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Evaluation of antimicrobial and cytotoxic activities of polar solvent extracts from leaves of *Ammi majus* used by the Omanis

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ABSTRACT

Ammi majus (*A. majus*) is an important medicinal plant traditionally used in Oman for the treatment of mouth ulcers. The objective of this study was to determine the antimicrobial and cytotoxic activities of different leaf extracts of *A. majus* using an established protocol. Different extracts were prepared using increasingly polar solvents. The antimicrobial activity of the extracts was determined using the disc diffusion method, and the cytotoxic activity of the extracts was assessed by the brine shrimp lethality bioassay. Exposure of the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*, Haemophilus influenzae and *Proteus* spp. bacterial strains to the different extracts revealed potential antimicrobial activity measured by zones of inhibition in the range of 0–20 mm. The cytotoxicity results showed an incremental rise in shrimp larvae mortality depending on the extract concentration, reaching 100% mortality at a concentration of 1000 μg/ml for all extracts. The methanol extract had the lowest of all LC_{50} values at 45.75 μg/ml. The LC_{50} values in ascending order for the chloroform, hexane, ethyl acetate, water and butanol extracts are 179.41, 288.31, 305.19, 417.94 and 570.02 μg/ml, respectively. In conclusion, *in vitro* and *in vivo* studies of the isolated leaf extracts are needed to determine the therapeutic potential of this plant.

1. Introduction

Plants are a valuable source of secondary metabolites, which are used as pharmaceuticals, flavours, fragrances, colours, biopesticides and food additives. Indeed, plants are a natural gift to human beings to promote health [1]. More than 80% of the world's population relies on plant-based medicine for primary healthcare, a system that developed over time by dynamic interactions between people and their environment [2]. Currently, plants and their products are important in traditional, botanical and pharmaceutical medicine [3].

Ammi majus belongs to the family *Umbellifereae* (*Apiaceae*) and is indigenous to Egypt, where it grows well in the Nile Delta and Valley, especially in Behira and Fayoom. These plants are found in oases in the Mediterranean region, West and North Africa, the Middle East, Europe, in some regions of Iran and in the mountains of Kohaz [2–6]. The plant is also available in Oman, where it is locally known as Khillah, Killah shaytani or Killah bariah [2–7]. *A. majus* is a branching annual plant 1.5–2.0 m high with whitish tap-roots and slender glabrous stems with fine longitudinal striations. Its leaves are alternate with a long petiole, and its flowers are whitish actinomorphic or zygomorphic, bisexual, pentamerous and bracteates [8]. The plant contains different bioactive compounds such as coumarins and flavonoids with different important biological activities [2]. Recently, this plant was shown to contain even more compounds such as marmesin, isoimperatorin, heraclenin, isopimpinellin, nonhydroxylic coumarins, ammirin, alloimperatorin, khellin, visnagin, acetylated flavonoids and essential oils [5,6,9,10]. Not only these compounds but also β-sitosterol, 6-hydroxy-7-methoxy-4 methyl coumarin and 6-hydroxy-7-methoxy coumarin were isolated from *A. majus* [9]. Other than the leaves, which are the object of our study, ripe fruit is used traditionally for treatment of skin disorders, psoriasis and vitiligo [7]; additionally, it is used for regulating

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menstruation and as a diuretic, as well as in the treatment of leprosy, kidney stones and urinary tract infections [2,3]. Chinese herbal medicine specifically uses the plant as a diuretic, as a carminative and to treat angina pectoris and asthma [3–11]. The Egyptians used it for leucoderma, melasma and pityriasis alba [11,12]. Currently, the plant is cultivated across India and in other countries because of its medicinal importance [9]. In Oman, *A. majus* is traditionally used for treatment of mouth ulcers [12]. Therefore, our objective was to determine the antimicrobial and cytotoxic activities of different leaf extracts from *A. majus* using an established protocol.

2. Materials and methods

2.1. Materials

Methanol, hexane and acetone were obtained from AnalaR, Normapur Company, India. Chloroform was obtained from Daejung Company, Korea. Ethyl acetate was obtained from Carbon Group Company, UK. Butanol and amoxicillin were obtained from Sigma Aldrich Company, Germany. Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific Company, UK. Filter paper was obtained from Whatman. Other reagents used in this study were obtained from E. Merck, Germany. The instruments used in this experiment were a rotary evaporator obtained from Yamato Company (Japan), an incubator obtained from VWR Company (UK), and an oven obtained from Memmert.

2.2. Microorganism

The bacterial strains *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, *Haemophilus influenza (H. influenza)* and *Proteus* spp. were collected from Nizwa Hospital, Nizwa, on 23 January, 2015. All the collected bacterial strains were stored at −20 °C.

2.3. Sample collection

Samples of *A. majus* were collected from Salalah, Dhofar (South Tawi Ettair City) during the month of November 2014 at around 9 am and were identified by Professor Dr. Mizanur Rahman, Department of Botany, University of Dhaka, Bangladesh. The specimen was deposited at the Bangladesh National Herbarium (BNH). The healthy leaves were separated from the stems and packed in a polyethylene bag for transportation to the Natural Product Lab, University of Nizwa, Nizwa, Sultanate of Oman, for drying and extraction.

2.4. Extraction

The leaves were washed with water and dried at room temperature under shade for three days, followed by grinding into powder using a blender. The powdered sample (42.33 g) was subjected to hot extraction with methanol (300 ml) for 20 h, and the methanol was subsequently evaporated using a rotary evaporator at 24 °C under reduced pressure. After evaporation, the extract (19.61 g) was suspended in 200 ml of water and shaken to dissolve. The mixture was transferred to a separatory funnel and extracted successively with hexane, chloroform, ethyl acetate and butanol. The solvents were then evaporated from their respective liquor vehicle to give extracts of hexane (3.49 g), chloroform (3.58 g), ethyl acetate (0.044 g) and butanol (3.38 g). Finally, a water extract was obtained after evaporation of the water, yielding 8.16 g [13].

2.5. Antimicrobial activity

All collected fractions, i.e., hexane, ethyl acetate, chloroform, butanol, methanol and water extracts of *A. majus*, were tested for antimicrobial activity using the disc diffusion method [14,15]. In this study, four microorganisms were used. Standard amoxicillin (30 μg/ml) and blank filter paper discs (6 mm, diameter) were used as positive and negative controls, respectively. Each extract (10 mg) and a parallel standard were dissolved each in 10 ml of DMSO to form stock solutions corresponding to 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml. Each concentration was tested for antimicrobial activity against *S. aureus*, *E. coli*, *H. influenza* and *Proteus* spp. on agar plates using the disc diffusion method [14,15]. For the test, filter paper discs were soaked in an extract and placed on the agar plate for incubation at 37 °C for 24 h. The zones of inhibition were measured for each of the tested bacteria [14,15].

2.6. Cytotoxic activity

The brine shrimp lethality bioassay was used to measure cytotoxicity [15]. A stock concentration of 1000 μg/ml in DMSO was prepared for each extract and subsequently used to make dilutions of 500 μg/ml, 100 μg/ml and 10 μg/ml. Brine shrimp eggs were kept over 24 h to hatch in sea water inside a covered chamber of a duo compartment plastic container. Active nauplii were separated from the eggs and used for the cytotoxicity bioassay. Nauplii viability was measured in each extract by adding 50 μl of each dilution to premarked test tubes containing 5 ml of sea water and 10 nauplii. After 24 h, the surviving nauplii in each tube were counted, and the lethality percentage was calculated for each dilution of each extract.

2.7. Data analysis

Data were analysed using Microsoft Excel 2010 to generate graphs and linear regression equations to calculate the LC_{50}.

3. Results

The disease-free leaf samples were collected from Salalah and extracted with methanol for preparation of different extracts as described above. These extracts were used for the determination of antimicrobial activity by the disc diffusion method and for cytotoxicity by the brine shrimp lethality bioassay [13–15].

3.1. Antimicrobial activity

For antimicrobial activity, the different extracts of *A. majus* were tested against one Gram-positive bacterium (*S. aureus*) and three Gram-negative bacteria (*E. coli*, *H. influenza* and *Proteus* spp.) using the disc diffusion method with amoxicillin as the standard (Table 1). All extracts of *A. majus* showed moderate antibacterial activity against all bacterial strains at all concentrations with an inhibition zone range of 0–20 mm, except for *H. influenza* where all extracts, excluding that of butanol, failed to inhibit growth for all concentrations. Nonetheless, the butanol extract had weak activity with a 0–7 mm zone of inhibition against *H. influenza* at all concentrations.

3.2. Cytotoxic activity

To measure the cytotoxic activity, the mean percentage mortality and LC_{50} of shrimp larvae exposed to different extracts of *A. majus* are shown in Table 2, as determined by the brine shrimp lethality assay [15]. All of the plant extracts killed every shrimp

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