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Original Article

Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant *Adenium obesum*

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ABSTRACT

Background: Medicinal plants constitute a natural reservoir for medicines worldwide. They serve mainstream therapeutics and are central in folklore medicine. In case of *Adenium obesum* (Lav, Apocynaceae), indigenous people of Oman use it for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pain as well as joint pain; yet, the active ingredients have not been classified. To screened the antioxidant and antimicrobial activities of an identified compound that we isolated from the highest active chloroform extract.

Methods: The antioxidant and antimicrobial activities of the extracts and the isolated compound were determined by diphenyl-1-picrylhydrazyl (DPPH) and disc diffusion methods. To characterize the compound, we used TLC, column, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, IR and MS.

Results: The highest antioxidant activity was found in chloroform extract with EC_{50} value of 28.32 μg/ml followed by water, methanol, butanol, ethyl acetate and hexane extracts, their IC_{50} being 29.95, 39.17, 42.40, 43.20 and 57.00 μg/ml respectively. All crude extracts and pure compound displayed moderate antimicrobial activity against one Gram positive *Staphylococcus aureus* and three Gram negative *Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris* within growth inhibition range of 0 −13 mm. The active metabolite was identified as 3,4-dihydroxycinnamic acid (*R*)-1-carboxy-2-(3,4-dihydroxyphenyl) ethyl ester which is a common plant ingredient known as rosmarinic acid.

Conclusion: The results indicate that walnut chloroform fraction may contain effective compounds with a broad spectrum of curative applications. This is the first report on isolation and characterization of a compound from chloroform crude extract of *A. obesum*.

1. Background

Adenium obesum (Lav, Apocynaceae) is a wild plant found in selected areas of Oman.[1] Plants in this family grow well in rocky and sandy soil.[2] Plants belonging to the genus *Adenium* occur mainly in dry bush land or woodland and wooded grassland up to 2100 m altitude. Now-a-day some species belonging to this family are commercially cultivated in Saharan Africa, Sudan, Kenya, Senegal and Swaziland due to their biological and medicinal importance.[2] Some rare species, including *A. obesum*, are available in the Arabian Peninsula. *A. obesum* is designated long-lived plant by virtue of its growth regulator being very slow. The plant is considered a small tree, as it grows up to four meters in height.[1] Some species of this plant have a fleshy taproot, and a stem swollen at its base reaching up to one meter in diameter. The bark is pale greyish-green, grey, brown, smooth, with sticky, clear or white latex, the branchlets glabrescent, pubescent at the apex. The leaves are arranged spirally, clustered at the end of branchlets.[3,4] The plant shows diversity of flower characteristics depending on environmental conditions such as rainfall, temperature, etc.[2] The shape, size and colour of flowers are completely different from each other where the plant grows.[1] A few species of this family are available in Oman being used by the local ethnic communities as a medicine for the treatment of different diseases.[5] Most of the species belonging to this family, including *A. obesum*, show medicinal values and they exude a milky sap.

A. obesum contains different chemical compounds such as alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and

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flavonoids.[3,6,7] As a medicine, the whole plant is used by different ethnic communities for the treatment of a variety of ailments including venereal diseases. The crude extracts from root and bark are used to prepare a lotion for the treatment of different skin diseases and to eliminate lice.[4] The latex is claimed to be a very good medicine for the treatment of decaying teeth and septic wounds[4] and in Somalia, it is traditionally used as nasal drops.[6] In Kenya, the stems and barks powder is used for eliminating skin parasites in camels and cattle[5] and in India, the bark of *A. obesum* is used as an abortifacient.[5,6] In Nigeria, the whole plant is used as antiplasmodial, anti-trypanosomal and anti-leishmanial drug.[1,2] However, Omani ethnic community use this plant for the treatment of venereal diseases, wounds, skin diseases, headaches, muscle pain and joint pain.[5] Limited information is available regarding the biological activity of this species since there is lack of extensive work on analysis of Omani species of *A. obesum*.

Therefore, in this paper, we describe the isolation and structure elucidation of a pure compound characterized as 3,4-dihydroxycinnamic acid (*R*)-1-carboxy-2-(3,4-dihydroxyphenyl) ethyl ester (**1**, rosmarinic acid) from stem-bark of *A. obesum* grown in Oman using different spectral techniques, showing antimicrobial activities using selected microbes. To our best knowledge, this is the first study to isolate and characterize this compound from the chloroform crude extract of stem-bark of *A. obesum*.

2. Methods

2.1. General

Chloroform, ethyl acetate, ethanol, methanol, butanol, DPPH (diphenyl-1-picrylhydrazyl), silica gel GF_{254}, were obtained from Sigma Chemical Company, UK. Silica gel (60–120 mesh), dimethyl sulphoxide (DMSO), potassium bromide (IR grade), deuterated chloroform and amoxicillin were collected from E. Merck, Germany. Evaporation was performed under reduced pressure on a rotary evaporator (Yamato Rotary Evaporator, Model RE 801, Japan). Melting point was determined on an electrochemical micro-melting point apparatus (Gallenkamp). ^{1}H-NMR spectra were recorded on a Bruker (600 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts δ, ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). IR spectra were recorded (KBr discs) on a FT-IR spectrometer, validation (υ_{max} in cm^{-1}). Mass spectra (MS) were recorded on Waters Quattro Premier XE Tandem Quadrupole system (Waters Inc. USA) with ESI^+ technique.

2.2. Microorganism

The microorganisms used in this study include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris* which were collected from Nizwa Hospital, Nizwa, Sultanate of Oman on March 14, 2014.

2.3. Plant materials

The stem-bark samples were collected from Al Mughsayl, Salalah, Sultanate of Oman during the month of November 29, 2013. The plant was identified by Ismail Al-Rashdi, Horticulture Senior Specialist, Ministry of Agriculture, Sultanate of Oman and voucher specimens No. 175 was deposited at Herbarium of this Ministry. The plant species was photographed for documentation and further taxonomic identification at Natural Product Laboratory, School of Pharmacy, University of Nizwa, Sultanate of Oman.

2.4. Processing of samples

The collected samples were washed and dried under sunlight for seven days and further dried under sunlight for seven days more after slicing to achieve complete dryness. The samples were then ground into a coarse powder by a ball mill. The powdered samples were preserved in clean polyethylene bags and kept away from light, heat and moisture until analyzed.

2.5. Extraction

Powdered dry stem-bark samples of *A. obesum* (70 g) were extracted with methanol by using Soxhlet extractor for period of 36 h. The extract was filtered through Buchner funnel with Whatman filter paper No. 1. After complete filtration, the methanol solvent was evaporated under reduced pressure at 24 °C using a rotary evaporator and the extract (9.17 g) was then suspended in water (150 ml). The whole mixture was transferred into a separatory funnel and extracted successively with differently polar solvents to give hexane (2.3 g), chloroform (2.68 g), ethyl acetate (1.51 g), butanol (1.32 g) and water (0.93 g).[5]

2.6. Antioxidant activity

Antioxidant activity in different polarities of stem-bark crude extracts of *A. obesum* was measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) with slightly modified methods of Hossain et al.[8] Approximately (2 mg) of each polarity crude extract of *A. obesum* was placed in a test tube and dissolved in methanol (10 ml). Different concentrations (12.5, 25, 50, 100 and 200 mg/L, respectively) were prepared by the addition of methanol. 0.004% of DPPH was prepared by addition of methanol. 300 μl of each concentration crude extract was taken in a separate test tube to which 3 ml of DPPH solution was added and shaken by hand. All test tubes were incubated in a dark place for one and half hour. The absorbance of all incubated concentrations was measured by UV-visible spectroscopy at wavelength 517 nm. The antioxidant activity of each concentration of crude extracts of *A. obesum* was calculated by using a standard formula.

$$\% \text{ Inhibition} = \text{A control} - \text{A extract/A control}^*100$$

2.7. Antimicrobial activity

Antibacterial activity of different polarities crude extracts and the isolated compound of stem-bark of *A. obesum* were measured against one Gram (+) bacteria *S. aureus* and three Gram (−) bacteria *E. coli, P. aeruginosa* and *P. vulgaris* on nutrient agar plates using disc diffusion method with modification.[5,9] Different concentrations (2, 1, 0.5, and 0.25 mg/ml) of each stem-bark crude extracts of *A. obesum* were prepared by the addition of DMSO solvent and for the pure compound two concentrations (0.5 and 1 μg/ml) were prepared by same solvent. Positive control was also prepared by the addition of DMSO solvent. Whatman filter paper was used as a disc of 6 mm diameter. The discs were impregnated with the prepared concentration of each polarity stem-bark crude extracts and the pure compound of *A. obesum* and then placed on the inoculated agar plates. The ready discs were incubated at 37 °C for 24 h. The diameter of the zones of inhibition against the tested bacteria was measured and compared with broad spectrum antibiotics amoxicillin. Each method in this experiment was replicated three times.

2.8. Isolation of antimicrobial compound from chloroform extract

The chloroform crude extract (2.50 g) was subjected to column chromatography on silica gel eluted with ethyl acetate-hexane