

Anatomical Study and Antimicrobial Activities of *Ammimajus*

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Received: 12 February 2022
 Accepted: 20 September 2022
 Published: 28 December 2022
 05 pages

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How to cite this article: Fathulla CN, Kamel FH, Rahman SI, Othman AS, Sadeq TW. Anatomical Study and Antimicrobial Activities of *Ammimajus*. PHARM. APPL. H. SCI. [Internet]. 2022 Dec. 10 ;1(2):22-26. Available from: <https://knu.edu.iq/ojs/index.php/phahs/article/view/12>

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ABSTRACT

Ammimajus is a plant species in the family Apiaceae, The purpose of this study was to assess anatomical and antibacterial and antifungal activities of *Ammimajus*, which taken from collage of Sciences/Salaheddin University/Erbil. Anatomical studies by the using paraffin method, have shown that the outline of stems, petiole lamina, and margin, they contain secretary canals with presence unicellular glandular and unicellular non glandular trichomes. The antimicrobial activity prepared by extract using ethanol (95%) method from stem, leaf and flower, then provide the different concentration of extract, which shown the exhibit antibacterial effects on some microorganisms as *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Candida albicans*.

Keywords: *Ammimajus*, Antimicrobial activity, Plant extracts, Antibacterial activity.

1. Introduction

Ammimajus is a plant species in the family Apiaceae, which includes many well-known plants such as celery, carrot, and parsley. It is native to India and is known for its medicinal properties.

The anatomical study of *Ammimajus* would involve examining the various structures of the plant, such as its leaves, stems, flowers, and roots. This could include examining the shape, size, and arrangement of these structures, as well as the types of cells and tissues that make them up. As for the antimicrobial activities of *Ammimajus*, there have been some studies that have shown that extracts

from the plant have antimicrobial properties against certain types of bacteria and fungi. For example, one study found that a methanol extract of *Ammimajus* had antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, two common types of bacteria that can cause infections. Another study found that an ethanol extract of the plant had antifungal activity against *Candida albicans*, a type of fungus that can cause infections in humans [1].

It is worth noting that the medicinal properties of *Ammimajus* have not been extensively studied and more research is needed to fully understand the plant's potential health benefits.

Plants are frequently employed as a source of medicines for over 87 percent of human diseases, including bacterial and fungal infections, cancer, and various disorders. Plants are the source of the majority of commercially accessible medications for the treatment of both curable and incurable diseases. Around 3000 species have been documented to have medicinal uses for both curable and incurable ailments around the world. Herbal remedies are used by more than 80% of people in both developed and developing countries for primary health care. The Arabian Gulf is known as a land rich in traditional medicine, and herbal treatments have long been the primary source of health care in the Gulf region's cities [2].

Secondary metabolites, which are utilized as medications, agrochemicals, tastes, perfumes, color, biopesticides, and food additives, are abundant in plants. Medicinal plants are nature's gift to humans, assisting them in maintaining a disease-free, healthy lifestyle. Humans have been using plants as medicines for thousands of years. Ammi species, which are members of the Apiaceae family, include bioactive chemicals such as coumarins and flavonoids, which have vital biological functions. *Ammimajus* is an Egyptian native that thrives in the Nile Valley, particularly in Behira and Fayoom. It can also be found in the Mediterranean Sea basin, West Africa, some parts of Iran, and the Kohaz highlands [3].

This plant, *Ammimajus*, contains several key chemical components, particularly coumarins and flavonoids, which have important biological functions. Apart from essential oils, marmesin, isoimperatorin, heraclenin, isopimpinellin, nonhydroxyliccoumarins, ammirin and alloimperatorin, khellin, visnagin, and acetylated flavonoids are discovered in great concentration in this plant [4].

Ammimajus is a common herb of cultivation in Iraq, growing in fields, gardens, and along the banks of rivers and streams. It's found in Kut, Baghdad, Hawija, and other places in Iraq, while *Ammivisnaga* is found in Erbil, Mosul, Baghdad, Sulaimania, and Kirkuk in the north [3]. This family includes economically important crops including carrot, parsnip, and celery, as well as seasonings like coriander, anise, caraway, cumin, parsley, and dill. Apiaceae species are well-known for their commercial value and diversity of essential oils [5].

The aim of this research is the anatomical study of *Ammimajus* and effect on bacteria.

2. Materials and Methods

2.1. Plant collection

Ammimajus was collected from the field of science college 25 April 2020 washed and dried it in the Lab.

2.2. Paraffin method

For 24 hours, the pieces of samples were combined in an FAA (Formalin-Acetic-Acid-Alcohol) solution made up of (90ml of 70% alcohol + 5ml of glacial acetic acid + 5ml of formalin). The samples were then dehydrated using a succession of alcohol concentrations (90%, 95%, and 100%) for 1 hour for 95% and 3-4 hours for other concentrations, before being put in xylene for 3-4 hours (Twice times). The sample was embedded in a mixture of xylene and paraffin (1xylene + 1paraffin at 60°C) for 30 minutes (twice), then transferred to pure paraffin and left in this wax overnight at 60°C.

Following that, paraffin blocks were formed and sections with an 8-micrometer thickness were prepared using a rotary microtome. The sections were then stained with safranin (1%) and either fast green or light green (1gm in 100ml ethanol). Finally, the sections were reorganized [6].

2.3. Media preparation and cultured

Use nutrient agar; prepared it and sterilized it in autoclave at 121 °C under pressure of 12-15 bar. Then inoculation of the media with *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Candida albicans*, then incubated at 37 °C for 24 hours, finally investigate the results.

2.4. Extraction methods

Weight (20g) of dry leaves and stems of plant sample, were grind in awarding blender for 1 minute, then add (200ml) ethanol 95%, then put inside the shaker, incubate the refrigerator for overnight, filtrated by sterilized goze, then dried the oven for 24 hours; the final product was stored in refrigerator.

2.5. Preparation the different concentration of the plant

The different concentration prepare(0.5-5%) from the plant sample by dilution with ethanol 95 % [7].

3. Results

The stem and petiole have a triangular shape and are made up of secretory cells with open vascular bundles and unicellular glandertrichomes in the stem. Accessory vascular bundles can be found in the stem and petiole. The midrib on the adaxial surface is tiny and humped, with unicellular, non-glandular trichomes, and the adaxial surface is "V" shaped, with two auxiliary vascular bundles. The lamina is made up of upper and lower epidermis, with trichomes that are unicellular and non-glandular on both levels, and a mesophyll layer that includes palisade and spongy layers. The trichomes are unicellular and non-glandular, and the edge is striate and semi-rounded. Finally, all secretory canals are of the schizogenus type, Fig. 1.

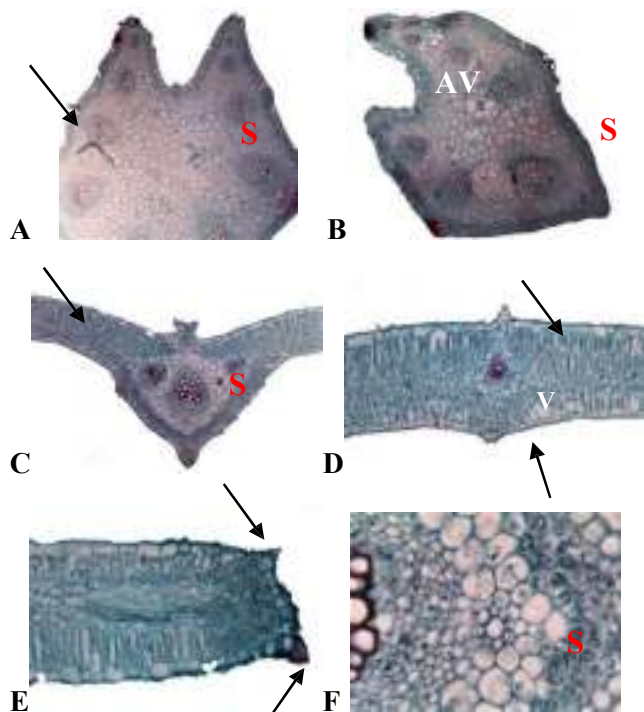


Fig. 1: TS of *Ammimajus*: A. stem, B. margin, C. midrib, D. lamina, E. margin, F. magnification power of secretory canals. V: vascular bundles, AV: accessory vascular bundles, S: secretory cells or canals, Trichomes (small black arrow). A,B=4X, C,D,E=10X, F=40X.

3.1. Anatomical characters

3.2. Antimicrobial activity

The effect on *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Candida albicans* was studied using varied concentrations (0.5-5%) of plant samples; leaves, stems, and flowers, Tables 1, 2, 3 and 4, and Figs. 2, 3, 4 and 5.

Table 1: Effect of different concentrations of *Ammimajus* extract on the growth of *Staphylococcus aureus* measured the dimension of zone of inhibited (cm).

Concentrations / Samples	0.5 %	1 %	2 %	3 %	4 %	5 %
<i>Ammimajus</i> stem extract inhibition zone	0.5cm	0.0	0.5cm	0.4cm	0.0	0.0
<i>Ammimajus</i> leaf extract inhibition zone	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ammimajus</i> flower extract inhibition zone	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Effect of different concentrations of *Ammimajus* extract on the growth of *Acinetobacter baumannii* measured the dimension of zone of inhibited (cm).

Concentrations / Samples	0.5 %	1 %	2 %	3 %	4 %	5 %
<i>Ammimajus</i> stem extract inhibition zone	0.0	0.0	0.0	0.55 cm	0.4 cm	0.5 cm
<i>Ammimajus</i> leaf Extract inhibition zone	0.0	0.0	0.0	0.0	0.45 cm	0.0
<i>Ammimajus</i> flower extract inhibition zone	0.4 cm	1.05 cm	0.15 cm	0.5 cm	0.0	0.0

Table 3: Effect of different concentrations of *Ammimajus* extract on the growth of *Pseudomonas aeruginosa* measured the dimension of zone of inhibited (cm).

Concentrations / Samples	0.5 %	1 %	2 %	3 %	4 %	5 %
<i>Ammimajus</i> stem extract inhibition zone	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ammimajus</i> leaf Extract inhibition zone	0.0	0.0	0.25 cm	0.0	0.0	0.0
<i>Ammimajus</i> flower extract inhibition zone	0.0	0.0	0.0	0.0	0.0	0.0

Table 4: effect of different concentrations of *Ammimajus* extract on the growth of *Candida albicans* measured the dimension of zone of inhibited (cm).

Concentrations Samples	0.5 %	1 %	2 %	3 %	4 %	5 %
<i>Ammimajus</i> stem extract inhibition zone	0.0	0.0	0.0	0.0	0.0	0.0
<i>Ammimajus</i> leaf Extract inhibition zone	0.75 cm	0.0	0.0	0.0	0.0	0.0
<i>Ammimajus</i> flower extract inhibition zone	0.85 cm	0.0	0.0	0.0	0.0	0.0

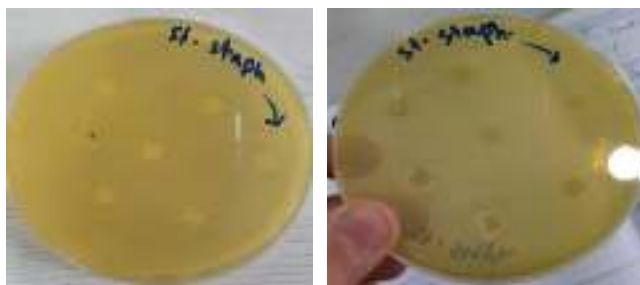


Fig. 2: Stem extracts effect on *Staphylococcus aureus*



Fig. 3: Leaf, Flower and Stem extract effect on *Acinetobacter baumannii*.



Fig. 4: Leaf extracts effect on *Pseudomonas aeruginosa*

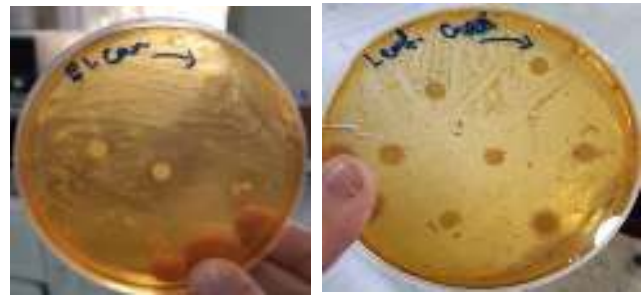


Fig. 5: Flower and leaf extract effect *Candida albicans*.

4. Discussion

The majority of natural antioxidants can be found in aromatic and therapeutic plants. They are, nevertheless, still underutilized in the medical, pharmaceutical, and food industries [8]. *Ammimajus* has long been used as an herbal medication in numerous nations to treat skin diseases, menstrual irregularities, and conditions that require diuresis. The purpose of this study was to determine the antioxidant, antibacterial, and cytotoxic effects of crude extracts of *Ammimajus* grown locally (*A. majus*). The antibacterial activity of all seed crude extracts from various polar solvents was evaluated using a slightly modified agar disc diffusion method. For this study, four bacterial strains were chosen *S. aureus*, *E. coli*, and *Proteusspp* [10]. The present work shows shape the outline of stem, petiole and margin with composed of leaf mesophyll layer. In leaf anatomy, it was observed that the shapes of epidermal cells are oblong to ovoid in both of the species. There are unicellular hairs on the leaf surface rarely in *C. aureum*. The leaves are amphistomatic and mesophyll is bifacial in both species [10].

The epidermal cells on the stem of the *Ammimajus* are square-rectangular in form. *Sclerenchymatous* cells line the ridges of the stem, and secretory canals run beneath this layer. The cortex is made up of parenchymatic cells and is quite large. Vascular bundles are seen in clusters and encircled by *sclerenchymatous* cells. The cells of the trachea are quite big. The parenchymatic pith fills a considerable portion of the stem. The leaf's epidermal cells are square-rectangular in shape and have a pronounced thickening, especially in the lower epidermis. Large *paranchymatic* cells make up the mesophylls. The mesophyll has a lot of vascular bundles (11).

The current study demonstrates the influence of ethanolic alcohol extracts of stem, leaf, and flower on the growth of bacteria such as *Staphylococcus aureus*, *Acinetobacterbaumannii*, *Pseudomonas aeruginosa*, and

Candida albicans. Bactericidal, fungicidal, insecticidal, larvicidal, molluscicidal, nematocidal, ovidical, viricidal, and herbicidal properties are all exhibited by furocoumarins. The antiviral efficacy of *Ammimajus*, coumarins against two mammalian viruses, HSV-1 and VSV, was tested using the end titration technique, which relies on the ability of plant extract dilutions to reduce the generated cytopathogenic impact. *Staphylococcus aureus*, *Leuconostocmesontrouide*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida tropicans*, and *Candida albicans* were used to assess the antibacterial properties of the ethanolic and aqueous extracts of *Ammivisnaga*. Ethanol extract, with a minimum inhibitory concentration (MIC) of 5 mg/ml against *Enterococcus faecalis*, was the most potent extract against Gram-positive bacteria. Furthermore, the same extract was found to have antibacterial action against Gram negative bacteria.

Several investigations have looked into the antibacterial properties of various *A. visnaga* extracts. The alkaloidal and sesquiterpene lactone fractions showed efficacy against *Candida* species, while ethanolic extracts of fruits inhibited *Mycobacterium tuberculosis* growth significantly. Antimicrobial activity was found in the same extract against Gram-negative bacteria *Escherichia coli* and *Klebsiellapneumoniae* [12]. Our observations revealed that the ethanolic alcohol crude extract of *A. majus* showed significant antioxidant activity.

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