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Antibacterial activity of ethanolic extract of (*Ammi visnaga*) seeds against some types of bacteria

Ammar H. Salman[1*] & Mohammed Mosleh Shwaish[2]

[1]Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Fallujah, Baghdad, Iraq

[2]Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Fallujah, Al-Fallujah, Iraq

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Abstract

Modern and traditional medicines both make use of the medicinal plant *Ammi visnaga* L. This species of plant belongs to the Apiaceae family and is originally from Asia, Europe and the North African Mediterranean. *A. visnaga* has many medicinal properties as well as active ingredients, including flavonoids, essential oils and furobenzopyrones (FBP). In the aim of this study, the antibacterial effect of *A. visnaga* seeds was tested against two bacteria, *Staphylococcus aureus* and *Klebsiella pneumonia*. The seeds were extracted using 70% ethyl alcohol and the method of agar diffusion was used to test the antibacterial effect. Several concentrations of the extract were prepared (6.25, 12.5, 25, 50, 100, 200, 400 and 800 mg/mL) and 3 plates were used for each concentration, each plate containing three pores. The results showed weak efficacy of seeds extract against the bacteria that was used in the study, as the zone of inhibition did not appear in concentrations below 600 mg/mL. The zones of inhibition formed at 800 and 400 mg/mL concentrations of extract were (1.8 ± 0.4) mm and (1.83 ± 0.11) mm, respectively, against *S. aureus* and (2.13 ± 0.15) mm and (1.83 ± 0.05) mm, respectively, against *K. pneumonia.* These results showed poor activity of plant seeds against the 2 types of bacteria used in this study.

Keywords

Ammi visnaga; Kella; *Staphylococcus aureus; Klebsiella pneumonia;* Iraq

Introduction

Throughout history, people have relied on natural resources to meet their most basic needs, such as food, housing, medicine, perfume, clothing, flavouring, fertiliser, and transportation. A large portion of the world's population relies on medicinal plants as a part of their healthcare system, especially in developing countries where herbal medicine has a long history of use. These medicinal plants have shown many economic and medicinal benefits and their usage has escalated in both rich and developing countries (1).

The use of medicinal plants is based on research that dates back hundreds to thousands of years, and it has recently been acknowledged that some of the therapeutic attributes associated with plants are not entirely accurate (1). Ancient Mesopotamian cuneiform records from around 2600 BC attest to the use of certain materials, including oils and active ingredients of many plants in medicine. These herbs are still used today to treat a wide range of ailments, from inflammation and parasitic infections to common cough and cold (2).

To this day, plants continue to play an essential role in medicine and hold great promise as a source for future, safe pharmaceuticals (3). Even if we now have access to a lot of current medicines, finding and developing novel therapeutic agents is still very important. Only about a third of all human diseases have a treatment that is considered appropriate. Hence, the battle against illnesses must be waged without cease. Because of their synergistic benefits and relatively low side effects, traditional plant medicines continue to have a prominent place in today's pharmaceutical companies.

The majority of the game-changing pharmaceuticals developed in the last half-century have originated from plants, either as isolated compounds or as delivered derivatives. The effects of these chemical components are similar to those of drugs found in animals and plants. Because they are more affordable, can withstand the test of time and are generally seen to be safer than contemporary synthetic treatments. Herbal medicine should be a part of national health care initiatives, according to the World Health Organization (4).

Exploring pharmacologically and biologically active compounds produced from screening natural sources, such as plant extracts, has led to the discovery of various pharmaceutically significant drugs that are crucial in treating human disorders (5). The synthetic medicine industry has been unable to compete with the new remedies developed via phytochemical-pharmacological research for a number of illnesses. Among them, studies on *Artimisia annua*, *Baccopa* spp., *Taxus* spp., *Cathranthus roseus*, *Lantana camara* and others rank highest in importance. Once thought to be either toxic or otherwise unproductive, these plants are now highly prized for their medical properties due to the discovery that they contain compounds with high pharmacological values.

Medicinal herbs have been an integral part of human society from the beginning, as shown in many religious and ceremonial practices (6). Many of today's medications, including aspirin, are derived indirectly from medicinal plants. Garlic is only one of several food crops that may be used medicinally. The secondary metabolites that plants produce are responsible for their therapeutic effects. A lot of people have taken an interest in studying natural product chemistry as a result of this. Reasons for this interest include the following: the need for treatments, the diversity of naturally occurring secondary metabolites in terms of chemical structure and biological activities, the possibility of novel bioactive natural compounds as biochemical probes, the development of sensitive methods to detect naturally occurring biologically active compounds, improvements in the separation, purification and structural characterization of these components and the need for solutions to the supply of complex natural products (7). In light of the importance of traditional medicine, the World Health Organization has established standards, procedures and goals for botanical treatments. Growing, processing and manufacturing herbal medicines all require the use of agro -industrial technologies (8). Many current medications are derived indirectly from plants, which are valuable resources for developing novel pharmaceuticals.

One such medicinal plant that belongs to the Apiaceae family is *Ammi visnaga* (L.) Lam. Native to the Mediterranean region, it has now spread as a species all over the globe (9). It is biennial or annual herb with height of up to 1.0 m, with dentate and striped leaves and erected, highly branched stems. The flowers are umbellate with ray figure and are highly swollen at the base, which becomes woody like toothpicks and is brown to pale brown in color. It is distributed in many regions worldwide like, Mediterranean region, Egypt, Tunisia, India, Argentina, Chile, North America, Mexico, Russian Federation and other regions (10). Kella, Khella, Chellah, Khella and Swak Al-Nabi are some of the old Arabic names for *A. visnaga*. The other names like, Pick tooth and Toothpick are the English names for it (11).

Ammi visnaga contains flavonoids, saponins, alkaloids, glycosides, phenols, turbines and tannins (12). *Ammi visnaga* is a densely packed herb that has many different components, including the main constituents khellin (0.3-1.2%) and visnagin (0.05-0.30%), as well as khellinol, ammiol, khellol, khellinin and fixed oils (up to 18%), γ-pyrones (furanochromone up to 4%), and coumarins (0.2-0.5%) (13). The hydro distillation of *Ammi visnaga* yielded a yellowish essential oil (1.3%). This oil was found to contain 21 components, which formed 97.3% of identified compound (14). 11 flavonoids were isolated from the plant, which included 3 methoxylated (isorhamnetin, rhamnetin and rhamnazin) and 1 hydroxylated (quercetin) (15). After aqueous extraction, the concentrations of the main active constituents, khellin and visnagin were found to be 2.88 and 1.72 mg/100 mg respectively (16).

Ammi visnaga was traditionally used to treat many disease conditions in humans. As a paste, it was used in the treatment of skin diseases like psoriasis and vitiligo; the fermented fruit was used to regulate menstruation and for renal and gallbladder stones, allergic rhinitis, depression, leucoderma, hypertension and rash was treated by Egyptian with the fruit of the plant (17). Water-alcohol extract showed activity of carminative, antiseptic, antispasmodic and diaphoretic effects. Many recent drugs, such as amiodarone (antiarrhythmic), cromolyn (mastocytosis) and nifedipine (unstable angina and hypertension) are derived from *Ammi visnaga* (18).

Evidence from clinical trials, *in vivo* studies, and alternative or complementary therapies suggests that herbal medications may be effective in the treatment of urolithiasis. The primary components of *Ammi visnaga* fruits that may help avoid kidney stones are the furanocoumarines, khellin and visnagin. Previous research by our group has shown that visnagin, khellin and an aqueous plant extract may decrease *in vivo* calcium oxalate deposition and *in vitro* cell damage caused by calcium oxalate. These findings suggest that visnagin may be somewhat more effective than khellin in preventing kidney stones. After giving rats a khella tea mixture, the

kidneys of the rats had less oxalate and calcium (19).

When trying to avoid kidney stones, it is crucial to know what causes them and what kind of stones are most common, such as crystal component super saturation of the urine. In most cases, a metabolic assessment should be conducted before patients are given dietary advice, such as increasing fluid intake and reducing sodium, protein and oxalate (20-22). Antioxidant activity has been shown in *A. visnaga* (23). We used the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique to find out how effective the butanolic extract of *A. visnaga* was as an antioxidant. Visnagin reduces the production of TNF-α, IL-1β and IFNγ, as well as the expression of messenger RNA. One possible mechanism by which visnagin inhibits inflammatory processes is via acting on transcription factors like AP-1 and NF-KB (24). One mechanism by which visnagin exerts its anti-inflammatory effects is via a neuroprotective action (20).

A. visnaga is widely used as an antidiabetic in various cultures, including those of Palestine, Morocco, and the Sefrou area (25). When given to diabetic rats, an aqueous *A. visnaga* sample had a significant hypoglycemic impact (26). This effect was seen in both regular and streptozotocin treated rats. The potential for a 51% reduction in blood glucose levels in normoglycemic rats was also shown by a decoction made from *A. visnaga* fruits when compared to a hypoglycemic drug (tolbutamide) (27).

Protein and total *A. visnaga* extracts exhibited immuno stimulatory effects. To evaluate the extracts on splenocytes, the MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay was used, either with or without concanavalin-A (Con-A) stimulation, a mitogenic drug used as a positive control (24).

The aim of the current study was to test the antibacterial effect of *Ammi visnaga* against two types of bacteria, gram-positive and gram-negative bacterium.

Materials and Methods

Plant extraction

Ammi visnaga seeds were obtained from local market of Fallujah City (Iraq). The seeds were ground to fine powder and extracted with 70% alcohol (500 g of seeds/1L of alcohol) (Fig. 1). After one week of continuous mixing, the solution was filtered through a glass funnel and alternate layers of cotton and gauze. The filtered solution was dried in a dryer to get solid brownish extract, which was grinded again and kept in tightly closed glass can in the refrigerator until use (28).

Preparation of stock solution and different extract concentration

Stock solution was prepared by adding 1600 mg of extract to 2 mL of distilled water in a test tube to get concentration of 800 mg/mL. Serial dilutions were made by taking 1 mL of stock solution and transferring it to another test tube which contained 1 mL of distilled water to get concentration of 400 mg/mL and so untill the following concentration was achieved: 800, 400, 200, 100, 50, 25, 12.5 and 6.25 mg/mL.

Fig. 1. Extraction of *Ammi Majus* with solvent by heating and stirring.

Antibacterial activity

To evaluate antibacterial activity of *Ammi visnaga* seeds, agar diffusion method was followed (12). 25 mL of nutrient agar was poured on the petri dish and after solidification, 3 wells of 7 mm were made. With equal distance between each other, one loopfull of bacteria was scratched on the top surface of the agar and after that, 50 µL of each concentration was added to the pours. The bacteria used were *Staphylococcus aureus* (*S. aureus*) as gram-positive bacteria and *Klebsiella pneumonia* (*K. pneumonia*) as gram-negative bacteria. The bacteria were obtained from Laboratory of Clinical Pathology, College of Veterinary Medicine, University of Fallujah. Three plates were used for each concentration of each type of bacteria.

Statistical analysis

The results were analyzed with T-test by using IBM® SPSS® Statistics version 26/USA.

Results and Discussion

The results showed poor activity of plant seeds against both types of bacteria. The inhibition zone against *S. aureus* was 1.8 ± 0.4 mm and 1.83 ± 0.11 mm for 800 mg/mL and 400 mg/mL, respectively (Fig. 2), while the inhibition zone against *K. pneumonia* was 2.13 ± 0.15 mm and 1.83 ± 0.05 mm for 800 mg/mL and 400 mg/mL, respectively (Fig. 3 and Table 1). The statistical analyses revealed that there were no significant differences ($p \geq 0.05$) between the inhibition zones of *S. aureus* and *K. pneumonia*. The variation of the results of this study from other studies may be due to many factors like plant harvesting, temperature, extraction methods, processing, types of bacteria, concentration of the extract and incubation (9).

Our results were not in agreement with previous reports considering similar experiments (29). This may be

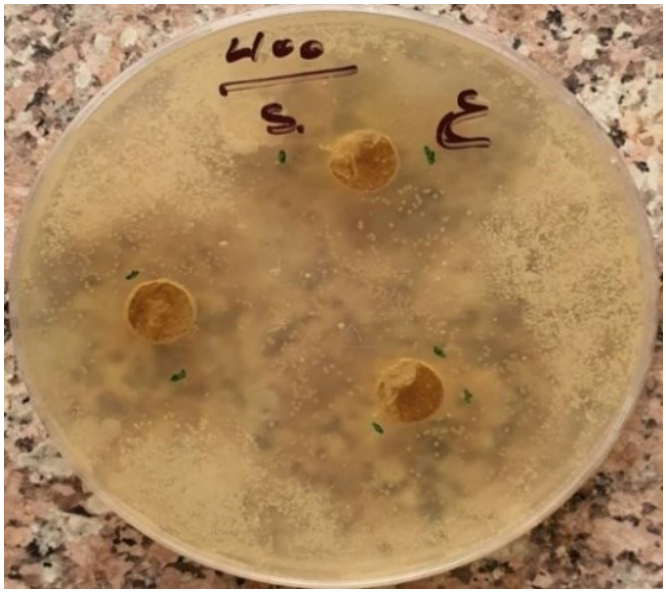

Fig. 2. The inhibition zone against *S. aureus* with concentration of 400 mg/mL.

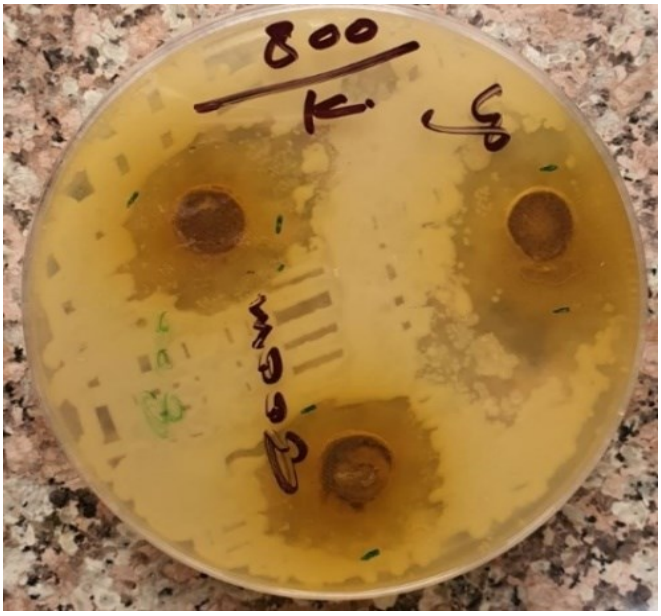

Fig. 3. The inhibition zone against *K. pneumonia* with concentration of 800 mg/mL.

because they used fruits of the plant and 95% ethanol, while in this study, the seeds were used and extraction was done with 70% ethanol. These differences may have led to differences in the components of extract and the concentration of its components. On the other hand, our result partially agreed with other research (30), where the effect of hydroalcoholic seed extract against *Streptococcus salivarius, Streptococcus mutans* and *Streptococcus sanguis* was studied. The result showed no activity against the first one, poor activity against the second one and good activity against the third one. These results prove that the type and species of bacteria are important factors in the sensitivity of plant seeds extract.

The results of this study agree with those found by other authors (30). In their study, the antimicrobial activity was tested against the plant seeds extracts obtained from different solvents. The activity against *S. aureus* was less than 2% and the MIC against *E. coli* was 800 ppm. This means that the type of solvent affects the activity of extract against bacteria. The antimicrobial activity of the plant, associated mainly with khellin and visnagin, is considered to have antibacterial, antifungal and antiviral effects. This revealed the reason for the antibacterial activity of the seed, but it appeared in high concentrations because of the crude extract used in this study (31).

Conclusion

The results showed poor activity of plant seeds against both types of bacteria. This may be due to the usage of crude extract which led to dilution of the active ingredients of the plant.

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Authors' contributions

AHS helped in writing and editing. MMS performed the practical work**.**

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Table 1. The zone of inhibition (Mean±SD) of different concentration against bacteria.

Conc. (mg/mL)	Zone of inhibition (Mean ± SD) mm							
Bacteria	800	400	200	100	50	25	12.5	6.25
S. aureus	1.8±0.4	1.83±0.11	-	-	-	-	-	-
K. pneumonia	2.13±0.15	1.83±0.05	-	-	-	-	-	-

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