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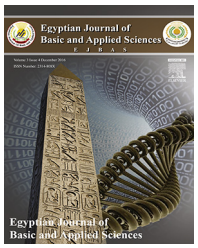

Full Length Article

Evaluation of antioxidant, antimicrobial and cytotoxic activities of seed crude extracts of *Ammi majus* grown in Oman

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ABSTRACT

Ammi majus has long been used as an herbal medicine in several countries for skin disorders, regulation of menstruation and for conditions in which diuresis is indicated. The present study was undertaken to evaluate the antioxidant, antimicrobial and cytotoxic activities of the crude extracts of locally growing *Ammi majus* (*A. majus*). Initially, methanol crude extract was prepared from powdered seed samples of *A. majus* by applying Soxhlet extraction method. Successive extracts were then derived by suspending methanol free crude extract in water followed by successive extraction with solvents of different polarities in an order of increasing polarity. The evaluations of antioxidant, antimicrobial and cytotoxic activities of seed crude extracts of *A. majus* were determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), agar disc diffusion and Artemia lethality methods, respectively. The highest antioxidant activity was observed in case of chloroform crude extract whereas the lowest activity was corresponding to methanol crude extract. However, the highest IC_{50} value was obtained with chloroform crude extract but the lowest result was observed in case of ethyl acetate crude extract. All crude extracts of *A. majus* displayed moderate antimicrobial activity against one Gram positive bacteria, *Staphylococcus aureus* (*S. aureus*), and three Gram negative bacteria, namely *Escherichia coli* (*E. coli*), *Haemophilus influenzae* (*H. influenzae*), and *Proteus spp* (*Proteus spp*), with growth inhibition zone of 0–15 mm. The cytotoxic activity results showed that all crude extracts of the seed had killed all the Artemia larvae at a concentration of 1000 µg/mL, being the highest for all extracts. However, regarding the overall magnitude of cytotoxic effect, chloroform crude extract showed the highest activity followed in a diminishing order by hexane, methanol, ethyl acetate, butanol and water crude extract. As such, the highest lethality was observed in case of chloroform crude extract where the lethal concentration (LC_{50}) was 49.16 µg/mL, whereas the lowest lethal concentration was seen with water crude extract where the LC_{50} was 652.38 µg/mL. The present study demonstrates that all seed crude extracts of *A. majus* have significant antioxidant, antimicrobial and cytotoxic effects. These results warrant follow up bioassay intended for isolation of the active compounds from this plant.

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1. Introduction

Plants as source of remedies are widely used for the treatment of almost 87% human diseases including bacterial and fungal infections, cancer and different disorders [1]. Most of the drugs commercially available currently for the treatment of curable and incurable diseases derive from plants [2]. About 3000 species reported worldwide have some medicinal use for curable and incurable diseases [3]. More than 80% of people in developed and undeveloped countries depend on herbal remedies for primary health care [3]. The Arabian Gulf is considered as the land of huge traditional medicine, and until recently herbal remedies have been the primary source of health care in urban areas in the Gulf region [4]. In Oman, there are about 1204 terrestrial plants, a large number of which is reported to be used in traditional medicine [5]. Terrestrial plants have been recognized since ancient times as potential sources of human pharmacopoeia and until today compounds derived from botanicals play an important role as a source of new chemical entities entering the market or clinical trials annually [1]. There is now an urgent need for novel and effective ingredient to remedy incurable diseases without any side effect.

Ammi majus L is a wild plant belonging to the family Apiaceae. It originates in Egypt and is widely distributed in Europe, Mediterranean and western Asia. It is now widely cultivated in India and other tropical countries due to its medicinal values [6]. The plant is an erect branching annual herb attaining a height of up to 1.5–2.0 metres. It has whitish tap-roots and an erect stem which is slender and glabrous with fine longitudinal striations. The leaves are alternate with long petiole and the flowers are whitish actinomorphic or zygomorphic, bisexual, pentamerous and bracteates [6,7]. Several major chemical compounds are present in this plant, especially coumarins and flavonoids, which possess important biological activities [8,9]. Other significant chemical compounds found at high concentration in this plant include marmesin, isoimperatorin, heraclenin, isopimpinellin, nonhydroxylic coumarins, ammirin and alloimperatorin, khellin, visnagin, acetylated flavonoids besides essential oils [8,9]. This plant is mainly used for regulating menstruation and as a diuretic [6,8,9]. It has recently been used in several countries for treating leprosy, kidney stones and urinary tract infections [6,8,9]. Traditionally, Omanis have been widely using it for the treatment of skin disorders (leukoderma), the regulation of menstruation as well as a diuretic [6,8,9]. One study was conducted on the cytotoxic and biological activities of the crude extract of this plant [6]; however, no wide scale work has been done on the crude extracts of the plant by different polar solvents, especially in Oman. Therefore, the present work was designed to evaluate the antioxidant, antimicrobial and cytotoxic activities of seed crude extracts of *A. majus*, native to the Sultanate of Oman by applying methods of DPPH, agar disc diffusion and Artemia lethality, respectively. The results of this study would furnish a baseline information on this plant species that could be used for the development of a new medicine of therapeutic importance.

2. Materials and methods

2.1. Chemicals and instrument

Different solvents, reagents and standards were used in this study. Most of solvents, especially dimethyl sulphoxide (DMSO), dichloromethane (DCM), acetone, hexane, chloroform, methanol and butanol were purchased from Fisher Scientific Company, UK. Sodium sulphate (Na_2SO_4) was obtained from Scharlau, European Union. Filter paper used as disc was purchased from Whatman (GE Healthcare Company, China, Catalogue 1001090). Artemia cysts, amoxicillin, sodium chloride, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), petri dishes and other chemicals were obtained from Sigma-Aldrich Company, USA. The UV spectroscopy system used to measure the absorbance of different concentrations of crude samples was procured from Japan (Model 1800 Shimadzu spectrophotometer, Japan).

2.2. Bacterial strains

Four bacterial strains, namely *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Haemophilus influenzae* (*H. influenza*), and *Proteus spp* (*Proteus spp*) used in this study were collected from Nizwa Hospital, Nizwa, Sultanate of Oman during the month of January 2015.

2.3. Sample collection

Selected seed samples from *A. majus* of different sizes were collected from the Northern part of Salalah, Sultanate of Oman during the month of September, 2014. The collected samples were transported to the Natural Products Lab, University of Nizwa for further processing. Morphological features and database of this plant are present in the website (http://www.uicnmed.org/nabp/database/HTM/PDF/p7.pdf).

2.4. Crude extract preparation

Collected seed samples were dried at room temperature under shade. For solvent extraction, the dried samples (300 g) were coarsely powdered to 60 mesh size using a blender. A powdered sample (142.00 g) was extracted with methanol (700 mL) using Soxhlet extraction method for 20 hours and then evaporated using a rotary evaporator under reduced pressure at 24 °C to yield crude extract (13.09 g, 9.21%). The residual crude extract (9.61 g) was suspended in 100 mL of water and partitioned successively with hexane, chloroform, ethyl acetate and butanol solvents, respectively (twice 30 mL and 20 mL of each solvent). All solvents were evaporated to dryness to give crude extracts of hexane (1.74 g, yield 18.15%), chloroform (1.97 g, yield 18.62%), ethyl acetate (0.472 g, yield 4.91%), butanol (1.69 g, yield 17.58%) and water (4.16 g, yield 43.28%), respectively [10].

2.5. Free radical scavenging activity by DPPH method

The antioxidant activity of the crude extracts of the seeds of *A. majus* was determined by the free radical scavenging by DPPH

method as described by Blois [11] with modification. Five different concentrations, 12.5, 25, 50, 100 and 200 μg/mL were used for each crude extract (hexane, chloroform, ethyl acetate, butanol, methanol and water). Gallic acid was used as a standard. Each concentration of each crude extract (4 mL) was placed in a clean test tube. DPPH solution (1 mL) was added to the same test tube and shaken vigorously manually. After adding DPPH solution, all the test tubes were kept at room temperature in a dark place for 45 min. After incubation, the absorbance was measured at 517 nm by a UV spectrophotometer. The control was prepared by the same procedure excluding any plant crude extract. The scavenging activity of each concentration crude extract was estimated based on the percentage of inhibition of DPPH using the following formula,

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

2.6. *Antimicrobial activity*

All seed crude extracts from differently polar solvents had their antimicrobial activity determined by a slightly modified agar disc diffusion method [12]. Four bacterial strains *S. aureus, E. coli, H. influenzae,* and *Proteus spp* were selected for this investigation. Amoxicillin was used as a standard, with DMSO as a control. Each crude extract was subjected to serial dilution using dimethyl sulphoxide (DMSO) to give 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL solutions. Sterile filter paper discs (5 mm diameter) were impregnated with each concentration of the crude extracts of *A. majus* and placed on the inoculated agar. The plates were incubated at 37 °C for 24 h. The antimicrobial activity was measured as the diameter of zone of inhibition against the four tested bacterial strains. Each method in this experiment was replicated three times.

2.7. *Cytotoxic activity assay*

Artemia lethality method [13] was used to investigate the cytotoxic activity of different seed crude extracts from *A. majus*. *In vitro* Artemia lethality test was carried out using Artemia cysts. Artemia cysts were hatched in covered chamber of duo compartment plastic container containing 3.8% NaCl solution. In one side of the compartment, a light source was placed in order to attract the nauplii. After hatching, the active nauplii were separated from the cysts and used for cytotoxicity assessment. DMSO was used as a control. Then 4 mg of each seed crude extract was measured accurately in a vial and dissolved in 4 mL of dimethyl sulphoxide (DMSO). Four test concentrations from each stock, 1000, 500, 100 and 10 μg/mL, were prepared in DMSO. From each test solution, 50 μl were added to pre-marked test tubes containing 5 mL NaCl solution. Ten Artemia nauplii were then placed in each test solution of the different crude extracts. As a control for each test concentration, the same volume of DMSO plus NaCl solution was completed to 5 mL with water. After 24 hours of incubation, the numbers of surviving nauplii in each test tube were counted using a magnifying glass. The percentage of mortality of brine shrimps was calculated for each concentration of sample.

2.8. *Statistical analysis*

All measurements were carried out in triplicate, and the results were presented as mean standard deviation (SD). The concentration that killed 50% of the nauplii (LC_{50}) was determined for each crude extract by Statistical Analysis Systems (SAS) computer programme (JMP version 8.0) [14]. It was determined by plotting a graph of percentage mortality of Artemia larvae against the logarithmic concentrations of extracts tested. The LC_{50} values were determined directly from probit analysis or calculated by substituting 50% for "y" into the curve equation in the graph.

3. Results and discussion

The selected seed samples were harvested according to our study guidelines and then dried at room temperature under shade. The dried powdered seeds of *A. majus* were extracted with methanol for 20 h. and then methanol was evaporated at 24 °C under reduced pressure. The methanol crude extract was fractionated with different solvents in an order of increasing polarity.

3.1. *Antioxidant activity*

Plant antioxidants have a remarkable capability to remove free radicals from the human body where they are responsible for some incurable diseases like cancer, diabetes, stroke, etc. Free radicals can accelerate chain reactions resulting in cell damage in the body and therefore plant antioxidants could play a significant role in protecting the body against these chain reactions. Free radical scavenging method (DPPH) was used for the determination of antioxidant activity of seed crude extracts of *A. majus*. In this method, phenols and flavonoids in the seed crude extracts convert reactive non stable DPPH free radical into stable non-reactive DPPH-H form by donating electron or hydrogen radical [15,16]. The scavenging activity of plant crude extracts demonstrated by DPPH-H was strongly comparable to the standard antioxidant gallic acid. The antioxidant activity obtained was due to the presence of phenols and flavonoids that are considered as secondary metabolites of plant origin [15,16]. All phenols and flavonoids have the ability to donate electron/hydrogen that results in converting highly reactive free radicals to non-reactive stable molecules. Should a significant content of total phenols and flavonoids be found in the crude extracts, this would strengthen the evidence for antioxidant activity. Our results showed that different concentrations of various seed crude extracts of *A. majus* had exhibited strong free radical scavenging activity (Table 1). The strong free radical scavenging activity in different crude extracts might be due to the high quantity of phenols and flavonoids present. This indicates that the seed crude extracts of *A. majus* possess a good potential source for natural antioxidants to prevent free radical oxidative damage. The crude extracts of the seeds were evaluated for their antioxidant activity by the free radical scavenging (DPPH) method with modification [11]. Among six seed crude extracts, the highest antioxidant activity result was observed in chloroform crude

Table 1 – Antioxidant activity of different polarities seed crude extracts of *A. majus*.

Seed crude extracts	Conc µg/mL	Log C	% Inhibition	IC_{50} µg/mL
Hexane	12.5	1.096	55.91 ± 0.10	
	25	1.397	47.92 ± 0.17	
	50	1.698	45.36 ± 0.31	123.651
	100	2	43.45 ± 0.16	
	200	2.301	32.26 ± 0.12	
Ethyl acetate	12.5	1.096	53.99 ± 0.18	
	25	1.397	47.92 ± 0.15	
	50	1.698	44.08 ± 0.33	236.246
	100	2	34.82 ± 0.54	
	200	2.301	22.68 ± 0.87	
Chloroform	12.5	1.096	56.23 ± 0.23	
	25	1.397	52.07 ± 0.24	55.97
	50	1.698	67.73 ± 0.10	
	100	2	50.79 ± 0.75	
	200	2.301	38.01 ± 0.08	
Butanol	12.5	1.096	48.88 ± 0.18	
	25	1.397	51.75 ± 0.10	
	50	1.698	46.96 ± 0.16	139.958
	100	2	43.45 ± 0.14	
	200	2.301	28.11 ± 0.19	
Methanol	12.5	1.096	48.88 ± 0.18	
	25	1.397	46.96 ± 0.25	
	50	1.698	50.79 ± 0.34	80.164
	100	2	41.21 ± 0.73	
	200	2.301	49.84 ± 0.29	
Water	12.5	1.096	54.95 ± 0.12	
	25	1.397	56.86 ± 0.17	
	50	1.698	55.59 ± 0.39	55.207
	100	2	53.35 ± 0.56	
	200	2.301	43.13 ± 0.87	

Values are given as mean ± SD ($n = 3$).

Table 2 – Antimicrobial activity of different polarities seed crude extracts of *A. majus*.

Seed crude extracts	Conc mg/mL	*H. influenza* (mm)	*S. aureus* (mm)	*E. coli* (mm)	*Proteus*
Methanol	Control	nd	10 ± 0.12	9 ± 0.17	6 ± 0.13
	2	nd	8 ± 0.17	11 ± 0.10	9 ± 0.16
	1	nd	7 ± 0.26	15 ± 0.17	7 ± 0.20
	0.5	nd	6 ± 0.15	12 ± 0.32	7 ± 0.54
	0.25	nd	7 ± 0.72	9 ± 0.09	9 ± 0.32
Hexane	Control	7 ± 0.10	10 ± 0.17	25 ± 0.09	13 ± 0.15
	2	8 ± 0.41	11 ± 0.13	10 ± 0.08	9 ± 0.17
	1	8 ± 0.18	13 ± 0.12	12 ± 0.12	9 ± 0.12
	0.5	7 ± 0.10	12 ± 0.15	9 ± 0.19	10 ± 0.12
	0.25	7 ± 0.11	14 ± 0.17	8 ± 0.13	10 ± 0.10
Chloroform	Control	7 ± 0.72	7 ± 0.25	24 ± 0.44	7 ± 0.17
	2	9 ± 0.16	9 ± 0.20	12 ± 0.10	8 ± 0.18
	1	8 ± 0.23	8 ± 0.42	10 ± 0.11	8 ± 0.12
	0.5	8 ± 0.53	10 ± 0.10	10 ± 0.17	8 ± 0.15
	0.25	7 ± 0.16	10 ± 0.09	8 ± 0.12	7 ± 0.12
Ethyl acetate	Control	6 ± 0.18	9 ± 0.13	24 ± 0.10	nd
	2	7 ± 0.09	8 ± 0.17	10 ± 0.42	nd
	1	6 ± 0.12	8 ± 0.18	10 ± 0.45	nd
	0.5	7 ± 0.14	9 ± 0.12	12 ± 0.23	nd
	0.25	7 ± 0.10	9 ± 0.15	10 ± 0.34	nd
Butanol	Control	7 ± 0.23	10 ± 0.12	23 ± 0.56	6 ± 0.10
	2	8 ± 0.43	9 ± 0.15	7 ± 0.23	9 ± 0.18
	1	8 ± 0.34	10 ± 0.10	7 ± 0.19	9 ± 0.22
	0.5	6 ± 0.67	14 ± 0.11	10 ± 0.10	8 ± 0.27
	0.25	7 ± 0.27	12 ± 0.43	10 ± 0.12	9 ± 0.13
Water	Control	9 ± 0.10	11 ± 0.35	22 ± 0.15	8 ± 0.09
	2	9 ± 0.19	7 ± 0.44	9 ± 0.10	8 ± 0.15
	1	7 ± 0.13	8 ± 0.17	8 ± 0.12	9 ± 0.18
	0.5	8 ± 0.11	8 ± 0.08	9 ± 0.18	8 ± 0.12
	0.25	8 ± 0.56	9 ± 0.12	10 ± 0.14	8 ± 0.19

nd, not detectable inhibition zone. Values are given as mean ± SD (n = 3).

extract whereas the lowest was in methanol and as such the antioxidant activity was in the order of chloroform > hexane>ethyl acetate > water>butanol > methanol crude extract (Table 1). The IC_{50} values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested crude extracts and the ordinate the average percentage of scavenging capacity. The concentrations of sample required to scavenge 50% of DPPH (IC_{50}) were determined. The lowest IC_{50} value was obtained with chloroform seed crude extract and the highest with ethyl acetate seed crude extract (Table 1). The lowest IC_{50} observed for chloroform crude extract indicates that this extract has the strongest ability to act as DPPH scavenger as observed in previous studies [6].

3.2. *Antimicrobial activity*

The disc diffusion assay is one of the most popular, inexpensive, and easiest methods for determining antibacterial activity against pathogenic bacterial strains, but not without limitations. A significant zone of inhibition was displayed by the plant crude extract against all the tested culture bacterial strains. Most antimicrobially active components that have been identified are not water soluble and thus organic solvent extracts proved to be more potent [17]. Therefore, it is impossible to determine the antibacterial activity of non-polar compounds accurately by the disc diffusion method; therefore, the antimicrobial activity of the crude extracts of the seed was evaluated by a modified disc diffusion method [12]. The results of antimicrobial activity of different crude extracts of seed of *A. majus* are presented in Table 2. The seed crude extracts of *A. majus* displayed significant antimicrobial activity against all four cultured bacterial strains (Gram positive and Gram negative) at all employed concentrations with a zone of inhibition in the range of 0–15 mm. However, methanol and ethyl acetate seed crude extracts did not show any activity against *H. influenzae* and *Proteus spp* bacterial strains at any of the employed concentration. Notably, most of the crude extracts obtained from seed powder displayed significant zone of inhibition against *S. aureus* and *E. coli* bacterial strains at all concentrations (Table 2). The results of this present study are in strong agreement with what has been reported earlier on antimicrobial activity of different crude extracts of *A. majus* [6–9].

3.3. *Cytotoxicity activity*

Artemia lethality method is the simplest technique for determining biologically active compounds having cytotoxic activity in the crude extract. This is a well-known method for the determination of different important pharmacological activities

Table 3 – Percentage of mortality and lethal concentration (LC_{50}) of different polarities seed crude extracts of *A. majus*.

Seed crude extracts	Conc µg/mL	Mortality (%)	LC_{50} (µg/mL)
Hexane	1000	100	
	500	100	
	100	60	50.818
	10	20	
	Control	0	
Ethyl acetate	1000	100	
	500	10	275.021
	100	40	
	10	20	
	Control	0	
Chloroform	1000	100	
	500	40	49.168
	100	30	
	10	20	
	Control	0	
Butanol	1000	90	
	500	20	312.982
	100	10	
	10	10	
	Control	0	
Methanol	1000	100	
	500	40	191.52
	100	50	
	10	20	
	Control	0	
Water	1000	100	
	500	20	652.382
	100	10	
	10	10	
	Control	0	

Values are given as mean ± SD ($n = 3$).

like enzyme inhibition, ion channel interference and cytotoxic activity [18,19]. In the present study, the seed crude extracts showed a rate of mortality in a concentration-dependent manner. The LC_{50} obtained for the extract was low, indicating that the extract is quite potent. All crude extracts obtained from the seed of *A. majus* exhibited potent cytotoxicity for Artemia; however, there was no earlier report available on cytotoxic activity of the selected seed crude extracts or its isolated pure compounds. Therefore, the effect of all crude extracts of *A. majus* warrants evaluation to reveal potential anticancer agents besides its wide use to regulate menstruation and as a diuretic [4–8]. The different polarities seed crude extracts of *A. majus* were evaluated for their cytotoxic activity by the simple Artemia lethality method reported by Weli et al. [13]. The cytotoxic activity results showed that all prepared crude extracts from seed have killed all the larvae a concentration of 1000 µg/mL, being the highest for all extracts; however, the mortality decreased with decreasing concentration of seed crude extracts. The mean percentage mortality of larvae exposed to different crude extracts of seed of *A. majus* is shown in Table 3. The lethal concentration (LC_{50}) of the crude extract samples after exposure for 24 hours was obtained by a plot of percentage of mortalities against the logarithm of the sample concentration (toxicant concentration). The highest lethal concentration (LC_{50}) of seed crude extracts was shown in chloroform crude extract having a value of 49.168 µg/mL whereas the lowest (LC_{50}) was in water crude extract, having a value of 652.38 µg/mL (Table 3). The chloroform seed crude extracts was most active, exhibiting LC_{50} value of 49.168 µg/mL. However, butanol crude extract was less active, exhibiting LC_{50} value of 312.982 µg/mL (Table 3). These findings are in agreement with the reported pattern by Balla et al. [7] on the cytotoxic activity of crude extracts of *A. majus*. Furthermore, other biological studies of different polarities seed crude extracts of *A. majus* are needed to confirm the activity.

4. Conclusion

Our observations revealed that the chloroform crude extract of *A. majus* showed significant antioxidant activity among the six extracts by the DPPH method which indicates the presence of polyphenolic compounds. All seed crude extracts at almost all concentration were found to inhibit the growth of several microbes in agar media with significant inhibition shown against *E. coli* and *S. aureus*. However, the chloroform and hexane crude extracts showed very good cytotoxic activity against Artemia cysts. The mortality rate was linearly correlated with the increase in concentrations. From this study, it is concluded that the screened plant has a potential antibacterial activity compared to the antibiotics which are commonly used. So, there is a possibility of generating new drugs from cheap and widely available source for the treatment of diseases.

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