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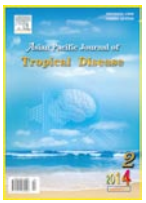

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Evaluation of different extraction methods on antimicrobial potency of *Adenium obesum* stem against food borne pathogenic bacterial strains in Oman

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ABSTRACT

Objective: To determine and compare the effect on antimicrobial potency of crude stems extract of *Adenium obesum* (*A. obesum*) by Soxhlet and maceration extraction methods.

Methods: The crude extracts were prepared from the coarse samples of stems with methanol by using Soxhlet and maceration extraction methods. Both the crude extracts from two extraction methods were dissolved in water and successively extracted by different polarities solvents with increasing polarities. *In vitro* antimicrobial potency of different polarities crude extracts obtained from Soxhlet and maceration methods was determined by agar gel diffusion method against different food borne pathogenic bacterial strains.

Results: The results for antimicrobial potency of different crude extracts were almost similar by Soxhlet and maceration and methods. The average range of inhibition potency of different polarities crude extracts was 0%–17% by Soxhlet method and inhibition potency 0%–24% by maceration method.

Conclusions: These results obtained from *in vitro* approach give promising basic information about this plant as well as some potential crude extracts can be used for the treatment of infectious diseases.

1. Introduction

Adenium obesum (*A. obesum*) is a tropical medicinal plant found all over of Oman. It belongs to the family Apocynaceae[1]. Various species are found all over the world. Among them two species such as *Beaumontia* and *A. obesum* are found in Oman. Both the *A. obesum* species in Oman are considered to be medicinal plants[1]. The species, belonging to this family, produce milky sap[2]. The milky sap contains toxic chemical compounds that can cause skin irritation[2]. These plants typically grow well in rocky and sandy soils[2]. Nowadays, it is cultivated in Saharan Africa, Sudan, Kenya, Senegal and Swaziland due to its medicinal values[3]. Some other rare species of *A. obesum* are found in Yemen and the Arabian Peninsula. The flower and fruits of these species completely depends on the environmental conditions such as rainfall, temperature, *etc*. This plant grows very slow therefore they are called a long–lived plant[4]. The plant is considered as a small tree, it grows up to two to four meters in height. Some of these plants have a fleshy taproot, and a stem swollen at its base up to one meter in diameter. The bark is pale greyish–green, grey, brown, smooth, with sticky, clear or white latex; branchlets glabrescent, pubescent at apex. Leaves are arranged spirally, clustered at the end of branchlets[4–6].

The different polarity crude extracts of *A. obesum*

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contains alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids[7]. It is known as a medicinal plant used in different countries around the world as poison on arrows[4]. Different ethnic communities in Oman and other countries use the whole plant for the treatment of a variety of ailments including venereal diseases[5,7]. The water extracts of crude root and bark are used to prepare lotion for the treatment of different skin diseases and to kill lice[5-9]. The latex of this plant is a very good medicine for the recovery of decaying teeth and septic wounds[5-9]. Traditionally, in Somalia, it is used for nasal drops[10]. In Kenya, latex is used to kill lice and stems powder is used for camels and cattle to kill skin parasites[5-9]. The bark of *A. obesum* is used as an abortifacient[11-13]. Therapeutically, in Nigeria, the whole plant has been used for its antiplasmodial, anti-trypanosomal and anti-leishmanial activities[12-15]. Traditionally, in Omani ethnic community, it is used for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pains and joint pains[2-5]. The literature survey indicated that there is no research that has been done on the Omani *A. obesum*. The present study was designed for the first time in Oman to evaluate and compare the antimicrobial potential of crude stems extract of *A. obesum* by different extraction methods, *i.e.* Soxhlet and maceration extraction methods.

2. Materials and methods

2.1. Chemicals and materials

Different polarities of solvents *e.g.*, hexane, chloroform, ethyl acetate and acetic anhydride were used in this study which were purchased from Scharlau, European Union. Methanol was purchased from Emsure, Germany. Dimethyl sulphoxide (DMSO), amoxicillin and agar gel were collected from Sigma-Aldrich Chemical Company, UK. Filter papers were used as a disc from Whatman, GE Healthcare Companies, China. The bacterial strains *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Vulgaris* used in this present study were collected on March 14, 2014 from Nizwa Hospital, Nizwa, Sultanate of Oman. The UV-visible spectroscopy (UV-1800 Shimadzu spectrophotometer, Japan) was used for measuring the absorbance of different polarities crude extracts of *A. obesum*.

2.2. Samples

The fresh stem samples were collected from Salalah on January 29, 2014. Salalah is in the southern part of Oman and its environmental conditions are completely different from other regions of Oman. This environment is suitable for the well growth of *A. obesum*. After collection of the stems, identification of this plant sample was done by the reference data which is available in the website. Instantly, the stems were separated from the barks by sharp knife. Then the stem samples were dried at room temperature.

2.3. Crude extracts preparation by Soxhlet method

The dried samples were cut into small pieces and powder by ball grinding machine. The powder samples (150 g) were extracted with polar methanol solvent (250 mL) by using Soxhlet extraction for 3 d. After complete extraction the methanol solvent was evaporated to obtain the methanol crude extract (12.750 g). Methanol crude extract (10 g) was dissolved in water (120 mL) and the whole water solution was transferred into a separatory funnel and extracted with different polarities of solvents with increasing polarities. First, hexane (30 mL) was used for the extraction and repeated twice with the same solvent. The other solvents with increasing polarities were used in twice for complete extraction. After extraction, the solvents was removed from the extracts and gave the hexane (1.732 g), chloroform (2.670 g), ethyl acetate (2.543 g), butanol (1.045 g) and water (1.620 g) crude extracts. All the crude extracts from the stems of *A. obesum* by Soxhlet extractor were used for the evaluation of antimicrobial study against four food borne pathogenic bacteria.

2.4. Preparation of crude extracts by maceration method

The same amount of powder samples of *A. obesum* (150 g) was soaked with methanol (250 mL) by using maceration method technique for 7 d. After 7 d, the methanol solvent was filtered by Bruckner funnel. The filtrate was evaporated to give the methanol crude extract (10.220 g). Methanol crude extract (10 g) was dissolved in water (120 mL) and the water solution was transferred into a separatory funnel and extracted with different polarities of solvents with increasing polarities. All the polar and non-polar solvents were used for the extraction and repeated twice. After removed the solvent from the crude extracts to

obtain hexane (1.412 g), chloroform (2.320 g), ethyl acetate (2.119 g), butanol (1.215 g) and water (1.700 g) crude extracts. All the crude extracts from the stems of *A. obesum* by maceration method were used for evaluation of antimicrobial study against food borne pathogenic bacteria.

2.5. Determination of antimicrobial potency by agar diffusion method

The antibacterial potential test of crude extracts of *A. obesum* stem by both methods were carried out using the agar disc diffusion method[16]. Four concentrations 2.00, 1.00, 0.50, and 0.25 mg/mL of crude stem extracts of *A. obesum* by both methods were prepared by DMSO solvent. The preparation of negative and positive controls were also used the same solvent. Inhibition growth were measured and compared with the antibiotics amoxicillin. Each prepared concentration of different crude extracts of *A. obesum* was tested for its antimicrobial potency against one Gram-positive bacterium (*S. aureus*) and three Gram-negative bacteria (*E. coli, Valgurais* and *P. aeruginosa*) on nutrient agar plates using disc diffusion method. Normal Whatman filter paper was cut into 6 mm diameter by punch machine. All the filter paper were used as discs for antimicrobial study. First, the disc was impregnated with methanol crude extract and its fractions of *A. obesum* and then placed them on the inoculated agar plate. When the plates were ready then the plates were incubated at 37 °C for 24 h. After the incubation, the growth of inhibition was measured by scale against the applied tested bacteria. Each method in this experiment was replicated three times.

3. Results

3.1. Extraction

The samples of *A. obesum* were collected from Salalah region and then started the process of extraction. The powder samples were extracted with methanol through Soxhlet and maceration methods. Same amount of powder samples and solvent were used for both extraction methods. The maximum amount of crude extracts was isolated by Soxhlet extractor rather than maceration methods (Table 1).

Table 1

Amount of crude extracts isolated from the stems of *A. obesum* by Soxhlet and maceration methods.

Extracts	Soxhlet (g)	Maceration (g)
Hexane	1.732	1.412
Chloroform	2.670	2.320
Ethyl acetate	2.543	2.119
Butanol	1.045	1.215
Methanol	12.750	10.220
Water	1.620	1.700

3.2. Antimicrobial potency of different crude extracts of A. obesum by different extraction methods

The antibacterial potency of different polarities crude extracts from the stem of *A. obesum* against four food borne pathogenic bacterial strains was evaluated. The presence or absence of inhibition zones was determined quantitatively through inhibition diameter. The different polarities crude stem extracts of *A. obesum* were exhibited different antibacterial potency against one Gram-positive (*S. aureus*) and three Gram-negative (*E. coli*, *P. aeruginosa* and *Valgurais*) bacterial strains at four different concentrations of 2.00, 1.00, 0.50 and 0.25 mg/mL. The crude extracts were dissolved with DMSO. The amoxicillin was used as a standard at 1 mg/mL with DMSO for this study. The different polarities crude extracts of *A. obesum* showed different antibacterial potency by Soxhlet method against four food borne pathogenic bacteria at all four concentrations. However, the crude extracts from maceration method inhibit almost similar antibacterial potency against all employed bacterial strains at all four concentrations (Table 2).

The results of antimicrobial potency of different polarity crude extracts of *A. obesum* against *S. aureus*, *E. coli*, *P. aeruginosa* and *Valgurais* were evaluated through agar gel diffusion method. The methanol crude stem extract of *A. obesum* and its different polarity crude extracts showed small antibacterial potency against Gram-positive and Gram-negative bacteria at the concentration of 2.00, 1.00, 0.50 and 0.25 mg/mL, respectively. The zones of inhibition of different polarities crude extracts by Soxhlet extractor method were in the range of 0-17 mm. However, methanol, hexane, chloroform, ethyl acetate, butanol and water crude stem extracts of *A. obesum* by maceration method also showed similar antibacterial potency against the employed bacterial strains and all working concentrations. The zones of inhibition of different polarities crude stem extracts by maceration method

Table 2

Antimicrobial activity of different crude stem extracts by different methods of extraction against food borne pathogenic bacterial strains.

Extract	Concentration (mg/mL)	*E. coli* (mm)		*P. aeruginosa* (mm)		*S. aureus* (mm)		*Valgurais* (mm)	
		Soxhlet	Meceration	Soxhlet	Meceration	Soxhlet	Meceration	Soxhlet	Meceration
Hexane	2.00	6.00±0.09	nd	nd	6.00±0.12	nd	nd	7.00±0.29	6.00±0.05
	1.00	nd	8.00±0.19	nd	nd	15.00±0.76	nd	nd	7.00±0.27
	0.50	nd	7.00±0.17	7.00±0.08	nd	17.00±0.13	nd	nd	7.00±0.67
	0.25	7.00±0.11	8.00±0.23	7.00±0.13	7.00±0.34	nd	nd	nd	7.00±0.34
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27±0.11	29.00±0.11	31.00±0.11	29.00±0.11
Ethyl acetate	2.00	7.00±0.21	7.00±0.25	7.00±0.21	8.00±0.09	6.00±0.12	nd	8.00±0.34	8.00±0.34
	1.00	6.00±0.07	nd	nd	6.00±0.77	8.00±0.17	nd	10.00±0.08	nd
	0.50	nd	7.00±0.09	7.00±0.23	nd	nd	7.00±0.23	7.00±0.45	nd
	0.25	nd	nd	nd	24.00±0.09	nd	nd	nd	7.00±0.08
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27.00±0.11	29.00±0.11	31.00±0.11	29.00±0.11
Chloroform	2.00	nd	7.00±0.34	nd	nd	nd	nd	nd	nd
	1.00	8.00±0.14	nd	nd	nd	23.00±0.13	nd	15.00±0.13	nd
	0.50	8.00±0.26	nd	8.00±0.07	nd	7.00±0.22	nd	nd	nd
	0.25	nd	7.00±0.22	7.00±0.78	nd	nd	nd	10.00±0.15	nd
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27.00±0.11	29.00±0.11	31.00±0.11	29.00±0.11
Butanol	2.00	6.00±0.13	7.00±0.15	nd	7.00±0.07	7.00±0.24	nd	7.00±0.07	nd
	1.00	nd	7.00±0.17	nd	7.00±0.11	9.00±0.47	nd	nd	nd
	0.50	nd	nd	7.00±0.23	7.00±0.53	nd	7.00±0.08	9.00±0.16	nd
	0.25	7.00±0.09	7.00±0.09	8.00±0.29	7.00±0.19	nd	nd	nd	nd
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27.00±0.11	29.00±0.11	31.00±0.11	29.00±0.11
Methanol	2.00	8.00±0.19	8.00±0.12	nd	nd	nd	nd	7.00±0.55	nd
	1.00	7.00±0.23	nd	8.00±0.09	nd	7.00±0.09	nd	7.00±0.13	nd
	0.50	7.00±0.12	7.00±0.08	nd	7.00±0.23	13.00±0.23	nd	nd	nd
	0.25	nd	nd	7.00±0.65	nd	nd	nd	35.00±0.78	nd
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27.00±0.11	29.00±0.11	31.00±0.11	29.00±0.11
Water	2.00	7.00±0.16	7.00±0.22	nd	nd	nd	nd	8.00±0.22	nd
	1.00	nd	7.00±0.19	nd	nd	9.00±0.17	nd	7.00±0.09	nd
	0.50	nd	7.00±0.11	7.00±0.25	nd	7.00±0.15	nd	nd	nd
	0.25	nd	7.00±0.08	7.00±0.09	nd	nd	nd	9.00±0.39	nd
	Control	19.00±0.11	20.00±0.11	23.00±0.11	23.00±0.11	27.00±0.11	29.00±0.11	31.00±0.11	29.00±0.11

nd: not detected.

were measured in the range of 0–24 mm. The hexane crude stem extract by Soxhlet and maceration methods showed almost similar antibacterial potency against *S. aureus*, *E. coli*, *P. aeruginosa* and *Valgurais* at the concentration of 2.00, 1.00, 0.50 and 0.25 mg/mL except *S. aureus* at concentration at 1.00 mg/mL and 0.50 mg/mL by Soxhlet method.

4. Discussion

All the extracts of the plants contain different chemical constituents such as alkaloids, flavonoids, terpenoids, saponins, *etc*. These chemical compounds are responsible for different kinds of activity such as antioxidant, antimicrobial, antifungal, anticancer, *etc*[17,18]. Particularly, the activities are depends on the secondary metabolic compounds through different mechanism of actions. The plant is considered as the main sources of secondary metabolic compounds and these compounds are isolated from the plants by polar solvents. Some bioactive compounds such as alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids were also presented in this plant[13–16].

The antimicrobial potency of plant crude extracts mainly depends on the dose used and the type of bacterial strains. Antibacterial potency was also responsible to the chemical components presented in the plant crude extracts[5–7,9,15]. Alkaloids, steroids, saponins, tannins and flavonoids are considered as secondary metabolic compounds, which were presented in the crude extracts of *A. obesum*. Therefore, due to these chemical compounds, all the crude extracts were shown the antimicrobial potency. The maximum antimicrobial potency means the maximum amount of active secondary components present in the crude extract[6,13,15–19].

These two concentrations showed the maximum antimicrobial potency against *S. aureus*. However, hexane crude extract from maceration method showed similar potency against all employed bacterial strains. All other crude extracts by Soxhlet and maceration methods have

shown similar potency against *S. aureus*, *E. coli*, *P. aeruginosa* and *Valgurais* at all concentrations. Most of the crude extracts of employed concentration by maceration method did not show any potency against employed bacterial strains. Almost similar antimicrobial potency results on *A. obesum* crude extracts were reported by other authors[13]. Further studies of the extraction, isolation, identification of bioactive compounds and *in vivo* studies are needed for the better understanding of the mechanisms of action.

The antimicrobial study of different crude extracts of *A. obesum* showed that all crude extracts from both extraction methods have almost the similar results against the employed food borne pathogenic bacteria. Further isolation and identification study are needed for active compound in the crude extracts which could be used as a medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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