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In vitro Antioxidant, Cytotoxic and Antibacterial Screening of the leaves of *Acridocarpous orientalis,* Native to Sultanate of Oman

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAA and HAM managed the laboratory work. Author AW designed the study, managed results analysis, and wrote the manuscript. Authors MSA, AH and SS guided the lab work, managed results analysis and wrote the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine antioxidant, cytotoxic and antimicrobial activities of organic extracts from leaves of *Acridocarpous orientalis* (qafas) from Sultanate of Oman.
Study Design: Brine shrimp test, DPPH assay and Disc diffusion method.
Place and Duration of Study: School of Pharmacy, University of Nizwa, Oman, December 2012.
Methodology: Hexane, chloroform, ethylacetate and hydroalcoholic extract were obtained by Kupchan's partitioning of ethanol extract isolated from leaves of *A. orientalis* by maceration. Antioxidant activity was determined by free radical scavenging of (2,2-diphenyl-1-picrylhydrazyl, DPPH). The antimicrobial activity was checked using agar disc diffusion method against Gram-positive bacteria (*Staphylococcus aureus)* and two Gram-negative bacteria *(Escherichia coli* and *Pseudomonus aeruginosa)*. Brine shrimp test was used to measure cytotoxic activity.
Results: All extracts demonstrated potential antioxidant activities, hydroalcoholic extract showed the strongest activity (RC_{50} = 6.11 μg/ml). The order of antioxidant activity was hydro alcohol > ethyl acetate > chloroform > hexane extract. Ethylacetate extract showed low activity against *Pseudomonus aeruginosa.* None of the extracts was found to be

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active against brine shrimp larvae.
Conclusion: *A. orientalis* could be considered as a good source of antioxidant compounds.

Keywords: Aridocarpous orientialis; qafas; DPPH; Oman; antimicrobial; antioxidant; cytotoxic.

1. INTRODUCTION

Acridocarpous orientalis A. Juss (qafas) is a plant species in Malpighiaceae family. It is endemic to the Arabian Peninsula and horn of Africa [1]. In Oman, the plant is fairly distributed in Central and Norhtern regions especieally in area surrounding Jabal Shams [2]. It is the dominant species over whole areas of the bare exposed south face of Jabal Shams growing in rocky pockets up to about 1500 meters altitude [3]. The oil and crude extract of qafas leaves are widely used for chronic headaches, massaging paralyzed limbs and for muscle and tendon pains [4]. Dried seeds of this plant are soaked in water to remove the outer covering. The seeds are then ground into a coarse powder. Then, salt is added and the mixture kneaded with some water to extract the crude oil. The oil is applied on the head for headache and on the limbs and joints to relieve the pain. Cosmetically the oil is applied on face and body to make the skin soft [5]. Apart from a recent preliminary pharmacological analysis of aqueous ethanolic extract of *A. orientalis* collected from Al-Ain and Oman [6], no other biochemical investigation has been carried on this species. Furthermore, *Acridocarpus* species are know to be good source of compounds with varying pharmacological activities [7-10]. In view of the above, the present study was aimed to evaluate antimicrobial, antioxidant and cytotoxic properties of *A. orientalis* grown in Oman.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

All solvent used were of analytical grade. Absolute ethanol, hexane, chloroform, ethyl acetate, and 2,2-diphenyl-1-picrylhydrazyl, (DPPH) were obtained from Sigma chemical company. Gram negative bacteria, *Escherichia coli* (ATCC 9637) *Pseudomonus aeruginosa* (ATCC 9027) and gram positive bacteria *Staphylococcus aureus* (ATCC 29213) were obtained from microbiology department, College of Arts and Sciences, Nizwa University. Filter paper discs (diameter 6 mm) were obtained from Whatmann Company, Catalogue number: 8174900. Nutrient agar and plastic Petri dishes were from Sharlau Chemie company. Brine shrimp eggs (ARTEMIA CYSTS) were purchased from GOAQUA, Taiwan. Sea Salt was obtained from Al-Qurum Muscat. Optical density was recorded using, Thermo spectronic, UV spectrometer Model Biomate (Great Britain). All solvent were evaporated using Yamato Rotary Evaporator, Model RE 801. Agar plates were incubated using Gen Lab incubator Model: MINO/75F.

2.2 Plant Collection

The leaves of *Acridocarpous orientalis* sample was collected from Rustaq, Sultanate of Oman. A photograph of the sampled plant was taken and a voucher specimen was deposited at the Department of Biology, Sultan Qaboos University, Oman. The sample was then taken to the laboratory, washed with running tap water to remove the dust and any

other foreign materials. The leaves were dried under shade at room temperature (25 ± 1ºC) for 2 weeks.

2.3 Extraction and Preparation of Organic Fractions

The dried leaves of *Acridocarpous orientalis* (295.11 g) were macerated in ethanol for 7 days. The solvent was then filtered out and evaporated at low pressure using rotatory evaporator to give viscous semi solid masses. The crude extract was suspended in ethanol:water mixture, 1:1 ratio and then extracted successively with hexane, chloroform, and ethyl acetate, All solvents were later removed under vacuum using rotatory evaporator.

2.4 DPPH Assay

Free radical scavenging activity of different organic extracts was estimated as described by Blois [11] with minor modification. Five concentrations 12.5, 25, 50, 100 and 200 µg/ml, for each of the four extracts (hexane, chloroform, ethyl acetate and hydro-ethanol) were prepared in methanol. An aliquot (4ml) from each concentration was placed in a test tube to which one milliliter of 0.1 M methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added and shaken vigorously. All the test tubes were then allowed to stand at 27ºC in dark place for 45 min. The control was prepared in the same way but without adding extract. The absorbance of the prepared samples was measured using UV spectrophotometer at 517 nm. Radical scavenging activity of the tested crude extracts samples was estimated as the percentage inhibition and was calculated by using the following formula,

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \text{ X } 100$$

Percent inhibition data were further analyzed using EXCEL to generate RC_{50} values (concentration that causes 50% reduction in absorbance).

2.5 Antibacterial Screening

The antibacterial test was carried out by agar disc diffusion method [12]. Each extract was subjected to serial dilution technique, using dimethylsulphoxide as a solvent to give concentrations of 2000, 1000, 500, and 250 µg/ml. Filter paper discs (6 mm diameter) were impregnated with each concentration and placed on the agar plates inoculated with the bacteria. Negative controls were prepared using the same solvents employed to dissolve the samples. The plates were incubated micro aerobically at 37ºC for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Each assay was done in triplicate.

2.6 Brine Shrimp Test

Brine shrimp (*Artemia salina* Leach) larvae were used as indicator animal for preliminary cytotoxicity assay as described by McLaughlin and his group [13].

Shrimp larvae were hatched in artificial sea water prepared by dissolving 38g of sea salt in distilled water (IL). The sea salt was placed in a small tank divided into two compartments by perforated polythene wall. About 50 mg of GOAQUA brine shrimp eggs were sprinkled at

one compartment which was later covered to create dark environment. The open compartment was illuminated to attract the shrimp larvae from the dark compartment once were hatched within 24 hours.

Solutions corresponding to 10, 100, 250, 500, 750 and 1000 mcg/ml were prepared in six vials by serial dilution of the stock samples (10mg/ml). Each experiment was done in triplicate. A total of 10 larvae were transferred in each vial and the solutions were diluted to 5 ml by adding the artificial sea water. Mean percent mortalities of the larvae were calculated after 24 hours of exposure.

2.7 Phytochemical Screening

Phytochemical analysis was done as described by Jones and Kinghorn [14]. Experimental procedures for each chemical group is as provided below.

Alkaloids: Presence of alkaloids was detected using the Dragendorff and Wagner reagents.
Flavonoids: Detection of flavonoids was afforded using Shinoda and sulfuric acid tests.
Tannins/Polyphenols: ferric chloride test.
Sterols: Sterols will be detected using Liebermann–Burchard test and Salkowski reaction.
Saponins: Analysis of saponins used frothing test.
Protien: Millions reagent test.
Carbohydrates: Molisch reagent test.
Anthraquionone: Borntrager test.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

Fig. 1 shows the DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical scavenging activity of the different successive concentration of extracts of *A. orientalis*. The activity is increasing with increase in the concentration of the sample extract (Fig. 1).

The RC_{50} values of each extract are shown in Table 1. The hydroalcoholic extract possess the highest scavenging activity (RC_{50} = 6.11μg/ml). Also presented in Table 1 are the amounts of each extract as percentage of the dry leaves materials. The plant leaves has low amount of medium polar compounds as compared to non-polar and polar materials. The amount of medium polar extracts (chloroform and ethylacetate) together is only half that of non-polar (hexane) or polar (hydro-alcoholic) extracts.

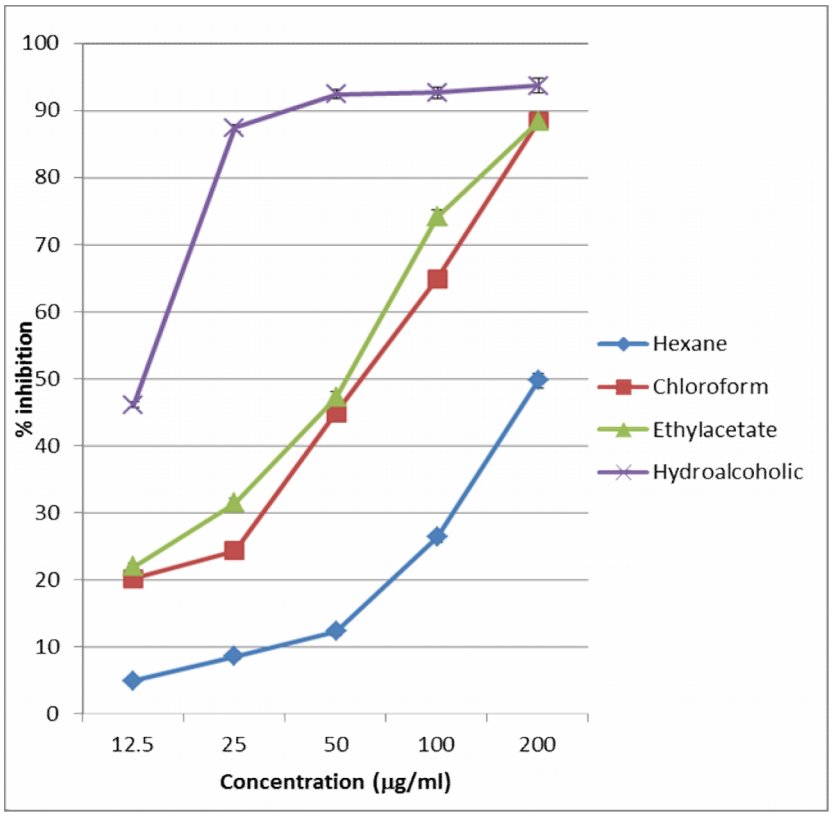

Fig. 1. Antioxidant activity of the different successive concentrations of extracts

Table 1. Antioxidant activity RC50 (µg/ml) and amount of organic materials of *A. orientalis* leaves extract

Concentration (µg/ml)	% inbibition			
	Hexane	Chloroform	Ethylacetate	Hydroalcoholic
12.5	5.01±0.3	20.22±0.5	22.04±0.5	46.17±0.4
25	8.60±0.5	24.35±0.6	31.46±0.7	87.44±0.5
50	12.34±0.7	44.95±0.7	47.27±0.8	92.45±0.9
100	26.39±0.8	64.95±1.0	74.16±1.1	92.67±1.0
200	49.75±1.1	88.37±0.9	88.38±1.2	93.72±1.1
RC_{50}	200.99	81.43	69.86	6.11
% yield	0.81	0.26	0.14	0.70

The principle of antioxidant activity in their interaction depends on oxidative free radicals. The main role of DPPH method is that the antioxidants react with the stable free radical i.e., α,α-diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-diphenyl-β-picrylhydrazine with discolouration. The gradually discolouration indicates the scavenging capacities of the crude sample antioxidant such as phenolic compounds and derivatives [11]. In the present study the four different crude extracts from the locally grown *Acridocarpous orientalis* were able to decolourise DPPH and the free radical scavenging capacities of the crude extracts were found to be in the order of ethanol extract > chloroform extract > ethyl

acetate extract >hexane extract. In present study may be it appears that the five different crude extracts from the leaves of *Acridocarpous orientalis* possess hydrogen donating capabilities to act as antioxidant. Our results are similar to what was reported by Ksiksi and Hamza on aqueous ethanolic crude extracts of *A. orientalis* collected from Al-Ain and Oman [6]. However, our samples displayed more potent antioxidant activity (RC_{50} = 6.11 μg/ml) than the Oman sample analyzed in their study, (RC_{50} = 32.44 μg/ml). This observation suggests that plant bioactivity is influenced by geographic location of the plant among other factors.

3.2 Antimicrobial and cytotoxic activities

In vitro inhibition of different microbial strains by various extracts of *A. orientalis* are presented in Table 2. Ethylacetate extract was active against *P. aeruginosa* with inhibition zones ranging from 6 – 9 mm at concentration of 500 – 2000 mM. Hexane, chloroform and hydroalcoholic extracts were inactive against strains of all tested organisms. Furthermore, none of the four extracts showed cytotoxic activity against the brine shrimp larvae.

Table 2. Inhibition of bacterial growth by *A. orientalis* extracts (inhibition zones, mm)

Extract	Concentration μg/ml	*E. coli*	*P. aeruginosa*	*S. aureus*
Hexane	2000	ND	ND	ND
	1000	ND	ND	ND
	500	ND	ND	ND
	250	ND	ND	ND
EtOAc	2000	8m	9mm	ND
	1000	ND	8mm	ND
	500	ND	6mm	ND
	250	ND	ND	ND
CHCl3	2000	ND	ND	ND
	1000	ND	ND	ND
	500	ND	ND	ND
	250	ND	ND	ND
Hydro-alcoholic	2000	ND	ND	ND
	1000	ND	ND	ND
	500	ND	ND	ND
	250	ND	ND	ND
Control	DMSO	ND	ND	ND

ND = Not Detectable, CHCl3 = Chloroform, EtOAc = Ethyl acetate

The *in vitro* antibacterial activity of hexane, chloroform, ethyl acetate and hydro-alcoholic extracts of *Acridocarpous orientalis* against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones. This is the first report on antimicrobial assay of organic extracts obtained from *A. orientalis*. Only ethylacetate extract of *A. orientalis* showed weak activity against strains of *P. aeruginosa*. These results are contrary to what has been documented with other Acridocarpus species. For instance, *A. socotranus* which is also endemic to the Arabian gulf, found only in the Soqotra island in Yemen displayed potential antimicrobial activities against a series of Gram-positive bacteria strains including *Micrococuss flavus* and *Staphylococcus aureus* as well as multiresistant *Staphylococcus* strains such as, *Staphylococcus epidermidis* 847, *Staphylococcus*

haemolyticus 535, and *Staphylococcus aureus* North German Epidemic Strain. Methanol and aqueous extracts of *A. socotranus* were reported to shown inhibition zones ranging from 10 – 22 mm [14].

Hexane, chloroform, ethyl acetate and hydro-alcoholic extracts obtained from *A. orientalis* showed no cytotoxic activity against brine shrimp larvae. These results are similar to what obtained from other Acridocarpus species growing in the region. For example *In vitro* assay of methanolic extract of *Acridocarpus socotranus* from Yemen demonstrated no cytotoxic activity against various cell lines [15]. However, other Acridocarpus speicies such as *A. vivy* from Madagascar are known to be active against some cancer cell lines [8].

3.3 Phytochemical Screening

Results for phytochemical screening of different extracts of *A. orientalis* are presented in Table 3. Phytochemical screening revealed the presence of both primary and secondary metabolites including, carbohydrates, phenolic compounds and taninns, flavonoids and saponins.

Table 3. Constituents identified in phytochemical screening

S. No.	Constituents	Results
1	Alkaloids	-ve
2	Carbohydrates	+ve
3	Phenolic compounds and taninns	+ve
4	Flavonoids	+ve
5	Proteins and amino acids	-ve
6	Saponins	+ve
7	Resins	-ve
8	Anthraquinones	-ve

-ve = absent; +ve = present

4. CONCLUSION

Organic extracts from a rare plant, *A. orientalis* from Oman possess potent radical scavenging activity. The extracts have also weak antimicrobial activity and were not displaying any cytotoxicity. *A. orientalis* might be a good source of antioxidant compounds. It is recommended to further investigate this species to isolate the active ingredients.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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