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Comprehensive analysis of phytochemistry, In Silico toxicity prediction, and antimicrobial efficacy across different fractions of *Ammi visnaga* L.

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ABSTRACT

Ammi visnaga L. is a dense source of bioactive compounds with potential antioxidant and antibacterial activities. Different fractions were prepared to determine their total phenolic content and antioxidant activities (TPC, TFC, TAC, and DPPH). While, agar disc diffusion, MIC, and MBC assays were used to examine the antimicrobial effects of different fractions. The phytochemistry of the umbel dry was performed using UHPLC. Aqueous extract registered the highest amounts of TPC, TAC, TFC, and DPPH compared to other extracts ($P<0.05$). Treatment of antimicrobial results against three bacterial strains (*E. coli*, *P. aeruginosa*, and *S. aureus*) and Candida albicans revealed that the aqueous extract exerted interesting activity against all microbes with diameter zones varying from 12 to 17 mm for bacteria and 25 mm for Candida albicans, while the hexane fraction registered the lowest antimicrobial activity against all microbes under study. The umbel contains 16 phenolic compounds whose majority components are isorhamnetin_3-O-rutinoside (50.18%), isorhamnetin_3-O-glucoside (19.35%) and kaempferol_3-O-glucoside (12.04%). Additionally, the in-silico toxicity prediction showed that the main phytochemicals did not possess any toxicity, including hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. The present findings indicate the possible application of *Ammi visnaga* L. as a useful antimicrobial agent that replaces chemical drugs that induce the emergence of resistance.

1. Introduction

Natural sources constitute an exhaustible mine of biologically active compounds. They are crucial components of biochemical and medical research that aims to find powerful antimicrobial agents. Isolation and purification the most potent phytochemical components for antimicrobial investigations is the most appropriate strategy for creating efficient antimicrobial drugs. Scientific experts have become aware of these natural substances to lessen the negative impacts of chemical agents.

Medicinal plants have been the keystone of traditional medicine across the world for thousands of years, and several plants are still used as the main source of health care for many purposes [1]. *Ammi visnaga* L. (AV) is known as Noukha (in Algeria), Bechnikha (in Morocco), or Khella (in parts of North Africa). It is classified in the Apiaceae family (Umbelliferae) [1]. Botanically, *Ammi visnaga* L. is a biannual or annual herbaceous plant with a height of approximately 1 m.

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Tetracyclic pentamerous flowers with radial symmetry have five stamens made up of two joined carpels and a lower ovary. A complex umbel of white flowers with a large base makes up the inflorescence, which eventually becomes woody and is used as toothpicks [2]. The principal components of the plant are furanochromones and coumarins. However, the most active compounds are khellin and visnagin, as previously evoked by Harvengt and Desager [3]. The secondary metabolites of the plant provide a major part of the plant's protection against different biotic and abiotic stresses. Bioactive compounds play an essential role in both human and animal health and are well recognized as having therapeutic potential for several diseases [4]. In Morocco, umbel is traditionally prescribed as an antidiabetic, antispasmodic and diuretic [5]. Furthermore, it has been reported that the antioxidant activity of the plant might be due to its dense chemical composition [6–9]. Phenolic compounds have an important characteristic and function as natural antiagents against a wide range of pathogenic microbes. Thereafter, the ethanolic and ethyl acetate extracts of *Ammi visnaga* were found to be effective against *S. aureus* (ATCC 25923), *S. mutans* (ATCC 25175), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 9027) and *C. albicans* (ATCC 10231) [10]. Additionally, the combination of lycopene and the *Ammi visnaga* L. extract showed an interesting antibacterial effect against three bacterial strains, including *E. coli, K. pneumonia* and *S. aureus* [11]. Finding the best way to extract the highest quantity of bioactive components from plant matrices has motivated ongoing research in the search for the most efficient bioactive compound extraction technology [12–15]. The current study was conducted to determine the total phenolic content, the total flavonoid content, the antioxidant, and the antibacterial properties of several extracts of inflorescence of *Ammi visnaga* L. while taking these characteristics into consideration.

2. Materials and Methods

2.1. Chemicals and reagents

Acetonitrile and formic acid were LC-MS grade solvents that were purchased from Fisher Scientific (Loughborough, UK) for use in LC-MS analyses. Deionization produced ultrapure water (Millipore, Billerica, USA). Sigma-Aldrich (Steinheim, Germany) provided the phenolic standards that were purchased.
per gram of dry weight.

2.2. Extraction procedure

A volume of 400 mL of mixed solvents (66.67% acetone, 16.67% water, and 16.67% methanol) was sonicated for 30 min to extract a 20 g mass of powder. The resulting mixture is then condensed using a rotary evaporator until dry [16].
After suspension of the extract in water and partitioned with n-hexane, dichloromethane, chloroform, and ethyl acetate (Figure 1), it was concentrated under decreased pressure at 40 °C using a vacuum rotary evaporator. With each solvent, the process was performed three times. Three extracts from each solvent were combined, evaporated using a rotary evaporator at reduced pressure, and suspended in ethanol.

2.3. Quantification of the total phenolic content

With a few minor adjustments, the Folin–Ciocalteu method was previously reported by Singelton et al. [17], was used to determine the total phenol content. The procedure involves mixing 50 mL of the fraction with 450 mL of Folin-Ciocalteu reagent (0.2 N) for 5 min before adding 450 mL of Na_2CO_3 (75 g/L) solution. The absorbance of each sample was measured at 760 nm using a Jenway 6505 UV/visible scanning spectrophotometer after incubation at room temperature in the dark for two hours. In an ethanolic solution of gallic acid, the calibration curve's concentration (y = 1,6021x + 0,0683, R2 = 0,997) ranged from 0.008 to 1 mg/mL. The results of the experiment, which was performed in triplicate, are presented as mg of gallic acid equivalent (GAE) per g of the dried plant.

2.4. Quantification of the total flavonoid content

Using aluminum chloride and the methodology developed by Ordonez et al., the TFC quantity measurement was evaluated [18]. 250 L of each extract was combined with 150 µL of $AlCl_3$ (10%), 75 µL of sodium carbonate (Na_2CO_3, 1M), and 500 µL of sodium hydroxide to create the reaction mixture. With the aid of distilled water, the volume was changed to 2.5mL. The incubation was maintained in the dark for one hour. A UV spectrophotometer was used to measure the optical density at 510 nm. The TFC amounts were given as mg QE/g dw, or milligrams of quercetin equivalent

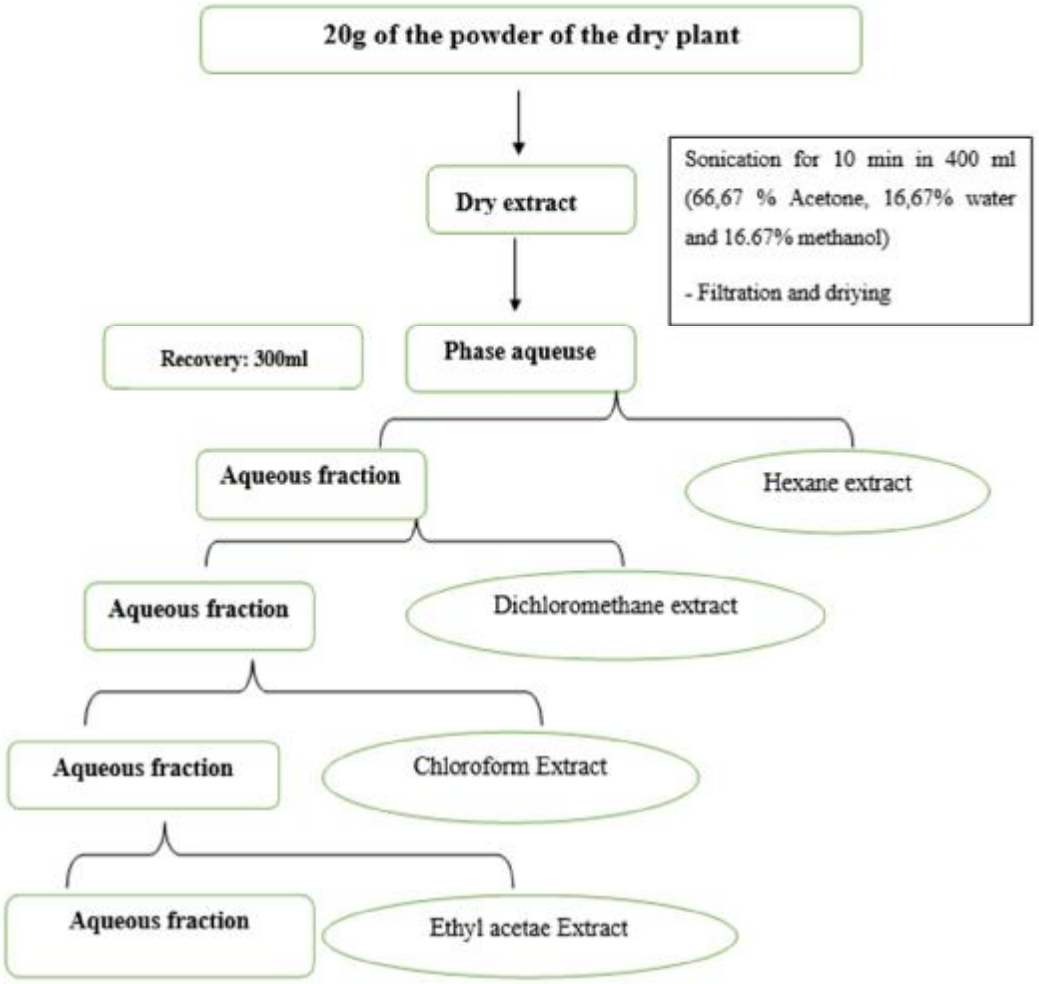

Fig. 1. Solvent fractionation scheme.

2.5. Total antioxidant capacity

According to the procedure described by Aazzaet al. [19], green phosphomolybdenum complex production was used to measure the total antioxidant capacity (TAC) of all samples. In Falcon 15 mL tubes, 25 µL of the sample solution and 1 mL of the reagent solution room temperature. The experiment was performed thrice, and the outcomes are expressed as mg of ascorbic acid equivalent (mg AAE/g dw).

(0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate) were mixed. The Falcon tubes were sealed and left to sit at 95°C in a water bath for 90 min. The absorbance of the mixture was measured at 695 nm against a blank in a Jenway 6505 UV/visible scanning spectrophotometer after the samples had cooled to

2.6. DPPH assay

The procedure for DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed according to Aazza et al.

[19]. 1 mL of a 60 mM methanolic solution of DPPH was mixed with 50µL of samples at various concentrations. After 60 min at room temperature, absorbance measurements were taken at 517 nm (A1). The negative control (A0) was the absorption of a blank sample that included the same quantity of methanol and DPPH solution. The concentration of the extract able to scavenge 50% of DPPH free radicals was evaluated by plotting the percentage inhibition [(A0-A1/A0) *100] against the sample or standard content.

2.7. Antibacterial activity

2.7.1. Sensitivity assay

The ability of different fractions prepared from *Ammi visnaga* L. was examined using the well diffusion test as previously described by [20]. Three bacterial strains and one fungus were subjected to antimicrobial potency. It consists of inoculating a suspension at a concentration of 10^8 CFU/ml for each microorganism including: *Eschershia coli* (CCCCCC X1), *Pseudomonas aeruginosa* (BSEAF X1), *Staphylococcus aureus* (BSEAF X2), and *Candida albicans* (LSEAF X3). Next, a disc of sterile Whatman paper (6 mm), soaked with the fractions studied (10 µl), on the Petri dishes that contain the PCA culture medium for the becterial strains and MT for the fungi. Finally, Petri dishes were incubated at 37 °C for 24 h for bacterial strains and 48h for the fungi. After incubation, the diameters of the inhibition zones were measured in mm.

2.7.2. Minimum inhibitory concentration (MIC)

The lowest inhibitory concentration (LIC) against *P. aeruginosa, E. Coli, S. aureus,* and *C. albicans*, was measured by the microdilution technique. A volume of 50 µL of the bacterial broth, diluted to 10^6 cells/mL using Luria-Bertani (LB) medium, and 50 µL of different concentrations of CH (3.90–1000 ppm) were added in a 96-well microliter plate, respectively, and further incubated for 20 h at 37 °C. The mixture of 50 µL bacterial solution and 50 µL of the sterile medium was used as a positive control. After incubation time, 15 µL of resazurin (0.015%) was put into the wells and then incubated for 2 h in order to visualize the change of color. The contents of wells containing ½ × LIC, LIC, 2 × LIC, and 4 × LIC were transferred onto agar plates and further incubated at 37 °C for 24 h [21].

2.7.3. Phenolic profile of *Ammi visnaga* L. umbels

The exploration of secondary metabolites of the plant under study was performed using a UHPLC system (ThermoFisher Scientific, Bremen, Germany). The system was equipped with a TSQ Quantum Acces Max triple-quadrupole mass spectrometer (ThermoFisher Scientific, Basel, Switzerland) and a diode array detector (DAD). The method was used according to the protocol previously described in our previous report [22]. The identification of phytochemicals was carried out by comparing standards with the database of the literature. The content of each detected compound was determined by the calculation of the peak areas.

2.8. In silico toxicity prediction

The prediction of toxicity of the main bioactive compounds found in Ammi visnaga L. was performed using an in silico assay. The ProTOX-II software was used to predict the toxicity of the main active components (https://tox-new.charite.de/protox_II/) [23].

2.9. Statistical analysis

Statistical analyzes of the results obtained were carried out using Pearson correlation coefficient (r) at a significance level of 99% (p<0.01). Then, the principal component analysis (PCA) was performed using PAST 3. The comparisons of different fractions were performed using Tukey test in Graph Pad Prism software

3. Results

3.1. Total phenol content (TPC)

Figure 2 displays the obtained results of the dosage of total phenolic content of different fractions prepared from *Ammi visnaga*. The analysis of the outcomes showed that the water was the most appropriate extractor solvent translated by the highest amount of TPC with value of 34.25 mg GAE/g dw. While, the lowest TPC value was found in the hexane extract (1.363 mg GAE/g dw).

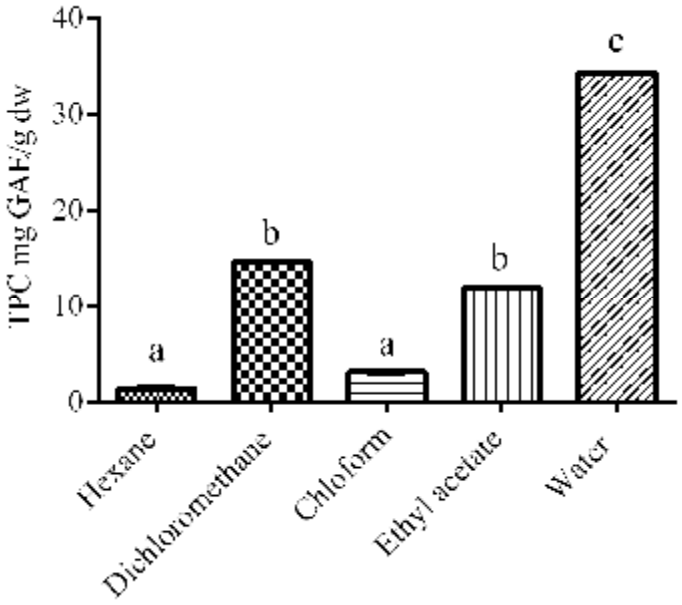

Fig. 2. Total phenolic content of different fractions obtained from *Ammi visnaga.*

3.2. Total flavonoid content

Figure 3 displays the obtained results of the dosage of TFC of different fractions of *Ammi visnaga* L. It is clearly seen that the fractions under study revealed different amounts of TFC that varying between 0.01 and 0.62 mg QE/g dw. The highest TFC value was registered in the water fraction, while the lowest amount was found in hexane fraction. The statistical comparison revealed that the TFC was significantly different (P<0.05).

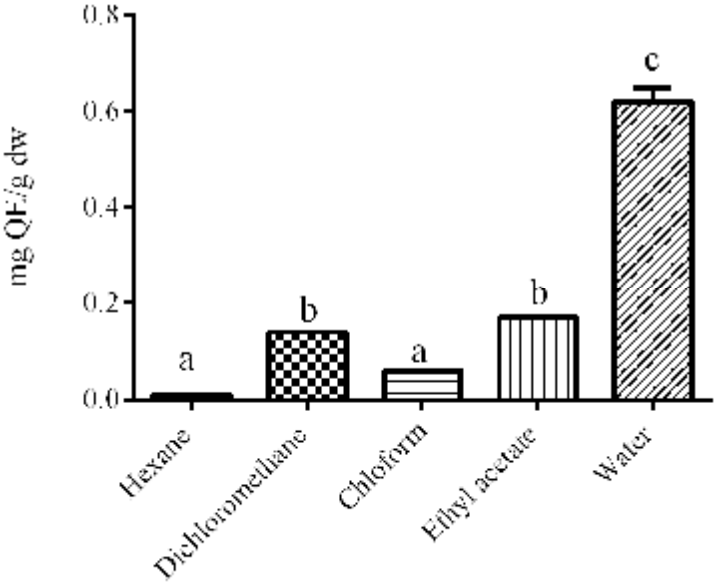

Fig. 3. Total flavonoid content of different fractions obtained from *Ammi visnaga*.

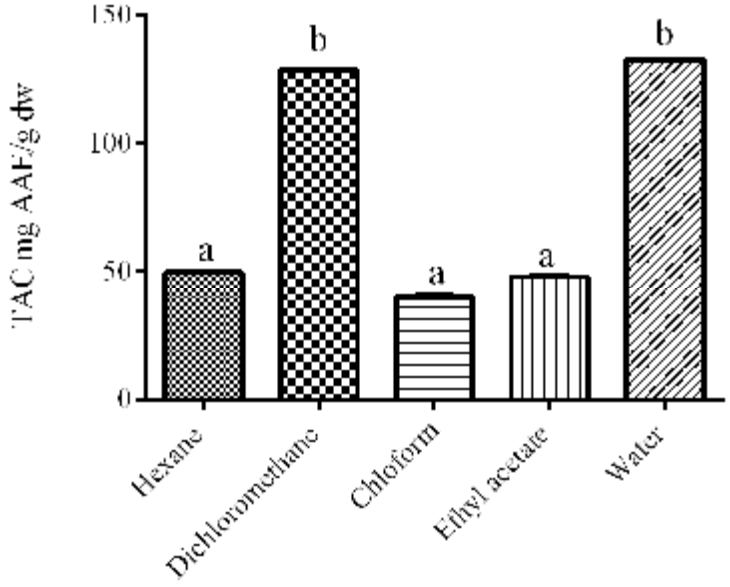

Fig. 4. Total antioxidant capacities of different extracts.

3.3. Total antioxidant activity/Phospho molybdenum assay (TAC)

Figures 4 displays the results of the determination of TAC. The treatment of data showed that dichloromethane and water extract were the most active and exhibited the highest total antioxidant capacities with the following values of 128.2 and 132.6 mg AAE/g dw, respectively.

3.4. DPPH Free Radical-Scavenging Activity

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidant can reduce it by donating an electron or hydrogen. The antioxidant ability of the different fractions under study (Hexane, dichloromethane, chloroform, ethyl acetate, and water) prepared from *A. visnaga* were evaluated using the DPPH assay, and the obtained results are displayed in Figure 5. The treatment of the obtained results revealed that the aqueous fraction exhibited the highest antioxidant activity, while the hexane fraction was the weakest fraction to scavenge the free radical.

According to the figure 5, all the fractions showed a dose dependant antioxidant activity. Based on the results obtained from this study, ethyl acetate fraction showed the highest antioxidant activity, followed by the dichloromethane fraction, while the n-hexane fraction exhibited the lowest antioxidant activity, showing that the antioxidant compounds in *Ammi visnaga* are not non polar. We can also notice that the antioxidant activity increases in the fraction with the increase of the solvent polarity.

3.5. Antimicrobial activity

Table 1 represents the obtained results of antibacterial activity of different extracts under study.
The analysis of the obtained results showed that all fractions were found to have a strong antimicrobial property to sabotage the normal development of different strains under study except the hexane fraction. The water fraction produced the best antimicrobial potency against *E. coli, P. aeruginosa* and *C. albicans* with an inhibition diameter 16.7± 0.2 mm, 14.1±0.1 mm and 25.23±0.1mm, respectively. While, the lowest DI values were registered for hexane fraction against all microorganisms under study.

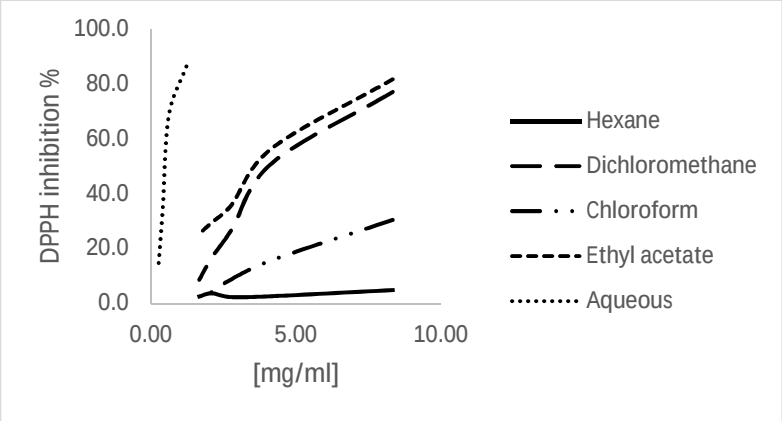

Fig. 5. DPPH free radical-scavenging activity of different extracts.

Table 1. Antimicrobial activity of different extracts.

	E.coli	*P. aeruginosa*	*S. aureus*	*C. albicans*
Water fraction	16.7± 0.2 [d]	14.1±0.1	13.23±0.1 [d]	25.23±0.1 [d]
Chloroform fraction	12.25±0.3 [a]	13.2±0.1 [ad]	12.23±0.3 [a]	14.16±0.1 [ad]
Ethyl acetate fraction	15.07±0.08 [b]	11.36±0.08 [b]	14.4±0.2 [b]	16.23±0.04 [b]
Hexane fraction	1.75±0.25 [c]	3.16±0.2 [c]	5.2±0.06 [c]	4.7±0.06 [c]
Dichloromethane fraction	16.2±0.1 [d]	13.2±0.1 [d]	13.06±0.04 [d]	14.47±0.08 [d]

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$).

3.6. Phenolic profile

To determine the phenolic profile of *Ammi visnaga* umbel dry related to its antioxidant and antimicrobial potencies, the phytochemicals were characterized using HPLC and the obtained results are displayed in Figure 6 and Table 3. The quantitative determination of AV umbel dry showed the presence of 16 compounds with different quantities. Individual phenolic components detected in the material under study with high amount are isorhamnetin_3-O-rutinoside (50.18%), isorhamnetin_3-O-glucoside (19.35%), kaempferol_3-O-glucoside (12.04%), and chlorogenic acid (4.93%). While, other compounds are detected in lowest amounts.

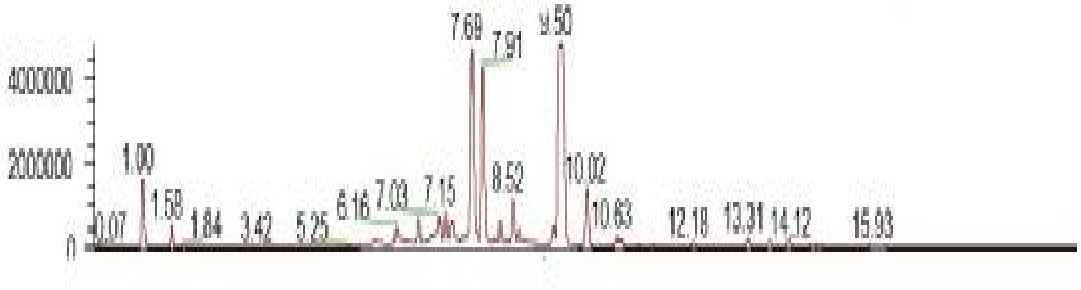

Fig. 6. HPLC chromatogram of the *Ammi visnaga* extract.

Table 2. Phenolic profile of dry umbel extract of *Ammi visnaga.*

Phenolic compound	Pourcentage (%)
Neochlorogenic acid	0.71
Chlorogenic acid	4.93
p-Hydroxybenzoic_acid	1.16
Caffeic_acid	1.11
Rutin	2.86
p-Coumaric_acid	2.26
Quercetin_3-O-glucoside	2.44
Isorhamnetin_3-O-rutinoside	50.18
Isorhamnetin_3-O-glucoside	19.35
Quercetin_3-O-rhamnoside	0.46
Kaempferol_3-O-glucoside	12.04
Quercetin	0.05
Naringenin	0.05
Kaempferol	1.16
Hispidulin	0.04
Isorhamnetin	1.14
SUM	**99.94**

1.1. *In silico toxicity prediction*

Toxicity prediction using an in silico assay of the main active compounds found in *Ammi visnaga* L. did not exhibit any toxicity, including hepatotoxicity, carcinogenicity, mutagenicity, or cytotoxicity. All tested molecules showed mild immunogenicity, except for p-coumaric acid (Table 5). The selected main active compounds of *Ammi visnaga* L. showed high lethal dosage 50 (LD50) concentrations, which could explain the safety of the plant extract.

Table 3. Toxicity prediction details by Protox-II of the main active compounds of *Ammi visnaga* L.

Compound	Predicted LD50 (mg/kg)	Toxicity class	Hepatotoxicity	Carcino-genicity	Immunotoxicity	Mutagenicity	Cyto-toxicity
Isorhamnetin_3-O-rutinoside	5000	5	0.81 (Inactive)	0.93 (Inactive)	0.99 (Active)	0.90 (Inactive)	0.52 (Inactive)
Isorhamnetin_3-O-glucoside	5000	5	0.83 (Inactive)	0.90 (Inactive)	0.93 (Active)	0.65 (Inactive)	0.58 (Inactive)
Kaempferol_3-O-glucoside	5000	5	0.82 (Inactive)	0.85 (Inactive)	0.64 (Inactive)	0.76 (Inactive)	0.69 (Inactive)
Chlorogenic acid	5000	5	0.72 (Inactive)	0.68 (Inactive)	0.99 (Active)	0.93 (Inactive)	0.80 (Inactive)
p-Coumaric_acid	2850	5	0.51 (Inactive)	0.50 (Active)	0.91 (Inactive)	0.93 (Inactive)	0.81 (Inactive)
Quercetin_3-O-glucoside	5000	5	0.82 (Inactive)	0.85 (Inactive)	0.66 (Active)	0.76 (Inactive)	0.69 (Inactive)

4. Discussion

Infectious diseases constitute a real challenge for animal and human health and chemical treatments presented some limitations. Historically, humans have sought to use different natural herbs to treat their ailments [24,25]. In fact, natural sources were found to contain effective antimicrobial agents that could neutralize antibioresistance acquired by the misuse of antimicrobial chemical drugs [26]. The bioactive compounds of medicinal plants are found to be effective and biorational molecules against several pathogen bacteria [27]. The failure of antibiotic spectra continues to increase, which prompted researchers to investigate the antimicrobial ability of medicinal plants, such as *Ammi visnaga* L.

The findings showed that the aqueous extract registered the highest amounts of total phenolic content, total flavonoid content, and remarkable antioxidant ability. The aqueous and dichloromethane fractions have a potent antimicrobial property against different bacterial strains (*E. coli, P. aeruginosa,* and *S. aureus*) and *Candida albicans*. The analysis of results revealed that the aqueous extract registered the highest amounts of phenolic and flavonoid contents, which are lower than those determined by Bitari et el. [28]. Karkouri et al. found that the phenolic content of *Ammi visnaga* L. ranged between 1.7 and 72.36 mg GAE/g [29]. Contents et al. documented the lowest values of TPC and TFC [30]. Those results can be explained by the fact that phenolic content and antioxidant activity of the extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity [31]. The most part of antioxidants compounds extracted from *Ammi visnaga* was very polar and stood the water fraction after liquid-liquid extraction with different solvent. It can be observed from these results; changes on solvent polarity alter its ability to dissolve antioxidant compounds. Similar results showing that water extracts were found to be the most efficient solvent to have antioxidant activity for three Mentha species [31]. The obtained results are in line with those reported by Ousaaid et al. who found that the water was the most extractant than other organic solvents [32]. Solvent polarity constitutes the main factor affecting phenolic content recovery. It has been found that the yield of phenolic extraction depends on the polarity of the extractant. The study conducted by Bui et el. revealed that dichloromethane was the lowest extractible solvent than chloroform and ethyl acetate [33]. Solvents with the highest polarity were preferred to recovery polar compounds such as phenolic components associated with multiple carbohydrates [34]. Secondary metabolites play a pivotal role in the plant protection against different biotic and abiotic stressors, including draught, salinity, fungi, viruses, and bacteria [35]. Phytoprotectants are found to be able to neutralize reactive oxygen species and act as natural antimicrobial agents with large spectrum of activities [36]. Bacteria and fungi can survive despite the application of different chemical agents acquiring the resistance. However, in the present study, we evaluated the potency of different fractions of *A. visnaga* to eradicate three pathogenic bacteria strains and one fungi. The finding revealed that the aqueous extract exerted an interesting activity against all microbes with diameter zones varying from 12 to 17 mm for bacteria and 25 mm for *Candida albicans*, while hexane extract registered the lowest antimicrobial activity against all microbes under study. The obtained results from this study are partially coherent with published articles reporting the antimicrobial potency of *A. visnaga* against *S. mutans, S. salivarius, S. sanguis* [37], *S. aureus, L. mesontroide, E. faecalis, E. coli, P. aeruginosa, K. pneumoniae, C. tropicans and C. albicans* [38] , *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853) [39]. The same finding were found for *A. majus* extracts against *S. aureus, E. coli, H. influenza,* and *Proteus* spp [40]. *A. visnaga* is considered to have remarkable antimicrobial properties, which are associated with its chemical composition. Several studies have demonstrated that the antimicrobial efficacy of natural products is highly related to their phytochemical composition [41–44]. The delve into the phytochemistry of *A. visnaga* revealed the presence of wide range of phytochemicals accounting 16 compound, including isorhamnetin_3-O-rutinoside (50.18%), isorhamnetin_3-O-glucoside (19,35%) and kaempferol_3-O-glucoside (12 ,04%) (Table 4). A published report on phytochemistry of *A. visnaga* documented that the main bioactive compounds found are khellin and visnagin [45]. While, Zaher et al. detected 46 compounds representing 89.89%, including edulisin III, binapacryl, khellin, and visnagin as the most abundant components [46]. The metabolomics of the bioactive compounds of *A. visnaga* revealed the presence of phenylpropanoids, flavonoids, isobenzofuranones, coumarins, and chromones [47]. It

has been documented that the main aglycones found in *A. visnaga* are quercetin, rhamnocitrin, rhamnetin, and rhmnazin [48], while, the most flavonols detected in our sample are conjugated with glucose, rutinose, and rhamnose. The obtained results are high concordance with those reported by Bencheraiet et al. [49]. The variability of the phytochemical profile of *A. visnaga* is highly dependent on various factors, viz the geographical origin, pedoclimatic conditions, extraction technique, and extractor solvent used [50,51].

An experimental study found that isorhamnetin can inhibit the hemolysis effect of pneumolysin produced by *S. pneumoniae*, which confirms its efficiency as an antivirulence agent against *S. pneumoniae* infection [52]. In the same context, Aruwa et al. reported that the application of isorhamnetin improved antibacterial activity by destabilizing bacterial membrane integrety [53]. Furthermore, isorhamnetin has been found to be effective in altering the morphology of *A. fumingatus* hyphal and the integrety of the membrane by diminishing the corneal fungal load and inhibiting neutrophil recruitment [53]. It has also been found that isorhamnetin could decrease the levels of mRNA and protein expression of TLR-2, TLR-4, Dectin-1, IL-1β, and tumor necrosis factor-α [54]. Furthermore, the interaction between these molecules forms different compounds that disturb the integrity of bacteria cells, which perturbs their stability, permeability, and normal growth. In addition, phenolic acids dissociate, altering cell membrane potential perturbing sodium-potassium pump [47]. They can interact with synthetic pathways of binding of DNA replication with topoisomerase and DNA gyrase [47]. *A. visnaga* contains several bioactive compounds that act synergistically to eradicate pathogenic bacteria. It has been found that khellin and visnagin exert their impact as inhibitors of the efflux pump (NorA) overproduced by *S. aureus* SA-1199B, which confers resistance against antibiotics [55]. In addition, the combination of khellin, visnagin, and antibiotics reduced the minimum inhibitory concentration of antibiotics [55]. Bioactive compounds have proved their ability to enhance the impact of antimicrobial drugs against resistant microbes [56].

The seven main active substances discovered in *A. visnaga* showed mild immunotoxicity in in silico test. Isorhamnetin-3-O-glucoside, one of the active substances evaluated, displayed an immunostimulatory effect and was recommended as a treatment for chronic granulomatous disease and insufficiency of neutrophil function [57]. In addition, it has been discovered that isorhamnetin-3-O-glucoside induced the transcriptional expression of cytokines [58]. On the other hand, the phytocompounds found in *A. visnaga* possessed an interesting hepatoprotective effect against numerous toxic agents [59–61].

Secondary metabolites constitute the main objective of several studies since their positive impact was announced on both human and animal health [62]. In fact, recent studies have deepened the scientific investigation of the beneficial properties of bioactive compounds isolated from *A. visnaga*. In the study by Ez-zahir et al., both furanochromones (khellin and visnagin) isolated from *A. vinsaga* L. exhibited an immunostimulating effect on the humoral response [63]. Furthermore, chlorogenic acid was tested to improve the meat quality of oxidatively stressed broilers, the obtained results showed that chlorogenic acid supplementation ameliorated the growth performance, meat quality, enhanced the antioxidant defense system [64]. The most interesting bioactive compounds are flavonoids well known for their wide range of biological properties, including anticancer, antidiabetic, antioxidative, antimicrobial, anti-inflammatory, and immunustimulant effects [63]. *A. visnaga* is a dense source of bioactive compounds that act synergistically on their positive impacts by affecting several physiological functions.

5. Conclusion

In the current work, different extracts of *Ammi visnaga*, especially aqueous extract, showed considerable phenolic and flavonoid contents, antioxidant activity, and adequate antibacterial and antifungal potency against different strains under study. These properties of *A. visnaga* could be due to its dense chemical composition, including phenolics, flavonoids, chromones, and coumarins. Ammi visnaga is a medicinal plant that could be used as a source of natural antimicrobial agents for the discovery of new antimicrobial drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] N. Khalil, M. Bishr, S. Desouky, O. Salama, Ammi Visnaga L., a Potential Medicinal Plant: A Review, Molecules 25 (2020) 301. https://doi.org/10.3390/molecules25020301.

[2] S. Hashim, A. Jan, K.B. Marwat, M.A. Khan, Phytochemistry and medicinal properties of Ammi visnaga (Apiacae), Pak J Bot 46 (2014) 861–7.

[3] C. Harvengt, J.-P. Desager, HDL-cholesterol increase in normolipaemic subjects on khellin: a pilot study., International Journal of Clinical Pharmacology Research 3 (1983) 363–366. https://europepmc.org/article/med/6678829 (accessed September 30, 2023).

[4] P. Ganesan, C.S. Kumar, N. Bhaskar, Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds, Bioresource Technology 99 (2008) 2717–2723. https://www.sciencedirect.com/science/article/pii/S0960852407005469 (accessed September 30, 2023).

[5] J.N. Amin, A. Murad, A.-M. Motasem, S.R. Ibrahem, J.M. Ass'ad, A.M. Ayed, Phytochemical screening and in-vitro evaluation of antioxidant and antimicrobial activities of the entire Khella plant (Ammi visnaga. L.) a member of palestinian flora, Int J Pharmacogn Phytochem Res 7 (2015) 137–143. https://staff-beta.najah.edu/media/published_research/2016/10/27/2015._Phytochemical_Screening__khella.pdf (accessed September 30, 2023).

[6] C. Ballester-Costa, E. Sendra, J. Fernández-López, J.A. Pérez-Álvarez, M. Viuda-Martos, Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth, Industrial Crops and Products 50 (2013) 304–311. https://www.sciencedirect.com/science/article/pii/S0926669013003890 (accessed September 30, 2023).

[7] M.L. Irakoze, E.N. Wafula, E.E. Owaga, Effect of Lactic Acid Fermentation on Phytochemical Content, Antioxidant Capacity, Sensory Acceptability and Microbial Safety of African Black Nightshade and African Spider Plant Vegetables, Bacteria 2 (2023) 48–59. https://www.mdpi.com/2674-1334/2/1/4 (accessed September 30, 2023).

[8] J. Tan, H. Jiang, Y. Li, R. He, K. Liu, Y. Chen, X. He, X. Liu, H. Liu, Growth, Phytochemicals, and Antioxidant Activity of Kale Grown under Different Nutrient-Solution Depths in Hydroponic, Horticulturae 9 (2023) 53. https://www.mdpi.com/2311-7524/9/1/53 (accessed September 30, 2023).

[9] Y. Huang, H. Xu, M. Ding, J. Li, D. Wang, H. Li, M. Sun, F. Xia, H. Bai, M. Wang, Screening of Rosemary Essential Oils with Different Phytochemicals for Antioxidant Capacity, Keratinocyte Cytotoxicity, and Anti-Proliferative Activity, Molecules 28 (2023) 586. https://www.mdpi.com/1420-3049/28/2/586 (accessed September 30, 2023).

[10] N. Fathallah, M.M. Raafat, M.Y. Issa, M.M. Abdel-Aziz, M. Bishr, M.A. Abdelkawy, O. Salama, Bio-guided fractionation of prenylated benzaldehyde derivatives as potent antimicrobial and antibiofilm from Ammi majus L. fruits-associated Aspergillus amstelodami, Molecules 24 (2019) 4118. https://www.mdpi.com/1420-3049/24/22/4118 (accessed September 30, 2023).

[11] D. Hamed, S. Keddari, M.Y. Boufadi, L. Bessad, Lycopene Purification with DMSO anti-solvent: Optimization using Box-Behnken's experimental design and evaluation of the synergic effect between lycopene and Ammi visnaga.L essential oil, Chem. Pap. 76 (2022) 6335–6347. https://doi.org/10.1007/s11696-022-02302-0.

[12] S.K.R. Namasivayam, S. Srinivasan, K. Samrat, B. Priyalakshmi, R.D. Kumar, A. Bharani, R.G. Kumar, M. Kavisri, M. Moovendhan, Sustainable approach to manage the vulnerable rodents using eco-friendly green rodenticides formulation through nanotechnology principles-A review, Process Safety and Environmental Protection (2023). https://www.sciencedirect.com/science/article/pii/S0957582023000617 (accessed October 1, 2023).

[13] N. Haq, M. Iqbal, A. Hussain, F. Shakeel, A. Ahmad, I.A. Alsarra, M.F. AlAjmi, A. Mahfooz, M.A. Abouzadeh, Utilization of Waste Biomaterial as an Efficient and Eco-Friendly Adsorbent for Solid-Phase Extraction of Pantoprazole Contaminants in Wastewater, Separations 10 (2023) 253. https://www.mdpi.com/2297-8739/10/4/253 (accessed October 1, 2023).

[14] M. Taghavijeloudar, P. Yaqoubnejad, A.K. Ahangar, S. Rezania, A rapid, efficient and eco-friendly approach for simultaneous biomass harvesting and bioproducts extraction from microalgae: Dual flocculation between cationic surfactants and bio-polymer, Science of The Total Environment 854 (2023) 158717. https://www.sciencedirect.com/science/article/pii/S0048969722058168 (accessed October 1, 2023).

[15] S.O. Essien, B. Young, S. Baroutian, Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials, Trends in Food Science & Technology 97 (2020) 156–169. https://www.sciencedirect.com/science/article/pii/S0924224419301116 (accessed October 1, 2023).

[16] Z. El Jabboury, S. Aazza, D. Ousaaid, O. Chater, W. Squalli, O. El Ghadraoui, M. Benjelloun, L. El Ghadraoui, Optimisation of Total Phenolic Compound Extraction and Antioxidant Activity from Dried Inflorescence of Ammi Visnaga Using Mixture Design and Triangular Surfaces., Jordan Journal of Biological Sciences 15 (2022). https://jjbs.hu.edu.jo/files/vol15/v4/Paper%20Number%2017.pdf (accessed October 1, 2023).

[17] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: Methods in Enzymology, Academic Press, 1999: pp. 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

[18] A.A.L. Ordonez, J.D. Gomez, M.A. Vattuone, Antioxidant activities of Sechium edule (Jacq.) Swartz extracts, Food Chemistry 97 (2006) 452–458.

[19] S. Aazza, B. Lyoussi, M.G. Miguel, Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds, Molecules 16 (2011) 7672–7690. https://www.mdpi.com/1420-3049/16/9/7672 (accessed October 1, 2023).

[20] D. Ousaaid, H. Imtara, H. Laaroussi, B. Lyoussi, I. Elarabi, An Investigation of Moroccan Vinegars: Their Physicochemical Properties and Antioxidant and Antibacterial Activities, Journal of Food Quality 2021 (2021) e6618444. https://doi.org/10.1155/2021/6618444.

[21] M. Elshikh, S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant, I.M. Banat, Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants, Biotechnol Lett 38 (2016) 1015–1019. https://doi.org/10.1007/s10529-016-2079-2.

[22] Z. El Jabboury, D. Ousaaid, U. Gašić, P. Janaćković, Z.D. Stevanovic, S. Kolašinac, M. Benjelloun, L.E. Ghadraoui, Unraveling the Phytochemical Profile Variability and Antioxidant Activities of Different Parts of Ammi visnaga (L) Collected from Taounate Region, Chemistry Africa (2023). https://doi.org/10.1007/s42250-023-00747-8.

[23] P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, ProTox-II: a webserver for the prediction of toxicity of chemicals, Nucleic Acids Research 46 (2018) W257–W263. https://doi.org/10.1093/nar/gky318.

[24] L. Lahrizi, F. Errachidi, H. Nekhla, L. El Ghadraoui, Ajuga iva L.: An overview of phytochemical profile and biological functionalities, Chemical Review and Letters 7 (2024) 31–44. https://doi.org/10.22034/crl.2024.413946.1241.

[25] U. Dandjlessa, B. Ezin, F.M. Assogba, N. Zossou, B. Glinma, E. Yayi Ladekan, J.D. Gbenou, A. Saïdoud, A. Ahanchede, Variation in the phytochemical composition of Chromolaena odorata (L.) King and Robinson (Asteraceae) across climatic zones in Benin (West Africa), Chemical Review and Letters 5 (2022) 193–199. http://www.chemrevlett.com/article_149189.html (accessed February 22, 2024).

[26] P.-C. Hsieh, J.-L. Mau, S.-H. Huang, Antimicrobial effect of various combinations of plant extracts, Food Microbiology 18 (2001) 35–43. https://doi.org/10.1006/fmic.2000.0376.

[27] D.C. Vodnar, L.F. Călinoiu, F.V. Dulf, B.E. Ştefănescu, G. Crişan, C. Socaciu, Identification of the bioactive compounds and antioxidant, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste., Food Chemistry 231 (2017) 131–140. https://doi.org/10.1016/j.foodchem.2017.03.131.

[28] A. Bitari, I. Oualdi, R. Touzani, M. Elachouri, A. Legssyer, Alpinia officinarum Hance: A mini review, Materials Today: Proceedings 72 (2023) 3869–3874. https://doi.org/10.1016/j.matpr.2022.10.080.

[29] J. El Karkouri, A. Drioiche, A. Soro, A. Ailli, N. Benhlima, A. Bouzoubaa, F. El Makhoukhi, H. Oulhaj, F.K. Elombo, T. Zair, Identification and antioxidant activity of Ammi visnaga L. polyphenols from the Middle Atlas in Morocco, Mediterranean Journal of Chemistry 10 (2020) 649. https://doi.org/10.13171/mjc10702007221459jek.

[30] K. Contents, I.N. Ammi, V.L. Lam, A.S. Mortada, K.M. Mohamed, Bulletin of Pharmaceutical Sciences EFFECT OF UV-C STRESS ON TOTAL PHENOLICS , TOTAL FLAVONOIDS, 45 (2022) 89–97.

[31] A. Barchan, M. Bakkali, A. Arakrak, R. Pagán, A. Laglaoui, Original Research Article The effects of solvents polarity on the phenolic contents and antioxidant activity of three Mentha species extracts, International Journal of Current Microbiology and Applied Sciences 3 (2014) 399–412.

[32] D. Ousaaid, I. Mansouri, H. Laaroussi, A. ElGhouizi, B. Lyoussi, I. ElArabi, Phytochemical Content and Antioxidant Activity of Flesh Fruits Rosa canina Extracts Collected from Ait Ayach Midelt, IJARe (2019). https://doi.org/10.18805/IJARe.A-494.

[33] N.T. Bui, T.-L.T. Pham, K.T. Nguyen, P.H. Le, K.-H. Kim, Effect of extraction solvent on total phenol, flavonoid content, and antioxidant activity of Avicennia officinalis, Res. Appl. Chem 12 (2021) 2678–2690. https://biointerfaceresearch.com/wp-content/uploads/2021/06/20695837122.26782690.pdf (accessed February 20, 2024).

[34] Q.D. Do, A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji, Y.-H. Ju, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica, Journal of Food and Drug Analysis 22 (2014) 296–302. https://www.sciencedirect.com/science/article/pii/S1021949813001348 (accessed February 3, 2024).

[35] M. Thakur, S. Bhattacharya, P.K. Khosla, S. Puri, Improving production of plant secondary metabolites through biotic and abiotic elicitation, Journal of Applied Research on Medicinal and Aromatic Plants 12 (2019) 1–12. https://doi.org/10.1016/j.jarmap.2018.11.004.

[36] A.G. Kurmukov, Phytochemistry of medicinal plants, Medicinal Plants of Central Asia: Uzbekistan and

Kyrgyzstan 1 (2013) 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4.

[37] H. Semyari, P. Owlia, S. Farhadi, S.M. Tabrizi, Evaluation of antimicrobial effect of "Ammi visnaga" against oral streptococci, Journal of Microbiology and Antimicrobials 3 (2011) 126–129.

[38] A.M. Ghareeb, T.H. Zedan, L.A. Gharb, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF Ammi visnaga EXTRACTS AGAINST PATHOGENIC MICROORGANISMS, Iraqi Journal of Science 52 (2011) 30–36.

[39] J.N. Amin, A. Murad, A. Motasem, S.R. Ibrahem, J. Mona, A.M. Ayed, 2015. Phytochemical Screening khella, 7 (2015).

[40] R.M.S. Al-Hadhrami, R.M.S. Al Muniri, M.A. Hossain, Evaluation of antimicrobial and cytotoxic activities of polar solvent extracts from leaves of Ammi majus used by the Omanis, Pacific Science Review A: Natural Science and Engineering 18 (2016) 62–65. https://doi.org/10.1016/j.psra.2016.08.002.

[41] T. Kebede, E. Gadisa, A. Tufa, Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes, PLoS One 16 (2021) e0249253. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0249253 (accessed February 21, 2024).

[42] B. Prakash, A. Kumar, P.P. Singh, L.S. Songachan, Antimicrobial and antioxidant properties of phytochemicals: Current status and future perspective, Functional and Preservative Properties of Phytochemicals (2020) 1–45. https://www.sciencedirect.com/science/article/pii/B9780128185933000014 (accessed February 21, 2024).

[43] I.A. Wani, T. Ahmad, A. Khosla, Recent advances in anticancer and antimicrobial activity of silver nanoparticles synthesized using phytochemicals and organic polymers, Nanotechnology 32 (2021) 462001. https://iopscience.iop.org/article/10.1088/1361-6528/ac19d5/meta (accessed February 21, 2024).

[44] I. Zalegh, M. Akssira, M. Bourhia, F. Mellouki, N. Rhallabi, A.M. Salamatullah, M.S. Alkaltham, H. Khalil Alyahya, R.A. Mhand, A review on Cistus sp.: phytochemical and antimicrobial activities, Plants 10 (2021) 1214. https://www.mdpi.com/2223-7747/10/6/1214 (accessed February 21, 2024).

[45] K. Günaydın, F.B. Erim, Determination of khellin and visnagin in Ammi visnaga fruits by capillary electrophoresis, Journal of Chromatography A 954 (2002) 291–294. https://doi.org/10.1016/S0021-9673(02)00168-1.

[46] A. Zaher, R. Aslam, H.-S. Lee, A. Khafouri, M. Boufellous, A.A. Alrashdi, Y. El aoufir, H. Lgaz, M. Ouhssine, A combined computational & electrochemical exploration of the *Ammi visnaga L.* extract as a green corrosion inhibitor for carbon steel in HCl solution, Arabian Journal of Chemistry 15 (2022) 103573. https://doi.org/10.1016/j.arabjc.2021.103573.

[47] S.S.T. Ahmed, J.R. Fahim, K.A. Youssif, A.M. AboulMagd, M.N. Amin, U.R. Abdelmohsen, A.N.E. Hamed, Metabolomics of the secondary metabolites of Ammi visnaga L. roots (family Apiaceae) and evaluation of their biological potential, South African Journal of Botany 149 (2022) 860–869. https://doi.org/10.1016/j.sajb.2022.01.011.

[48] J.B. Harborne, L. King, Flavonoid Sulphates in the Umbelliferae, 4 (1976) 111–115.

[49] A. Activity, L. Apiaceae, R. Bencheraiet, H. Kherrab, A. Kabouche, O. De Substances, D. De Chimie, Flavonols and Antioxidant Activity of Ammi visnaga L . (Apiaceae), (2011).

[50] L. Giupponi, V. Leoni, R. Pavlovic, A. Giorgi, Influence of Altitude on Phytochemical Composition of Hemp Inflorescence: A Metabolomic Approach, Molecules 25 (2020) 1381. https://doi.org/10.3390/molecules25061381.

[51] S. Mollaei, M. Ebadi, S. Hazrati, B. Habibi, F. Gholami, M.M. Sourestani, Essential oil variation and antioxidant capacity of Mentha pulegium populations and their relation to ecological factors, Biochemical Systematics and Ecology 91 (2020) 104084. https://www.sciencedirect.com/science/article/pii/S0305197820301708 (accessed February 22, 2024).

[52] Y. Zou, H. Wang, J. Fang, H. Sun, X. Deng, J. Wang, Y. Deng, G. Chi, Isorhamnetin as a novel inhibitor of pneumolysin against *Streptococcus pneumoniae* infection *in vivo/in vitro*, Microbial Pathogenesis 185 (2023) 106382. https://doi.org/10.1016/j.micpath.2023.106382.

[53] C.E. Aruwa, S.O. Amoo, N. Koorbanally, T. Kudanga, Laccase-mediated modification of isorhamnetin improves antioxidant and antibacterial activities, Process Biochemistry 112 (2022) 53–61. https://doi.org/10.1016/j.procbio.2021.11.019.

[54] X. Tian, X. Peng, J. Lin, Y. Zhang, L. Zhan, J. Yin, R. Zhang, G. Zhao, Isorhamnetin Ameliorates Aspergillus fumigatus Keratitis by Reducing Fungal Load, Inhibiting Pattern-Recognition Receptors and Inflammatory Cytokines, Investigative Ophthalmology & Visual Science 62 (2021) 38. https://doi.org/10.1167/iovs.62.3.38.

[55] D.F. Rodrigues, N.H.P.B. Borges, C.E.S. Nogueira, J.F. Tavares, D.D.R. Arcanjo, H.M. Barreto, J.P. Siqueira-Junior, Modulation of Drug Resistance by Furanochromones in NorA Overexpressing Staphylococcus Aureus, Evidence-Based Complementary and Alternative Medicine 2022 (2022). https://doi.org/10.1155/2022/9244500.

[56] M. Acharjee, N. Zerin, T. Ishma, Md.R. Mahmud, In-vitro anti-bacterial activity of medicinal plants against

Urinary Tract Infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics, New Microbes and New Infections 51 (2023) 101076. https://doi.org/10.1016/j.nmni.2022.101076.

[57] P. Akbay, A.A. Basaran, U. Undeger, N. Basaran, In vitro immunomodulatory activity of flavonoid glycosides from Urtica dioica L., Phytotherapy Research 17 (2003) 34–37. https://doi.org/10.1002/ptr.1068.

[58] G.-T. Kim, N.K.S. Tran, E.-H. Choi, Y.-J. Song, J.-H. Song, S.-M. Shim, T.-S. Park, Immunomodulatory Efficacy of Standardized *Annona muricata* (Graviola) Leaf Extract via Activation of Mitogen-Activated Protein Kinase Pathways in RAW 264.7 Macrophages, Evidence-Based Complementary and Alternative Medicine 2016 (2016) e2905127. https://doi.org/10.1155/2016/2905127.

[59] H. Oh, D.-H. Kim, J.-H. Cho, Y.-C. Kim, Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from Equisetum arvense, Journal of Ethnopharmacology 95 (2004) 421–424. https://www.sciencedirect.com/science/article/pii/S0378874104003861 (accessed October 1, 2023).

[60] Y. Wang, C. Tang, H. Zhang, Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from Carthamus tinctorius L. on CCl4-induced oxidative liver injury in mice, Journal of Food and Drug Analysis 23 (2015) 310–317. https://www.sciencedirect.com/science/article/pii/S1021949814001343 (accessed October 1, 2023).

[61] Y. Zang, D. Zhang, C. Yu, C. Jin, K. Igarashi, Antioxidant and hepatoprotective activity of kaempferol 3-O-β-d- (2,6-di-O-α-l-rhamnopyranosyl)galactopyronoside against carbon tetrachloride-induced liver injury in mice, Food Sci Biotechnol 26 (2017) 1071–1076. https://doi.org/10.1007/s10068-017-0170-7.

[62] G. Seth, D. Guthrie, Letters to the Editor, The Journal of Laryngology & Otology 51 (1936) 138–139. https://doi.org/10.1017/S0022215100042249.

[63] W.H. Talib, A. Abuawad, S. Thiab, A. Alshweiat, A.I. Mahmod, Flavonoid-based nanomedicines to target tumor microenvironment, OpenNano 8 (2022) 100081. https://doi.org/10.1016/j.onano.2022.100081.

[64] M.R. Preetha Rani, P. Salin Raj, A. Nair, S. Ranjith, K. Rajankutty, K.G. Raghu, In vitro and in vivo studies reveal the beneficial effects of chlorogenic acid against ER stress mediated ER-phagy and associated apoptosis in the heart of diabetic rat, Chemico-Biological Interactions 351 (2022) 109755. https://doi.org/10.1016/j.cbi.2021.109755.