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# A comparison of the antimicrobial effectiveness of different polarities crude extracts from the leaves of *Adenium obesum* used in Omani traditional medicine for the treatment of microbial infections

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### ABSTRACT

**Objective:** To study and compare different crude extracts from the leaves of *Adenium obesum* for antimicrobial potency and to perform different group tests.

**Methods:** After processed, the leaves samples were used for extraction with methanol solvent by Soxhlet extraction method. The methanol crude extract was prepared by evaporating the solvent. The obtained extract was defatted by water and fractioned into different solvents. All the fractionated crude extracts were evaporated and used for antimicrobial study by disc method against pathogenic bacterial strains (Gram-positive and Gram-negative). The antimicrobial results for all crude extracts obtained from the leaves of *Adenium obesum* were compared with a standard antibiotic amoxicillin.

**Results:** The calculated diameter of inhibition zone for all polarities crude extracts were in the range of 0–13 mm. The results showed that all crude extracts have strong potential against different pathogenic bacterial strains.

**Conclusions:** The isolated crude extracts by Soxhlet method could be used as herbal or pharmaceutical medicine for the treatment of infectious diseases.

## 1. Introduction

Recently medicinal plants and their products are considered as a great interest for the treatment of different infectious diseases[1]. The utilization of medicinal plants and their products as a medicines for the treatment of infectious diseases is quite safe[1]. Several biological and phytochemical screenings have been done by the authors on plants and have found different secondary metabolites responsible for anticancer, antimicrobial and antifungal activities[2,3]. The main secondary metabolites responsible for those activities are tannins, terpenoids, alkaloids, flavonoids and glycosides. Day-by-day these kind properties on medicinal plants are tremendously increasing

from different parts of the world. World Health Organization reported that 80% world's population used plants extract, plant products and active chemical ingredients for the treatment of different infectious diseases[4–7]. For the prevention as well as treatment of human curable and incurable diseases, plants and plant-based products play an important role from the ancient time[8]. Most of the developed and developing countries used folk and traditional medicine as a primary source of healthcare systems[1,9]. In the traditional medicine systems plants and herbal products are well documented for their curative potential[10]. More than 60% natural products based new drugs had been innovated during the year 1981–2002[11]. Almost all developed drugs from plant sources have been used successfully for the treatment of different diseases especially the infectious disease and cancer[8]. Nowadays, the innovation rate of new drugs is declining due to the lack of trained manpower[11]. A large number of scientists all over the world have studied the effectiveness of plant crude extracts and their products against different bacterial

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strains<sup>[12]</sup>.

Tropical medicinal plant *Adenium obesum* (*A. obesum*) belongs to genus *Adenium* and family Apocynaceae. Most of the species in this family are found in the Arabian countries<sup>[13]</sup>. Only few species of this family is found in the Sultanate of Oman. Among them *A. obesum* and *Tabernaemontana* are most commonly found in the Sultanate of Oman; they are used as medicine for the prevention and treatment of curable and incurable diseases. All these plant species can produce milky sap. The milky sap contains some toxic chemical compounds. In contact with skin it can produce skin irritation due to toxic compounds. Generally the suitable place for *A. obesum* growth is in rocky and sandy soils<sup>[14]</sup>. Nowadays many countries commercially cultivated this plant due to its medicinal importance<sup>[14]</sup>. In Yemen and Arabian Peninsula it is found that some rare species belong to this family. The flowers belong to this family are completely different from other. It depends on the environmental conditions such as rainfall, temperature etc. The growth regulator of this family including *A. obesum* is very slow. Due to slow growth rate they are called a long-lived plant. The plant looks like a small tree up to 4 m in height. The leaves are green and arranged spirally, clustered at the end of branchlets<sup>[15–17]</sup>. The bark is pale greyish-green, grey and brown. Different phytochemicals such as alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids are present in the plant crude extracts of *A. obesum*<sup>[18]</sup>.

Among many countries the whole plant is used as a medicine for the treatment of a variety of ailments including venereal diseases. Omani national still use the same for the prevention and treatment of various infectious diseases. One important lotion prepared from the root and bark crude extracts is used for the treatment of different skin diseases as well as to kill lice<sup>[17]</sup>. Traditionally, latex of this plant is used as a medicine for the recovery of decaying teeth and septic wounds<sup>[18]</sup>. In Kenya, it is used to kill lice and stems powder is used for camels and cattle to kill skin parasites<sup>[19]</sup>. In Somalia, they use this plant for nasal drops<sup>[19]</sup>. The bark of *A. obesum* is used as an abortifacient<sup>[20,21]</sup>. Therapeutically, the extracts from the whole plant has been used as antiplasmodial, anti-trypanosomal and anti-leishmanial<sup>[22]</sup>. Traditionally, Omani people used it for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pain and joint pain<sup>[14,23]</sup>. There is no such research on antimicrobial activity of Omani *A. obesum* species so far in the current literature. Therefore, the aim of this study was to prepare different crude extracts from the leaves of *A. obesum* native to Oman by Soxhlet extraction method using different polarities solvents and compare their antimicrobial study through agar gel method.

## 2. Materials and methods

### 2.1. Chemicals

Different polarities solvents such as hexane, chloroform, ethyl acetate, butanol and methanol from E. Merck, Germany

were used in this experiment. Amoxicillin was used as a standard from Sigma–Aldrich Company, UK. Agar gel and other materials for the preparation of Petri dishes were collected from the Department of Biological Sciences, College of Arts and Sciences, University of Nizwa, Sultanate of Oman.

### 2.2. Plant samples

The leaves samples of *A. obesum* used for the investigation of antimicrobial activity were obtained from Dhofar region, Sultanate of Oman. The samples were collected during the month of November 13, 2013. The morphological identification was done by the website. The leaves samples separated from the stems were washed with water and cut into small pieces. The samples were dried under shade for two weeks. The dried samples were crushed and blended to make it powder.

### 2.3. Source of pathogenic microorganism

Cultures of pathogenic bacterial strains such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Proteus vulgaris* (*P. vulgaris*) were collected from Nizwa hospital, Nizwa, Sultanate of Oman. The collected organisms were subcultured in nutrient agar plates and kept at 4 °C until needed for use.

### 2.4. Preparation of methanol crude extract

The leave powder samples (50 g) were extracted with methanol (250 mL) by using Soxhlet extractor for 3 d. After 3 d, the samples were filtered and the filtrate was evaporated through rotary evaporator to give methanol crude extract (4.58 g). Water (100 mL) was used to defatted the methanol crude extract (4.0 g). The defatted extract was transferred to a separatory funnel for fractionation by different solvents. First 30 mL of hexane solvent was added to it for extraction and repeated twice. According to the polarity then chloroform, ethyl acetate and butanol were used for extraction from the defatted crude extract. All collected fractions by different solvents were evaporated manually to give hexane (0.65 g), chloroform (1.098 g), ethyl acetate (0.867 g) and butanol (0.492 g). The obtained different polarities of crude extracts were used for antimicrobial study through disc diffusion method.

### 2.5. Antimicrobial assay

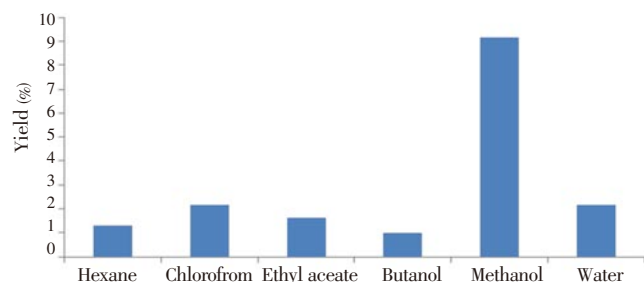
The antimicrobial study for the crude extracts obtained from the leaves of *A. obesum* was done by agar gel diffusion method<sup>[24]</sup>. Standard amoxicillin was used to compare the activity with the crude extracts of *A. obesum*. Dimethyl sulfoxide was used as a negative control. Whatman filter was used as disc for this study. All the crude extracts were dissolved separately and diluted with dimethyl sulfoxide to produce solution having the concentrations 2.00, 1.00, 0.50 and 0.25 mg/mL. In this method, different polarities crude samples of *A. obesum* were sucked by the paper discs. Then

the paper disc was placed on the nutrient agar plate seeded with the organism. When the plates were ready, they were incubated at 37 °C for 24 h. After incubation of the plates, growth of inhibition zone was measured by scale against the applied tested bacteria. Each method in this experiment was replicated three times.

### 3. Results

#### 3.1. Percentage of yield

Methanol crude extract was obtained by the evaporation of solvent. The yield of extraction was 9.16%. Then the methanol crude extract (4 g) was used to be defatted by water. Different solvents such as hexane, chloroform, ethyl acetate and butanol were used for preparation of extract of different bioactive compounds which is responsible for antibacterial activity in Figure 1. After extracted by the solvents the residual fraction is considering as water extract.



**Figure 1.** Percentage of 50 g dry samples of *A. obesum* extracted with different polarities of solvents.

#### 3.2. Antimicrobial activity

The different crude extracts were obtained from the leaves of *A. obesum* by Soxhlet method. These all crude extracts were used to determine for their activity by comparing their inhibition zone against four Gram-positive and Gram-negative pathogenic bacterial strains by agar gel method present in Table 1. Amoxicillin is an antibiotic used as comparison for antimicrobial activity. All the crude extracts showed antimicrobial activity against all employed bacterial strains at almost all concentrations. Among them all the crude extracts of *A. obesum* showed strong potential against *E. coli* and *P. vulgaris* (Table 1).

**Table 1**

Antimicrobial activity of different crude extracts.

Extract	Concentration (mg/mL)	<i>E. coli</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>S. aureus</i> (mm)	<i>P. vulgaris</i> (mm)
Hexane	2.00	12.00±0.12	11.00±0.09	11.00±0.10	10.00±0.18
	1.00	8.00±0.72	9.00±0.15	7.00±0.09	15.00±0.13

ND: Not detectable.

**Table 1, Continued**

Antimicrobial activity of different crude extracts.

Extract	Concentration (mg/mL)	<i>E. coli</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>S. aureus</i> (mm)	<i>P. vulgaris</i> (mm)
Hexane	0.50	8.00±0.33	10.00±0.17	7.00±0.11	11.00±0.22
	0.25	8.00±0.09	10.00±0.10	ND	10.00±0.33
	Control	28.00±0.18	27.00±0.16	28.00±0.17	30.00±0.42
	2.00	9.00±0.10	10.00±0.12	ND	10.00±0.10
Ethyl acetate	1.00	8.00±0.17	9.00±0.14	ND	12.00±0.15
	0.50	7.00±0.29	11.00±0.34	ND	12.00±0.19
	0.25	8.00±0.09	8.00±0.32	ND	10.00±0.42
	Control	24.00±0.15	25.00±0.12	23.00±0.09	25.00±0.33
Chloroform	2.00	9.00±0.16	10.00±0.10	12.00±0.11	10.00±0.10
	1.00	9.00±0.06	11.00±0.19	7.00±0.17	13.00±0.18
	0.50	10.00±0.54	11.00±0.15	7.00±0.19	8.00±0.44
	0.25	9.00±0.32	9.00±0.12	ND	12.00±0.23
Butanol	Control	23.00±0.10	27.00±0.12	28.00±0.08	23.00±0.22
	2.00	9.00±0.19	7.00±0.16	11.00±0.13	10.00±0.32
	1.00	8.00±0.14	7.00±0.18	ND	10.00±0.10
	0.50	9.00±0.52	ND	8.00±0.15	8.00±0.19
Methanol	0.25	7.00±0.22	ND	9.00±0.52	11.00±0.31
	Control	29.00±0.34	27.00±0.12	25.00±0.22	23.00±0.33
	2.00	8.00±0.23	ND	ND	9.00±0.26
	1.00	8.00±0.10	8.00±0.72	ND	9.00±0.10
Water	0.50	10.00±0.15	ND	8.00±0.42	10.00±0.09
	0.25	8.00±0.12	7.00±0.23	ND	10.00±0.54
	Control	25.00±0.18	21.00±0.10	26.00±0.10	27.00±0.72
	2.00	10.00±0.43	7.00±0.10	9.00±0.77	9.00±0.47
Water	1.00	8.00±0.09	ND	8.00±0.12	9.00±0.17
	0.50	8.00±0.11	ND	7.00±0.75	8.00±0.15
	0.25	9.00±0.14	ND	10.00±0.09	10.00±0.76
Control	24.00±0.17	22.00±0.07	25.00±0.12	24.00±0.52	

ND: Not detectable.

### 4. Discussion

Different polarities of solvents were used for the extraction from the power leaves samples of *A. obesum*. The percentages of yield obtained from *A. obesum* were 9.16% (methanol), 1.30% (hexane), 2.18% (chloroform), 1.63% (ethyl acetate), 0.98% (butanol), and 2.16% (water) respectively. The highest yield from the leaves of *A. obesum* was in methanol and the lowest yield was in butanol. The order of yield rate was methanol>chloroform>water>ethyl acetate>hexane>butanol.

Six crude extracts obtained from the leaves of *A. obesum* by fractionation method was used for antimicrobial study against different Gram-positive and Gram-negative pathogenic bacterial strains by disc diffusion method. All the different polarities crude extracts at different concentrations showed different activity against bacterial strains. Four pathogenic bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus* and *P. vulgaris* were used in the present study for the evaluation of antimicrobial activity. Different concentrations such as 2.00, 1.00, 0.50 and 0.25 mg/mL and standard amoxicillin were used in this experiment. All crude extracts isolated from the leaves of *A. obesum* showed moderate activity against *E. coli* at all concentrations. The zone of inhibition range was of 0–12 mm. Similarly, almost all the extracts also showed

moderate activity within the range of 0–11 mm against *P. aeruginosa* except in butanol, methanol and water crude extracts. Among them butanol extracts at 0.50 and 0.25 mg/mL, methanol extracts at 2.0 and 0.5 mg/mL and water extracts at 1.00, 0.50 and 0.25 mg/mL did not show any activity against *P. aeruginosa*. Water, hexane, butanol and chloroform crude extracts showed good potential activity within the range of 0–12 mm at most of the concentrations against *S. aureus*. However, ethyl acetate crude extract did not show any activity against *S. aureus*. All crude extracts from the leaves of *A. obesum* at all concentrations showed a very strong significant activity against *P. vulgaris* bacterial strain. The inhibition range for all crude extracts against *P. vulgaris* bacterial strain was of 0–15 mm. Our findings showed that the antimicrobial activity was increased with the increase of the polarity of solvents. It could be implied that the bioactive secondary metabolic compounds have strong antimicrobial activity<sup>[25–27]</sup>. In addition, comparing with each employed bacteria, all the crude extracts at all concentrations showed highest activity against Gram-negative bacterial strain. Similar results from several authors were reported in the literatures<sup>[25–27]</sup>.

From the above mentioned findings it can be concluded that water and chloroform crude extracts have a potential antimicrobial activity and support the traditional use of this plant as medicine. However, it is needed to investigate further for isolation and characterization of different active ingredients from the leaves of *A. obesum* and to test different biological activities.

### Conflict of interest statement

We declare that we have no conflict of interest.

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