

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Original article doi: 10.1016/S2222-1808(15)60879-9

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Determination of total phenolics, flavonoids and antioxidant activity of root crude extracts of *Adenium obesum* traditionally used for the treatment of bone dislocations and rheumatism

Mouza Khamis Nasser AL-Ghudani, Mohammad Amzad Hossain*

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P.O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

ARTICLE INFO

Article history:

Received 15 Sep 2014

Received in revised form 13 Oct, 2nd

revised form 28 Oct 2014

Accepted 23 Apr 2015

Available online 4 Jun 2015

Keywords:*Adenium obesum*

Roots

Maceration and Soxhlet methods

Total phenolics

Total flavonoids

Antioxidant activity

UV-visible spectroscopy

ABSTRACT

Objective: To prepare different polarities crude extracts from the roots of *Adenium obesum* (AO) and to evaluate their total phenolics, total flavonoids and antioxidant activity by Folin-Ciocalteu reagent, aluminum chloride and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) methods.

Methods: Different polarities crude extracts were prepared through Soxhlet extractor and maceration methods by using different polarities organic solvents, and were used for the determination of antioxidant activity by using DPPH method with modification. The determination of total phenolics and flavonoid contents of the AO root crude extracts was conducted through Folin-Ciocalteu reagent and aluminum chloride methods.

Results: Different extraction methods led to different results. By Soxhlet method, the highest total phenolics content of different crude extracts was equivalent to the gallic acid in ethyl acetate while the lowest equaled to the gallic acid in methanol. However, by maceration method, the highest phenolics content was found in methanol and the lowest was in hexane. The maximum amount of total flavonoids through Soxhlet method was found in hexane and the minimum was in methanol. On the contrast, in maceration method, the maximum amount of total flavonoids was found in methanol and the minimum was in butanol crude extracts. In the case of antioxidant activity of different crude extracts from AO, by Soxhlet method, the highest one was equivalent to DPPH in methanol and the lowest one was in butanol. Dissimilarly, by maceration method, the highest antioxidant activity was in ethyl acetate while the lowest was in chloroform extracts.

Conclusions: In conclusion, the crude extracts from the roots of AO show the high amount of total phenolics and flavonoids contents and it could be used as medicines.

1. Introduction

Adenium obesum (AO) is a species of flowering plant belonging to Apocynaceae family[1]. It is an important medicinal plant which is found in Oman and native to Sahel regions, south of Sahara, Africa. It grows well in tropical and subtropical regions of eastern and southern parts of Africa and Arabia. Several common names are available all over the world such as Sabi star, kudu, mock azalea, impala lily and desert rose[2,3]. It is a small tree up to 3

meters height. It has thick or swollen trunk, sometimes with fleshy tap roots. The bark is smooth green to pale brown. Stems exude milky sap. Leaves are dark green, deciduous, fleshy and arranged in alternate spiral. Flowers are showy, funnel-shaped, with five distinct pinkish or light red lobes[4]. The roots are susceptible to water molds which thrive in water-logged soils. Prevention is the best strategy because most fungicides are ineffective against this group and the few that work are expensive. Use of a well-drained medium and careful watering prevents most roots from rotting. The flowering and fruiting period in Oman starts from August to October but the plant also blossoms from July to August. The fruit is mature during the month of October[5]. In Omani tradition, AO bark is crushed and soaked in warm water to be used for treating bone dislocations, rheumatism, sprains, paralysis, swellings, wounds and skin infections[6]. However, in Saudi Arabia and Yemen, the juice from the stem and crushed bark

*Corresponding author: Mohammad Amzad Hossain, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman.

Tel: +968 99708496

Fax: +968 25446236

E-mail: hossainabi@gmail.com

Foundation Project: Supported by University of Nizwa, Sultanate of Oman (Grant No. Ref.No. A/13-14-UoN/04/CPN/IF).

is applied on wounds[7]. In Sahel Africa, roots of AO alone or in combination with other plants are used for treatment of venereal diseases[2]. In addition, root or bark extract is used as bath or lotion for skin diseases and lice[8]. Latex of AO is used for decaying teeth and septic wounds[9]. In Somalia, root decoction is used as nose drops for rhinitis[2,8]. In Kenya, the latex is rubbed on the head for lice[2,8-11]. In Kenya, the bark is also used for ethno-veterinary control of lice and fleas in livestock[10]. The stem powder is used for skin parasites of camels and cattle and in magic potions[12]. Several cardiac glycosides and pregnanes have been reported from the species[9]. The main glycoside constituent in this plant is oleandrogenin B-gentiobiosyl-B-D-theretoside[12]. AO also contains various groups of chemical compounds such as alkaloids, steroids, *etc*[2,8-11]. The literature survey reveals that no work has been done on the roots of Omani AO species. Therefore, the aim of this present work is to prepare different polarities crude extracts by using different polarities of solvents from the roots of AO and to determine their total phenolics, total flavonoids and antioxidant activity by FCR, $AlCl_3$ and DPPH methods.

2. Materials and methods

2.1. Chemicals

Hexane, chloroform, ethyl acetate, butanol and methanol collected from E. Merck, Germany were used in this experiment. Folin-Ciocalteu reagent (FCR), aluminum chloride ($AlCl_3$), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and quercetin were collected from Sigma-Aldrich Chemical Company Ltd, Germany. Other necessary chemicals used in this experiment were from BDH, UK. Sodium hydroxide pellets, sodium nitrate, sodium carbonate were obtained from Scharlau-Spain. UV-visible spectroscopy (Thermo Spectronic spectrophotometer, Great Britain, UK, Model No. Biomate) was used for the determination of absorbance of the selected plant crude samples.

2.2. Plant samples and extraction

The roots of AO were collected during the month of January 2014 from Salalah, Dhofar, Sultanate of Oman. The collected root samples were brought to the Natural Products Lab, University of Nizwa, Nizwa, Sultanate of Oman. The samples were cleaned with water to remove soil. The root was too hard to be cut into small pieces with knife. The small pieces of samples were dried at room temperature for 3 weeks. At last, the dried samples were ground to powder by Blinder machine. The root powder samples (147.5 g) were deposited in a 2-liter beaker and 350 mL of methanol was added. The sample beaker was kept for 4 days in the lab at room temperature until the extraction process was completed. The whole mixture was stirred every day for complete extraction and filtered under pressure by using Buchner funnel. The filtrate was evaporated by rotary evaporator to give methanol extracts (12.89 g). The methanol crude extract was defatted by using 120 mL of distilled water and transferred into separated funnel. 30 mL of hexane solvent was added to the separatory funnel and shaken by hand for 10 min. After 15-30 min, the extract began to separate into

two layers. Then the whole process was repeated with 20 mL of hexane. The two obtained parts of hexane were combined together and evaporated the hexane to give the hexane crude extract. Similarly, the polarities chloroform, ethyl acetate and butanol solvents were used to prepare chloroform, ethyl acetate and butanol crude extracts. The remaining water part in the separatory funnel was evaporated to give water crude extract.

2.3. Total phenolics assay

2.3.1. Preparation of chemicals

A volume of 10 mL of Folin-Ciocalteu reagent (FCR) was put in a 100 mL volumetric flask and then 90 mL of distilled water was added. 3 g of sodium carbonate (Na_2CO_3) was deposited in a 50 mL volumetric flask and 50 mL of distilled water was added.

2.3.2. Preparation of gallic acid

Gallic acid was used as the standard for the calculation of total phenolics in the prepared plant crude extracts. 2 mg of gallic acid was put in a 10 mL volumetric flask and diluted with 10 mL of methanol. Then, the solution was diluted with methanol followed by serial dilution technique to prepare 200, 100, 50, 25 and 12.5 μ g/mL. 200 μ L of each concentration was separately taken in a test tube and 1.5 mL of FCR solution was added. The solution was kept for 5 min in a dark place. At last, 1.5 mL of Na_2CO_3 was added to each test tube. The tubes were shaken by hand and covered by aluminum sheet and kept in a dark place for 2 h. The absorbance was measured by UV-visible spectroscopy at the fixed wavelength of 760 nm. The standard curve was prepared by using the obtained data.

2.3.3. Total phenolics content

4 mg of each prepared crude extract from the roots was taken in a separate test tube and 4 mL of methanol was added. From there, 200 μ L of each sample was taken in another new test tube and 1.5 mL of FCR was added. Then, the mixture was kept for 5 min in the dark. At last, 1.5 mL of Na_2CO_3 was added; and the mixture was covered by aluminum sheet and kept for 2 h in the dark. The absorbance was measured by using UV-visible spectrophotometer at the wavelength of 760 nm.

2.4. Total flavonoids assay

2.4.1. Preparation of chemicals

2.5 g of $NaNO_3$ was taken in a 50 mL volumetric flask and 50 mL of distilled water was added. 2 g of NaOH was taken in a 50 mL volumetric flask and 50 mL of distilled water was added. 1 g of $AlCl_3$ was taken in a 10 mL volumetric flask and 10 mL of distilled water was added.

2.4.2. Preparation of quercetin

Quercetin was used as the standard for the calculation of total flavonoids. 2 mg of quercetin was taken in a 10 mL volumetric flask and diluted with 10 mL of methanol. The prepared concentration of quercetin was diluted followed by serial dilution technique to prepare 200, 100, 50, 25 and 12.5 μ g/mL. 250 μ L

of each concentration was taken in a test tube added with 125 μ L of water and 75 μ L of sodium nitrate solution with stirring. The solution was kept for 6 min in the dark. Then 150 μ L of aluminum chloride was added to each test tube and kept in a dark place for 5 min. Finally, 500 μ L of sodium hydroxide and 275 μ L of water were added. The absorbance was recorded by UV-visible spectroscopy with a fixed range at 510 nm wavelength. The standard curve was prepared by using the obtained data.

2.4.3. Procedure for total flavonoids content

Each plant sample extract (250 μ L) (4 mg crude extract in 4 mL of methanol) were taken in a separate test tube. Then 125 μ L of water and 75 μ L of sodium nitrate solution were added to each test tube with stirring. The solution was then left for 6 min in a dark place. Later, 150 μ L of aluminum chloride was added to each test tube and kept in a dark place for 5 min. At the end of the procedure, 500 μ L of sodium hydroxide and 275 μ L of water were added. The absorbance was measured by UV-visible spectroscopy at the fixed wavelength of 510 nm.

2.5. Antioxidant activity assay

Each crude extract such as hexane, chloroform, ethyl acetate, butanol, methanol and water crude extract (2 mg) was taken in separate test tube and dissolved with 10 mL of methanol. Different concentrations such as 200, 100, 50, 25 and 12.5 μ g/mL were prepared by using serial dilution technique. 3.3 g of DPPH was taken in a 100 mL volumetric flask and dissolved with 100 mL of methanol, after which 2.5 mL of DPPH solution was added to all test tubes and shaken gently by hand and put in dark place for an hour and half. The absorbance of the samples was recorded by UV-spectrophotometer at 517 nm wavelength. Finally, antioxidant activity of the crude extract samples was calculated by using the following formula:

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

3. Results

Different AO root crude extracts from different solvents by maceration and Soxhlet methods used for the determination of total phenolics content were presented in Table 1. The results showed that the total phenolics content through maceration method were the highest in methanol (0.0195 mg of GAE/g of powder crude extract) and the lowest in hexane (0.0082 mg of GAE/g of powder crude extract) followed by chloroform, ethyl acetate and butanol crude extract. By comparison, the total phenolics content through Soxhlet method was the highest in ethyl acetate (0.0196 mg of GAE/g of powder crude extract) and the lowest in methanol (0.0102 mg of GAE/g of powder crude extract) followed by butanol, water, hexane and chloroform root crude extracts.

The determination of total flavonoids content of different crude extracts from AO roots by maceration and Soxhlet methods was presented in Table 2. The results revealed that by maceration method, the highest amount of flavonoids content from AO roots

was obtained in methanol (0.014 mg QE/g dry plant material) and the lowest was in butanol crude extract (0.0009 mg QE/g dry plant material). However, by Soxhlet method, the highest amount of flavonoids content was obtained in hexane (0.008 mg QE/g dry plant material) while the lowest were in chloroform and methanol crude extracts (0.001 mg QE/g dry plant material).

Table 1

Total phenolics contents of different polarities roots crude extracts of AO.

Crude extracts	Maceration (mg of GAE/g of powder crude extract)	Soxhlet (mg of GAE/g of powder crude extract)
Water	-	0.016
Chloroform	0.015	0.011
Methanol	0.020	0.010
Hexane	0.008	0.013
Ethyl acetate	0.014	0.020
Butanol	0.011	0.019

Table 2

Total flavonoids contents of different polarities roots crude extracts of AO.

Crude extracts	Maceration (mg QE/g dry plant material)	Soxhlet (mg QE/g dry plant material)
Water	-	0.002
Chloroform	0.0020	0.001
Methanol	0.0140	0.001
Hexane	0.0010	0.008
Ethyl acetate	0.0040	0.004
Butanol	0.0009	0.003

All crude extracts from the roots of AO were used for the determination of antioxidant potential by maceration and Soxhlet methods and the antioxidant activities were determined by using well established DPPH method[3]. The results of antioxidant activity of different crude extracts from the roots of AO by Soxhlet method were equivalent to DPPH in these extracts in the same order: methanol > chloroform > hexane > ethyl acetate > water > butanol crude extract.

Similarly, the antioxidant activity of crude extracts by maceration method was equivalent to DPPH in these extracts in the same order: ethyl acetate > methanol > hexane > butanol > chloroform extract (Tables 3 and 4).

Table 3

Antioxidant potential of hexane, ethyl acetate, chloroform, butanol, methanol and water crude extracts from root samples of AO by Soxhlet method.

Concentration of crude extract (μ g/mL)	Water	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
200	0.45	0.86	0.52	0.47	0.59	0.36
100	0.41	0.68	0.37	0.39	0.40	0.35
50	0.43	0.59	0.43	0.39	0.41	0.38
25	0.33	0.44	0.31	0.34	0.32	0.32
12.5	0.34	0.47	0.34	0.86	0.35	0.36

Table 4

Antioxidant potential of hexane, ethyl acetate, chloroform, butanol and methanol crude extracts from root samples of AO by maceration method.

Concentration of crude extract (μ g/mL)	Methanol	Hexane	Ethyl acetate	Chloroform	Butanol
200	11.08	10.61	11.28	4.80	9.25
100	29.84	6.92	18.42	12.22	11.99
50	4.65	11.80	11.26	11.72	11.05
25	29.02	11.34	14.54	4.03	28.87
12.5	12.93	13.51	9.49	11.62	14.34

4. Discussion

Secondary metabolites phenolic compounds are widely presented in all plant kingdoms. The total phenolic contents of different crude extracts from the roots of AO are revealed (Table 1)[1]. The highest phenolics content among the six different root crude extracts through both methods was in methanol and ethyl acetate while the minimum was found in hexane and methanol respectively (Table 1). However, the total flavonoid contents were determined by aluminum chloride method. The highest flavonoids content among the six different root crude extracts through both methods was in methanol and hexane crude extracts while the minimum was found in butanol and methanol as well as chloroform respectively (Table 2)[1]. Almost, the similar total phenolic and flavonoid results were obtained by the other authors from the root crude extracts of AO[13].

The antioxidant activity of each six different crude extracts from roots of AO was evaluated by free radical scavenging activity (DPPH) method[1]. The activity of the different polarities crude extracts through DPPH method are presented. The results of antioxidant activity of different crude extracts from the roots of AO by Soxhlet method were equivalent to DPPH in these extracts in the same order: methanol > chloroform > hexane > ethyl acetate > water > butanol. Similarly, the antioxidant activity of crude extracts by maceration method was equivalent to DPPH in these extracts in the same order: ethyl acetate > methanol > hexane > butanol > chloroform extract. It has been found that this plant contains several bioactive compounds such as glycosides, alkaloids, steroidal saponins, flavonoids, oligosaccharides, and amino acid derivatives[10-15]. Their color gradually was faded by DPPH and finally completely decolourised by α, α -diphenyl- β -picrylhydrazyl by their hydrogen donating ability[2,8-11]. From the results, it appears that the six crude extracts from the roots possess hydrogen donating capabilities and it will be acting as an antioxidant. The difference of obtained antioxidant results might possibly be the results of the different extraction solvents and methods. Maybe in the process of the sample handling and the sample drying, some volatiles in the active compounds were destroyed or evaporated from the samples.

The crude extracts from the roots of AO showed the high quantity of total phenolics and flavonoids contents. All the crude extracts from this plant also showed very high percentage of antioxidant activity by DPPH method. Therefore, all the crude extracts from this plant could be used as a medicine for the treatment of different diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Prof. Dr. Nafsiah Binti Shamsudin, Dean, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman for her continuous encouragement during the

work. Thanks to Sommya Saif Said Al Riyani, Lab Technicians, Natural Product Lab, University of Nizwa for her continuous help during the experiment. Authors wish to thank University of Nizwa, Sultanate of Oman for providing financial support (Grant No. Ref. No. A/13-14-UoN/04/CPN/IF) for the completion of this project.

References

- [1] Hossain MA, AL-Raqmi KA, AL-Mijizy ZH, Weli AM, AI-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac J Trop Biomed* 2013; **3**(9): 705-10.
- [2] Tijjani A, Sallau MS, Sunus I. Synergistic activity of methanolic extract of *Adenium obesum* (Apocynaceae) stem-bark and oxytetracycline against some clinical bacterial isolates. *Bayero J Pure Appl Sci* 2011; **4**(1): 79-82.
- [3] Hossain MA, AL-Mijizy ZH, AI-Rashdi KK, Weli AM, AI-Riyami Q. Effect of temperature and extraction process on antioxidant activity of various leaves crude extracts of *Thymus vulgaris*. *J Coast Life Med* 2013; **1**(2): 130-4.
- [4] Tijjani A, Ndukwue IG, Ayo RG. Studies on antibacterial activity of *Adenium obesum* (Apocynaceae) stem-bark. *Cont J Microbiol* 2011; **5**(1): 12-7.
- [5] Ghazanfar SA. *Handbook of Arabian medicinal plants*. Florida: CRC Press; 1994.
- [6] McLaughlin J, Garofalo J. The desert rose, *Adenium obesum*: nursery production. Florida: Miami-Dade Cooperative Extension; 2002.
- [7] Dimmitt MA, Hanson C. The genus *Adenium* in cultivation. Part 1: *A. obesum* and *A. multiflorum*. *Cact Succt J* 1991; **63**(5): 223-5.
- [8] Akharaiyi FC. Antibacterial, phytochemical and antioxidant activities of *Datura metel*. *Int J PharmTech Res* 2011; **3**(1): 478-83.
- [9] Anyasor GN, Ogunwenmo KO, Oyelana OA, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae). *Afr J Biotechnol* 2010; **9**(31): 4880-4.
- [10] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process* 2011; **89**(3): 217-33.
- [11] Hossain MA, Nagooru MR. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydalis terminalis* L. Kunth. *Pharmacogn J* 2011; **3**(24): 25-30.
- [12] Igbinsosa OO, Igbinsosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afri J Pharm Pharmacol* 2009; **3**(2): 58-62.
- [13] Mitchell MGW, Breyer-Brandwijk. *The medicinal and poisonous plants of Southern and Eastern Africa*. 2nd ed. Edinburgh: E & S Livingstone Ltd; 1962, p. 941-50.
- [14] Malebo HM, Tanja W, Cal M, Swaleh SAM, Omolo MO, Hassanali A, et al. Antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants. *Tanzan J Health Res* 2009; **11**(4): 226-34.
- [15] Ebrahim N, Kershi RM, Rastrelli L. Free radical scavenging activity and anthocyanin in flower of *Adenium obesum* collected from Yemen. *J Pharm Photother* 2013; **1**(5): 5-7.