Botanical source	Common medicinal uses	Active constituents
Family: Umbelliferae (Apiaceae) Scientific name: *Ammi visnaga* Used part: Fruits English name: Khella, Bishops weed, tooth pick.	Hypoglycemic Bronchodilator Vasodilator and muscle relaxant	Coumarins

Fruits of *Ammi visnaga* used in this study were authenticated by the University herbarium / College of science, University of Baghdad. The

authenticated Specimens of the plant was dried to be used in this study.

Preparation of plant ethanolic extract: Plant material was dried in the shade at room temperature and powdered using an electric mill. Two hundred and fifty grams of plant powder were soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature. The mixture was shaken daily for regular infusion. On the sixth day, the extract was filtered using Whatman filter paper No. 1. Dry crude extract was produced by evaporating ethanol under low pressure using a rotary vacuum evaporator at 60°C. The final crude extract was stored in labeled sterile glass vials at −20C° until used for the antimicrobial activity test [16].

Preparation of plant aqueous extract: The air dried fine powdered plant material (100 g) were infused in distilled water until complete exhaustion. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried using either a rotary evaporator at 60 °C or a freeze drier [17]. The final dried samples were stored in labeled sterile bottles and kept at −20C°.

Microbial test suspensions: Test organisms used in this study are Gram-positive bacteria (*Staphylococcus aureus*, *Leuconostic mesontroide*, *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and yeast (*Candida tropicans*, *C. albicans*). The Source and identified clinically resistant strains were obtained from the Microbiology Laboratory at Department of Biotechnology, University of Al-Nahrain, Iraq, and very carefully identified using standard microbiological methods. The microorganisms were maintained on slants of nutrient agar (NA) at 4°C. The inoculums were incubated overnight in nutrient broth at 37°C to produce dense microbial suspension of approximately 10^6cfu/ml (tube no.6) by diluting fresh cultures and comparing with McFarland density (cells/ml) [18] and confirmed the concebtration by spectrophotometer after dilution.

Screening for antimicrobial activity: Hole-plate diffusion method was used for studying the antimicrobial activity and determining the minimum inhibition concentrations (MICs) [19]. Each inoculum from dense bacterial suspension containing 10^6 bacterial cells/ml was spread on the surface of Mueller-Hinton Agar while the *Candida tropicans* and *Candida albicans* were spread on the surface of potato dextrose agar (PDA). Three holes were made on the media using 6 mm diameter sterile cork-borer. The dried plant ethanolic extract was dissolved in dimethylsulfoxide (DMSO) to provide a stock solution with the final concentration of 50 mg/ml while the plant aqueous extract was dissolved in distilled water to provide a stock solution with the same final concentration of ethanolic extract. A range of serial dilutions were prepared from the stock solution to provide 0.05, 0.1, 0.15, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 12.5, and 25 mg/ml. Each hole diameter (6mm) was filled with (50µl) from diluted of plant extract. The inoculated agar plates were incubated at 37°C for 24 hr. After the incubation period, bioactivity was determined by the measurement of the diameter of inhibition zone around each hole in mm. The inhibition zone was recognized as the area surrounding the hole with no growth of the tested pathogens.

Control plates received only DMSO in Mueller-Hinton Agar and PDA without plant ethanolic extract while the control plates in aqueous extract only distilled water in Mueller-Hinton Agar and PDA and was run following the same procedure as mentioned earlier. The values reported for diameter of inhibition zone were the average of three replicates.

Results and Discussion

Organic and Aqueous extracts obtained from *Ammi visnaga* was tested against (*Staphylococcus aureus*, *Leuconostic mesontroide*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida tropicans* and *C. albicans* (Table 2 and Table 3). Antimicrobial activity was determined by measuring the diameter of inhibition zones in mm and the minimum inhibitory concentration (MIC in ppm) over a range of concentrations 0.05, 0.1, 0.15, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 12.5, 25 and 50 mg/ml. Plant extract showed different antimicrobial activity against the tested pathogens and the diameter of inhibition zone was directly proportional to the increase in plant extract concentration reaching a plateau.

A. visnaga required a concentration of (MIC) 12.5 mg/ml to reach diameter of inhibition zone ranging from (12-13) mm for *E. coli* and *K. pneumoniae* while for *P.aeruginosa* which inhibited at MIC (25 mg/ml) to give inhibition zone (15mm).

Table (2) shows the inhibitory effects of ***A. visnaga*** ethanolic extract at various

concentrations against pathogenic microbes used in the study (5mg/ml) with inhibition zone 15mm. *A. visnaga* inhibited *S. aureus* and *L. mesontroide* required higher concentration of MIC(50 mg/ml) with inhibition zone 18 and 24mm respectively. The extract of A. visnaga effect at MIC of 5 mg/ml and inhibition zone 9mm against *E.faecalis*. The growth of *C. albicans* was considerably inhibited at MIC 50mg/ml (inhibition zone = 21 mm), followed by *C.tropicans* at MIC (50 mg/ml) (inhibition zone=18 mm). Overall MICs of active plant extract and inhibition zones are shown in table (2).

The inhibition activity of the plant ethanolic extract on test strains was in decreasing order according to the minimum inhibition concentration as follows:

A. visnaga: E. faecalis < K. pneumoniae < E. coli<P. aeruginosa < C. tropicans<S. aureus<C. albicans<L. mesontroide. while in table (3) show the inhibition aqueous extracts at the same concentrations against *E.coli* required MIC 25mg/ml to reach inhibition zone 30mm, against *P. aeruginosa* (50mg/ml) to reach inhibition zone 25mm, while for *K. pneumoniae* 25mg/ml to reach inhibition zone 30mm, and MIC of 25mg/ml to reach inhibition zone 8mm for *S. aureus* and inhibited *L. mesontroide* at MIC 50mg/ml with inhibition zone 25mm, highly effected *E. faecalis* with MIC 50mg/ml with inhibition zone 44mm finally the effect on *C. albicans* and *C. tropicans* were in MIC 25mg/ml and 50mg/ml at inhibition zone 12mm and 20mm respectively.

From table (3) the inhibition activity of plant aqueous extract on test strains was in decreasing order according to the MIC's as follows:

A. visnaga : *S. aureus*< *C. albicans* < *E. coli* and *K. pneumoniae* < *C. tropicans*< *P. aeruginosa* and *L. mesontroide*< *E. faecalis.*

Table 2: Antimicrobial activity (diameter of inhibition zone mean in mm) of crude *Ammi visnaga* organic extract at various concentrations against pathogenic microbes used in the study.

Conc. / Strains	Control (DMSO)	0.05mg/ml	0.1mg/ml	0.15mg/ml	0.2mg/ml	0.3mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	12.5mg/ml	25mg/ml	50mg/ml
Escherichia coli	0*	0	0	0	0	0	0	8	8	9	10	10	12	12	13*	13	13
Pseudomonas aeruginosa	0	0	0	0	0	0	7	9	9	9	10	10	10	11	11	15*	15
Klebsiella pneumoniae	0	0	0	0	0	0	0	0	8	8	9	9	10	10	12*	12	12
Staphylococcus aureus	0	0	0	0	8	8	12	12	12	12	13	13	15	17	17	17	18*
Leuconostic mesontroide	0	0	0	0	0	0	0	0	0	8	8	10	13	15	20	20	24*
Enterococcus faecalis	0	0	0	0	0	0	0	0	0	0	7	7	7	9*	9	9	9
Candida albicans	0	0	0	0	0	0	0	8	9	13	19	19	19	19	20	20	21*
Candida tropicans	0	0	0	0	0	0	0	0	7	9	9	10	15	15	16	18*	18

Diameter zone (mean in mm)
(*) mean MIC

Table 3: Antimicrobial activity (diameter of inhibition zone mean in mm) of crude Ammi visnaga aqueous extract at various concentrations against pathogenic microbs used in the study.

Conc. / Strains	Control (D.W)	0.05mg/ml	0.1mg/ml	0.15mg/ml	0.2mg/ml	0.3mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	12.5mg/ml	25mg/ml	50mg/ml
Escherichia coli	0*	0	0	0	0	7	7	7	8	9	9	12	12	12	25	30*	30
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	8	8	8	10	10	11	15	15	25*
Klebsiella pneumoniae	0	0	0	0	0	0	0	0	7	8	10	10	12	12	25	30*	30
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	8*	8
Leuconostic mesontroide	0	0	0	0	0	0	0	0	9	12	12	12	13	13	17	23	25*
Enterococcus faecalis	0	0	0	0	0	0	0	0	0	0	7	7	8	10	20	30	44*
Candida albicans	0	0	0	0	0	0	0	0	0	0	0	0	8	8	10	12*	12
Candida tropicans	0	0	0	0	0	0	0	0	0	0	8	9	10	10	13	15	20*

Diameter zone (mean in mm)
(*) mean MIC

The plant extracts showed antimicrobial activity against most tested microorganisms. Table (4) showed the ethanolic extract from ***A. visnaga*** fruits with strongest activity was seen against *Enterococcus faecalis* (MIC=5mg/ml) while *E.coli* and *K.pneumoniae* was inhibited in (MIC=12.5mg/ml). *P. aeruginosa* and *C.tropicans* inhibits; 25mg/ml. *S. aureus, L. mesontroide* and *C. albicans* were inhibited by using highest ethanol extracts concentration 50mg/ml.

Table 4: minimum inhibitory concentration and diameter inhibition zone of crude *Ammi visnaga* extract against pathogenic microorganisms used in the study.

Strains	Ethanolic		Aqueous	
	MIC (mg/ml)	DIZ (mm)	MIC (mg/ml)	DIZ (mm)
E. coli	12.5	13	25	30
Pseudomonas aeruginosa	25	15	50	25
Klebsiella pneumoniae	12.5	12	25	30
Staphylococcus aureus	50	18	25	8
Leuconostic mesontroide	50	24	50	25
Enterococcus faecalis	5	9	50	44
Candida albicans	50	21	25	12
Candida tropicans	25	18	50	20

DIZ » Diameter of inhibition zone (mean in mm)
MIC»Minimum inhibition concentration (mg/ml)

Aqueous extract from ***A. visnaga*** fruits revealed close results with less action compared with fruit ethanolic extracts,which was active against all the tested microorganisms using high concentration (MIC=25 and 50mg/ml).

All the extracts were able to inhibit the growth of one or more of the tested microorganisms. The gram positive bacteria *Enterococcus faecalis* was the most sensitive strain to the ethanolic extract of ***A. visnaga*** (MIC=5mg/ml), while for gram negative bacteria *E.coli* and *K.pneumoniae* made(MIC=12.5mg/ml) using the same extract.

The best MIC value against most microorganisms was the ethanol extract from the plant followed by aqueous extract which were inhibited by highest concentration of plant extracts against gram negative bacteria, gram positive bacteria and yeast

The tested plant extract demonstrated broad spectrum antimicrobial activity against tested microorganisms with variable inhibitory effect, antifungal effect at all tested concentrations. This could be related to the variations in the quality and quantity of active compounds in the plant extract (Table 1), this indicated that antimicrobial activity of plant extracts is related to the presence of different chemical agents including essential oils, flavonoids, anthocy-anins and terpenoids in addition to other compounds of phenolic nature or containing free

hydroxyl groups [20], Fungitoxic, the plant differed significantly in the activity against tested microorganisms. These differences could be attributed to structural nature of the microorganisms and plant constituents [21]. The optimal effectiveness of medicinal plants may not be due to one active constituent, but to the combined actions of different plant constituents [7]. Moreover, the differences observed in antimicrobial activities of the investigated plant extract suggest the susceptibility variations of microorganisms to various chemical components. Earlier results support our findings that the composition of essential oil depends on the plant species, the chemotypes and the climatic conditions, which lead to variation of their antimicrobial activities [2].

From the above findings it could be concluded that the tested plant extracts exhibit a broad spectrum of activity against various microorganisms. Further investigations to determine bactericidal, bacteriostatic, fungicidal or fungistatic effects are recommended with emphasis on the identification of the active antimicrobial chemical constituents of these commonly used Iraqian medicinal plants. Results of the present study should be considered for the possible application of plant extracts as natural bacteriostatic and bactericidal component in various products and as natural preservatives extending the pharmaceutical and dietary products shelf life, as we believe is of great importance.

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