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Evaluation of *in vitro* antioxidant potential of different polarities stem crude extracts by different extraction methods of *Adenium obesum*

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PEER REVIEW

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Comments

This study done by the author is very good for the scientific community. The present study on antioxidant activity of different stem crude extracts of *A. obesum*is gives valuable brief and scientific preliminary information about this plant.
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ABSTRACT

Objective: To select best extraction method for the isolated antioxidant compounds from the stems of *Adenium obesum*.
Methods: Two methods used for the extraction are Soxhlet and maceration methods. Methanol solvent was used for both extraction method. The methanol crude extract was defatted with water and extracted successively with hexane, chloroform, ethyl acetate and butanol solvents. The antioxidant potential for all crude extracts were determined by using 1, 1-diphenyl-2-picrylhydrazyl method.
Results: The percentage of extraction yield by Soxhlet method is higher compared to maceration method. The antioxidant potential for methanol and its derived fractions by Soxhlet extractor method was highest in ethyl acetate and lowest in hexane crude extracts and found in the order of ethyl acetate>butanol>water>chloroform>methanol>hexane. However, the antioxidant potential for methanol and its derived fractions by maceration method was highest in butanol and lowest in hexane followed in the order of butanol>methanol>chloroform>water>ethyl acetate>hexane.
Conclusions: The results showed that isolate antioxidant compounds effected on the extraction method and condition of extraction.

KEYWORDS

Adenium obesum, Extraction, *In vitro* antioxidant potential, DPPH, UV-visible spectroscopy

1. Introduction

Adenium obesum (*A. obesum*) is a most important medicinal plant belongs to the family Apocynaceae[1]. Several species are available worldwide. Among the species *Beaumontia*, *Carissa*, *Allamanda*, *Mandevillea*, *Nerium*, *Plumeria*, and *Tabernaemontana* are tropical species. Almost all of these species produce milky sap which can cause skin irritation or more serious internal poisoning. All of the family species grow well on rocky and sandy soils[2]. *A. obesum* grows well in Saharan Africa, Sudan, Kenya, Senegal and Swaziland. It is also found in Yemen and Arabian peninsula. The flora of this plant depends on the environment condition such as rainfall, temperature *etc*. They are called long lived plant and the growth is very slow. The plant is a small tree up to 4 m tall. Sometimes with a fleshy taproot, stem swollen at base up to 1 m in diameter. The bark is pale greyish-green, grey or brown, smooth, with sticky, clear or white latex; branchlets glabrescent, pubescent at apex. Leaves arranged spirally, clustered at the end of branchlets[3-5].

Phytochemical study of the crude extracts showed the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids[6]. It is known as

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a medicinal plant worldwide used as poison on arrows[3]. It was mainly used for the treatment of a variety of ailments including venereal diseases. The crude extracts from the root or bark are used as lotion to treat different skin diseases and kill lice[5]. The latex from this plant is used to recover decaying teeth and septic wounds[6]. In Somalia, it is used for nasal drops[7]. In northern Kenya, the latex is used to kill lice and the powder of stems is used for camels and cattle to kill skin parasites[7]. The bark of *A. obesum* is used as an abortifacient[8,9]. The whole plant of other *A. obesum* species from Nigeria has been shown to possess antiplasmodial, anti-trypanosomal and anti-leishmanial activities[10-12]. Traditionally it is used by the Omani community for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pain and joint pain[2,13,14]. The literature search reveals that still nobody works on the Omani species. Therefore, the present study was undertaken for the first time to evaluate antioxidant potential of methanol crude extract and its derived fractions from the stems of locally grown *A. obesum*. The aim of this work is to isolate the antioxidant compounds from the traditionally used stem of locally grown *A. obesum* by different methods and evaluate their antioxidant potential by free radical scavenging method.

2. Material and methods

2.1. Chemicals

Organic solvents such as hexane, chloroform, ethyl acetate, butanol and methanol were used in this present work from BDH, UK. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was from Sigma-Aldrich, Germany. The other chemicals were analytical grade from BDH, UK. The absorbance of the tested *A. obesum* samples was recorded on Thermo Spectronic spectrophotometer (Model No. Biomate, Great Britain, UK) Ultrospeck in methanol (λ_{max} in nm). Rotatory evaporator was used (Yamato Rotary Evaporator, Model RE 801, Japan).

2.2. Plant materials

The fresh stem samples of *A. obesum* were collected from Moscil, Salalah, Sultanate of Oman during the month of January 2014. The morphological identification of the plant sample was determined by the reference data available in the website. The collected samples were packed in a polyethylene bag and carried to the hotel at Salalah, Sultanate of Oman. The stems were separated from the barks instantly by knife. The separated stems samples were dried at room temperature under shade. After drying, the stems samples were cut into small pieces by knife and powder the samples by using grind machine. The powdered stem samples were preserved in clean polyethylene bag and kept away from light, heat and moisture until use.

2.3. Extraction by Soxhlet extractor

The powder samples of *A. obesum* (120 g) were extracted with methanol (3 L) by using Soxhlet extractor method for 72 h. After complete extraction, the methanol solvent was evaporated by rotary evaporator. The weight of methanol crude extract was 10.75 g. The methanol crude extract (5.24 g) was defatted with distilled water (150 mL) and successively extracted with hexane, chloroform, ethyl acetate and butanol. The water was evaporated from the remaining water part. The extraction was carried out using six different polarities of solvents and their antioxidant potentials were evaluated and compared.

2.4. Extraction by maceration extractor

The stem powder samples of *A. obesum* (120 g) were macerated in methanol solvent (3 L) at room temperature and kept in the laboratory for one week. The whole samples and methanol were stirring up and down every day for complete extraction. After seven days, the methanol solvent were separated from the powder samples by using buncher funnel to get clear methanol extract. Then the methanol solvent was evaporated by using rotatory evaporator to give semi solid mass crude extract (8.67 g). The methanol crude extract (3.22 g) was suspended in distilled water (120 mL) and then finally extracted successively with hexane, chloroform, ethyl acetate and butanol. The water was also removed from water part by using rotary evaporator. The extraction was carried out using the same solvents as mentioned in Soxhlet method and their antioxidant potentials were evaluated and comparee in the same way.

2.5. Free radical scavenging activity by DPPH method

The free radical scavenging assay by DPPH method was first described by Blois[12]. This method was modified later by several researchers. This is one of the most effective method used worldwide for the determination of antioxidant potential for plant samples. The evaluation of antioxidant potential of different crude extracts from the stems of *A. obesum* was determined by free radical scavenging method described by Hossain *et al.*[13,14] with modification. Five different concentrations such as 12.5, 25.0, 50.0, 100.0 and 200.0 μg/mL were used for each crude extract (hexane,

chloroform, ethyl acetate butanol, methanol and water). 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 by hand. After adding DPPH solution, all the test tubes were kept at room temperature in a dark place for 45 min. The blank sample was prepared by following the same procedure without adding any plant crude extract. After incubation, the absorbance was measured for all tested samples at fixed wave length 517 nm by using UV-visble spectrophotometer. The percentage of inhibition of each concentration plant crude extract was estimated and the antioxidant potential of each concentration plant crude extracts was calculated by using the following formula:

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

3. Results

3.1. Comparison of crude extracts by Soxlet and maceration methods

Both extraction methods of the *A. obesum* powder stem samples were extracted with methanol. After complete extraction, methanol was evaporated by rotary evaporator. The obtained methanol crude extracts by both methods was defatted with distilled water separately. Then the defatted crude extract was extracted successively with hexane, chloroform, ethyl acetate and butanol. The water was removed. The weights of each extracts from Soxhlet and maceration methods are presented in Table 1.

Table 1

Weight of different crude extracts by soxlet and maceration methods

Crude extracts	Soxhlet method (g)	Maceration extract (g)
Water	0.98	1.29
Methanol	5.24	3.22
Hexane	0.68	0.28
Ethyl acetate	0.43	0.29
Chloroform	3.29	0.32
Butanol	0.96	0.63

3.2. Comparison of antioxidant potential of crude extracts from Soxlet and maceration methods

The antioxidant potential of all crude extracts by Soxhlet and maceration methods were determined through using well established DPPH method[15]. The inhibition percentage of stems crude extracts by Soxhlet method is higher than maceration method. The antioxidant potential for methanol and its derived fractions from Soxhlet extractor method was highest in ethyl acetate and lowest in hexane crude extracts and found in the order of ethyl acetate>butanol>water>chloroform>methanol>hexane. However, the antioxidant potential for methanol and its derived fractions from maceration method was highest in butanol and lowest in hexane followed in the order of butanol> methanol>chloroform>water>ethyl acetate>hexane (Table 2 and Table 3).

Table 2

Antioxidant potential of hexane, ethyl acetate, chloroform, butanol, methanol and water crude extracts from stem samples of *A. obesum* by Soxhlet method (%).

Concentration of crude extract (μg/mL)	Water	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
12.5	86.75±0.34	86.22±0.77	84.09±0.87	88.29±0.23	86.36±0.33	88.22±0.44
25	89.08±0.21	88.75±0.54	85.56±0.14	92.21±0.19	89.35±0.27	98.07±0.56
50	93.87±0.14	98.13±0.12	88.42±0.25	98.40±0.05	96.40±0.11	98.66±0.26
100	92.54±0.09	98.73±0.43	92.54±0.55	98.66±0.17	98.20±0.42	98.93±0.12
200	98.60±0.22	99.26±0.13	98.60±0.23	98.73±0.27	98.73±0.89	99.13±0.23

Table 3

Antioxidant potential of hexane, ethyl acetate, chloroform, butanol, methanol and water crude extracts from steam samples of *A. obesum* by maceration method (%).

Concentration of crude extract (μg/mL)	Water	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
12.5	91.21±0.22	98.80±0.11	84.23±0.27	87.82±0.12	98.60±0.23	99.06±0.19
25	97.73±0.31	98.66±0.26	85.89±0.45	91.68±0.39	98.40±0.14	98.93±0.89
50	98.07±0.21	98.60±0.34	88.02±0.33	97.53±0.13	97.93±0.27	98.86±0.77
100	98.40±0.11	98.53±0.33	93.28±0.17	98.20±0.44	97.53±0.44	98.53±0.67
200	98.46±0.49	98.13±0.29	97.73±0.89	98.33±0.51	97.87±0.38	98.13±0.90

4. Discussion

The yield of the two extraction methods for each crude extract of *A. obesum* were shown in the above. The yield of extraction by Soxhlet and maceration methods with methanol were 10.75 and 8.67 g. In this present study, the extraction yield was higher by Soxhlet extractor method and the lowest by maceration method. The difference of extraction yield from two method might be due to hot and cold extraction. In Soxhlet extraction method hot extraction process was used so that the extraction yield was high compared to maceration method. In addition, during the Soxhlet extractor method, the high molecular and less soluble antioxidant compounds extracted more due to recycle of the hot solvent[16]. However, in maceration method the yield of extraction was low, which might be due to cold extraction and extraction time. Both factors highly influenced the extraction efficiency, so that the extraction yield was different[14]. The Soxhlet extraction could provide comparable or even better results than the maceration method for extracting polyphenolic compounds and showed a significant advantage in extraction time and solvent consumption over the two methods[16].

Our present study showed that different concentration and different crude extracts from the stems of *A. obesum*

exhibited strong free radical scavenging activity. The strong free radical scavenging activity in different stems crude extracts might be due to high quantity of poly phenolic compounds. The high quantity poly phenolic compounds indicated that the stem crude extracts of *A. obesum* is a good potential source for natural antioxidants to prevent free radical oxidative damage. Few explanations could be available to support for the decreasing antioxidant potential of the stem crude extracts of *A. obesum* during the process of drying. May be during the drying process, some of antioxidant compounds were deactivated or enzymatic degradation[15]. DPPH is considered as a stable free radical. It has been used for the determination of free radical scavenging potential of plant crude extracts/antioxidants. The antioxidant results of the present study showed that methanol crude extract and derived fractions of stem crude extracts of *A. obesum* by Soxhlet extraction method was slightly decreasing in *in vitro* DPPH method. It is suggested that stems crude extract of *A. obesum* contains more secondary metabolic compounds with strong antioxidant potential. The antioxidant potential of natural and synthetic antioxidants are believed to be responsible for their beneficial effects during treatment of DPPH. Phenolic and poly phenolic compounds of the crude extract of *A. obesum* are may be involved in their free radical reactions by decreasing the stable DPPH radical to change the color of diphenyl picrylhydrazine derivative. However, *A. obesum* has also been reported to have anti-DPPH-activity[16]. In our present study, in Soxhlet extractor method, the highest antioxidant potential was in ethyl acetate crude extract and the lowest was in hexane crude extract and followed in the order of ethyl acetate>butanol>water>chloroform > methanol>hexane. However, in maceration method, the highest potential was in butanol and lowest in hexane followed in the order of butanol>methanol>chloroform> water>ethyl acetate>hexane.

The result was found that the methanol extracts from Soxhlet extraction showed the highest antioxidant potential compared to maceration method. Hot organic solvent treatment was used in Soxhlet method, which confirmed higher antioxidant potential than without heat treatment extraction by maceration method. In addition, ethyl acetate crude extracts of *A. obesum* showed higher antioxidant activity than other crude extracts obtained from the same method. Similarly butanol crude extract of *A. obesum* showed higher antioxidant potential compared to other crude extracts obtained by the maceration method. Very good correlation was obtained for antioxidant potential between two extraction methods. In Soxhlet method, the temperature 70 °C was used, it may have effect on antioxidant potential due to the release of polyphenolic compounds from the stem samples of *A. obesum*. Because the best quality of the crude extract depends on the maximum amount of solvent used for the extraction and also less time consuming. Methanol crude extract from Soxhlet method has proven to be the best and suitable method for extraction from the stems of *A. obesum* for its antioxidant compounds. Similar several reports were available for other plant species[17-20].

In present study, similar pattern of antioxidant potential was obtained from the stems of *A. obesum* crude extracts by both methods assayed through DPPH method. This similar antioxidant potential of crude extract by both extraction methods is in accordance with the amount of antioxidant compounds that was present in the stems crude extract of *A. obesum*. The present study found that the Soxhlet extractor is the best method to isolate more biologically active antioxidant compounds compared to maceration method. Further studies should be designed for the isolation and identification of individual phenolic compounds and also *in vivo* studies are needed for better understanding their mechanism of action as antioxidant.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

A. obesum and their species are considered as a medicinal plant worldwide. It grows well in Arabian Peninsula due to the environmental condition. It is belonging to the family Apocynaceae. Mainly it is used for the treatment of a variety of ailments including venereal diseases. Its crude extracts from the root or bark is also

used as lotion to treat different skin diseases and to kill lice. The literature search reveals that there is no work has been done on Omani *A. obesum* species.

Research frontiers

The objective is to prepare different crude extracts using different extraction method from the stem of *A. obesum* and to evaluate their antioxidant activity.

Related reports

The literature search reveals that there is no work has been done on Omani *A. obesum* species.

Innovations and breakthroughs

Traditionally different community used plants as a medicine. It is new medicinal plant grown in Arabian Peninsula. So the experimental work done by the author is very important and the data generated from this plant will be useful to the scientific community.

Applications

This plant is considered as a medicinal plant worldwide. Its main used for the treatment of a variety of ailments including venereal diseases. The crude extracts from the root or bark is used as lotion to treat different skin diseases and to kill lice.

Peer review

This study done by the author is very good for the scientific community. The present study on antioxidant activity of different stem crude extracts of *A. obesum*is gives valuable brief and scientific preliminary information about this plant.

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