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Research Article
Antimicrobial, Antioxidant and Cytotoxic Activity of *Maytenus dhofarensis* Growing in Oman

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Abstract

Background and Objective: The emergence of resistance to the presently used anti-infective and anticancer agents by microbials and neoplasm raises an urgent need to search for new and more potent chemotherapeutics that will work in different mechanisms. Investigation reported in this document is a contribution to the global effort towards that goal. To investigate the antimicrobial, antioxidant and cytotoxic activity of organic extracts of *M. dhofarensis*. **Materials and Methods:** Powdered stem bark of *M. dhofarensis* was macerated in ethanol to give crude extract that was Kupchan partitioned into hexane, chloroform, ethylacetate and hydroalcoholic fractions. Antimicrobial activity was determined using disc diffusion method against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Cytotoxic activity of the extracts was tested using brine shrimp test while that of pure compounds were assayed against Ovarian (MCAS) and breast (MDA MB231) cancer cell lines using Alamar blue assay. Pure compounds were isolated by chromatographic methods. Antioxidant activity was determined using DPPH assay. Total phenolic and flavonoid contents of each extract were evaluated using standard methods. **Results:** All extracts showed cytotoxic activity against brine shrimp larvae, hexane extract was the most active (LC_{50} = 5.15 μg mL^{-1}). However, they showed only mild antimicrobial activity against tested micro-organisms. Furthermore, all extracts possessed radical scavenging activity against DPPH. The order of scavenging activity was hydroalcoholic>ethyl acetate>chloroform>hexane. The highest concentration of phenols was seen in the ethyl acetate (167.65 μg of gallic acid/mg of dried extract). The major metabolite isolated from hexane extract was identified as taraxerol. However, this compound showed weak *in vitro* cytotoxic activity against the tested cancer cell lines. **Conclusion:** Organic extracts from *M. dhofarensis* possess excellent cytotoxic activity and strong radical scavenging activity. A further investigation on this species to isolate the bioactive metabolites is worthwhile.

Key words: *Maytenus dhofarensis*, *Sherbith*, cytotoxic, antimicrobial, antioxidant, taraxerol

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The field of chemotherapy is among the main stay for control of human maladies and reducing world health burden. Resistance to antibiotics and cytotoxic drugs is currently a great challenge in control of cancer and infectious diseases. In this regard there has been global effort aimed to provide new, effective and safe therapy to affected population worldwide to improve their quality of life. Secondary metabolites are among the major sources of new chemical entities for development of new drugs including antimicrobial and anticancer agents[1]. Terrestrial plants have been recognized as among the main sources of bioactive metabolites based on their ethnomedical history[2].

Maytenus dhofarensis (sherbith) is among the perennial plant of Dhofar that go under the common name of spike thorn belonging to the family Celastraceae[3]. Biomedical studies on the genus Maytenus have revealed various biological properties in materials obtained from its species. Pharmacological activities of species belonging to this genus include cytotoxicity, antimicrobial, antiprotozoal, anticoagulant, anti-inflammatory, antioxidant, proto scolicidal and insecticidal activities[4]. Like many Celastraceae species, *Maytenus* plants are popularly used in traditional medicine for treatment of many diseases, including chest pains, rheumatism, snakebites, diarrhea, eye infection and dyspepsia (indigestion). They are also used as an antibacterial, antimicrobial and antibiotic. Preparations from the roots and barks of *Maytenus* species are used for severe headaches, an analgesic, for skin rashes, muscle spasms, excessive sweating, fevers, parasitic intestinal infections, as an anti-inflammatory, for arthritis and muscle pain, for nausea, vomiting and diarrhea. The leaves are used for malaria, yellow fever and trypanosomiasis. They are also used for fertility problems, venereal diseases, pneumonia and epilepsy, as well as tonic, for asthma and as aphrodisiac[5,6].

Maytenus dhofarensis is endemic to Dhofar and is traditionally used in Oman for wound healing, as antiseptic, ophthalmic defects, as deodorant as well as soap[7]. This plant is in the 'International Union for Conservation of Nature and Natural Resources' IUCN Red List of Threatened Species. It is threatened by habitat loss[8]. Despite the popular use of this species as a medicinal plant, there are no data about its phytochemical or pharmacological actions. This study reports the evaluation of antibacterial, antioxidant and cytotoxic activities of *M. dhofarensis* as a contribution to the global efforts on search of new anticancer and anti-infective agents.

MATERIALS AND METHODS

Materials: All chemicals and reagents were of analytical grade thus were used without further purification. Chloroform, methanol and dimethyl sulfoxide (DMSO, purity 99%) were obtained from Fisher Chemical. Hexane was obtained from Daejung, Korea. Ethyl acetate was obtained from Carbon group (Ireland). The 2,2-diphenyl-1-picrylhydrazyl, Folin Ciocalteu, ferric chloride, sodium acetate, sodium carbonate, quercetin and gallic acid were obtained from Sigma Aldrich 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 Laboratory, College of Arts and Sciences, University of Nizwa. Filter papers were obtained from Whatman, UK. Silica gel and TLC plate were obtained from E. Merck Germany. Brine shrimp eggs (ARTEMIA CYSTS) were purchased from GOAQUA, Taiwan. Sea salt was obtained from Al-Qurum, Muscat.

Plant collection: The stem bark of *M. dhofarensis* was collected on 28th September 2014 from Salalah, Dhofar governorate, Sultanate of Oman. The plant was identified by Dr. Amina Al-Farsi a taxonomist at Life Science Unit, Department of Biology, Sultan Qaboos University (SQU). A specimen of the plant with voucher number Al-Farsi, A. 566 has been deposited at the Herbarium of SQU. The barks were transported to the lab washed with tap water to remove dust and dirt, then dried in shade at room temperature for 1 week. The dried bark was ground to give coarse powder.

Preparation of extracts: The powdered stem bark (59.14 g) was soaked twice in ethanol for 7 and 2 days, respectively. The filtrates obtained at the end of each soaking were combined and the solvent was removed at low pressure to give crude ethanol extract. The crude extract was later Kupchan's partitioned in aqueous ethanol to give four fractions including hexane, chloroform, ethyl acetate and hydroalcoholic.

Antibacterial assay: The antibacterial activity was determined by using agar disc diffusion method against *E. coli*, *P. aeruginosa* and *S. aureus*. Each extract was tested at four concentrations including 2, 1, 0.5 and 0.25 mg mL^{-1}. Cloxacillin 0.03 mg mL^{-1} was used as a standard solution. All preparations were done in DMSO. Filter paper discs (6 mm) impregnated with different concentrations of the extracts were placed on agar plates that were inoculated with different

strains of the selected Gram-positive and Gram-negative bacteria. The agar plates were then incubated for 24 h at 37°C. Antibacterial activity was evaluated as inhibition zone against the tested bacteria and was measured as diameter of inhibition minus the disc diameter[9].

Radical scavenging assay: The antioxidant activity of the extracts was determined by DPPH (2,2-diphenyl-1-1-picrylhydrazyl) using the method of Brand Williams with some modifications[10]. Each extract was assayed at five concentrations viz. 40, 60, 80, 100 and 200 µg mL^{-1} prepared in methanol. Radical scavenging activity of the tested concentrations was estimated as the inhibition percentage by using the following formula:

$$RSA\ (\%) = \frac{A_o - A_s}{A_o} \times 100$$

where, A_o is absorbance of control and A_s is absorbance of test sample.

Determination of total phenolic contents: The concentration of phenolic compounds in plant extracts was estimated using Folin-Ciocalteu's method as described by Cindric *et al.*[11].

Determination of total flavonoid concentrations: Romanian Pharmacopoeia method was used for the determination of the flavonoid contents in the different plant extracts. Quercetin was used as reference compound. In each experiment, 1 mL of plant extract was mixed with 5.0 mL of sodium acetate (100 g L^{-1}), 3.0 mL of aluminium chloride (25 g L^{-1}) and diluted with dist. water to make 25 mL in a graduated volumetric flask. The absorbance was then recorded at 430 nm after 15 min. The blank sample was prepared in the same manner without plant extract. Standard quercetin solutions (0.02-0.2 mg mL^{-1}) were prepared in the same pattern for generating a calibration curve. The concentrations of total flavonoids were hence, determined as quercetin equivalents[12].

Cytotoxicity assay: Cytotoxicity assay was done using brine shrimp (*Artemia salina*), commonly known as 'sea- monkeys' as indicator organisms[13]. Each extract was tested at four different concentrations including. The LC_{50} value of each sample was determined by FinneyProbit analysis of the percent mortality values concentrations 1000, 500, 250 and 125 microgram per milliliter after the word including[14].

Isolation and bioassay of taraxerol: Gradient elution of hexane extract (0.9 g) on silica gel column using hexane with increasing percent of ethylacetate gave 63 fractions. Vials 14-26 which were collected while eluting the column with 5% EtOAc/hexane gave a white precipitate upon evaporation of solvent. The precipitate was recrystallized using hexane ethyl acetate mixture to give pure taraxerol as white crystals. M.p. = 283°C, IR (KBr): vmax3310, 2945, 2867, 2852, 1463, 1360, 1190, 1035, 995 cm^{-1}, 1H-NMR ($CDCl_3$) and 13C-NMR ($CDCl_3$) data (Table 4), HRESIMS m/z 425.3760 $[M-H]^+$, Calcd m/z 425.3778. The isolated compound was tested against Ovarian (MCAS) and breast (MDA MB231) cancer cell lines using Alamar blue assay as we describe previously[15].

RESULTS AND DISCUSSION

Yields of extracts: The amount of the crude ethanol extract was 9.88 g (16.7%) of the dried stem bark (59.14 g). The yields of different fractions obtained from the ethanol extract in comparison to the dried stem bark (59.14 g) are shown in Table 1. Out of the four prepared fractions the hydroalcoholic extract gave the highest yield 10.59%.

Antimicrobial activity: Results for antibacterial activities of hexane, chloroform, ethyl acetate and hydroalcoholic extracts of the stem bark of *M. dhofarensis* are shown in Table 2. In general all extracts showed little antimicrobial activity against strains of the tested bacteria, maximum inhibition zone (IZ) was 10 mm at the highest concentration of 2 mg mL^{-1}. Gram-positive bacteria *S. aureus* was more susceptible to different concentration of extracts compared to other bacteria strains Table 2. Furthermore, hexane and chloroform extracts were the most active, the responding strains were susceptible to all concentrations of these extracts (IZ = 6-10 mm). Antibacterial results obtained in the present investigation were contrary to many antimicrobial studies on species belonging to the genus *Maytenus*[16]. Probably, extracts from *M. dhofarensis* lack compounds that were responsible for antimicrobial activity of other *Maytenus* species, corresponding to the fact that chemical constituents of plant species may vary depending on geographic locality.

Table 1: Comparison of the yields of different fractions prepared from ethanol extract to the dried stem bark of *M. dhofarensis* (59.14 g)

Extracts	Amount(g)	Yield (%)
Hexane	0.98	1.66
Chloroform	1.87	3.16
Ethyl acetate	0.30	1.07
Hydro-alcoholic	6.26	10.59

Table 2: Antimicrobial activity of different extracts from stem bark of *M. dhofarensis*

Extracts	Concentration (µg mL^{-1})	Inhibition zones (mm)		
		S. aureus	*E. coli*	*K. pneumoniae*
Hexane	2000	10	ND	6
	1000	8	ND	6
	500	7	ND	ND
	250	7	7	ND
Chloroform	2000	7	7	6
	1000	7	ND	ND
	500	7	ND	ND
	250	6	ND	ND
Hydroalcoholic	2000	ND	ND	ND
	1000	ND	ND	ND
	500	ND	ND	ND
	250	ND	ND	ND
Ethyl acetate	2000	10	ND	6
	1000	8	ND	6
	500	ND	ND	ND
	250	ND	ND	ND
Cloxacillin	30	28	28	25

*ND: Not detectable

Antioxidant property, total flavonoid and total phenolic contents: The results for radical scavenging activity of different extracts from stem bark of *M. dhofarensis* against DPPH radical are shown in Fig. 1. All four crude extracts with different polarities were able to gradually discolor and reduce the DPPH stable free radical. The scavenging potentials of these extracts were found to be in the order of hydroalcoholic = ethyl acetate>chloroform>hexane. The results indicated that the bark of *M. dhofarensis* contained high level of antioxidant compounds. Antioxidant property of hydroalcoholic and ethyl acetate extracts were almost similar to that of the standard compound, quercetin.

The amount of total phenolic content in each of the four extracts is shown in Fig. 2. As expected the highest amount of phenolic compounds were found in hydroalcoholic and ethyl acetate extracts (total phenolic contents varied from 16-167 µg Gallic acid/mg of DE). Furthermore, concentration of phenolic compounds in each extract correlated well with its radical scavenging property. Phenolic compounds can scavenge the DPPH free radical by donating the hydrogen that is required to reduce it to a neutral substituted hydrazine. The high radical scavenging activity of hydroalcoholic extract might also be attributed to presence of flavonoids in this fraction. The amount of total flavonoid in hydroalcoholic extract was 12.75 µg quercetin/mg of DE. Flavonoids can also scavenge free radicals by mechanisms similar to that of phenolic compounds. Apart from hydroalcoholic extract flavonoids were not detected in other prepared extracts (Fig. 2).

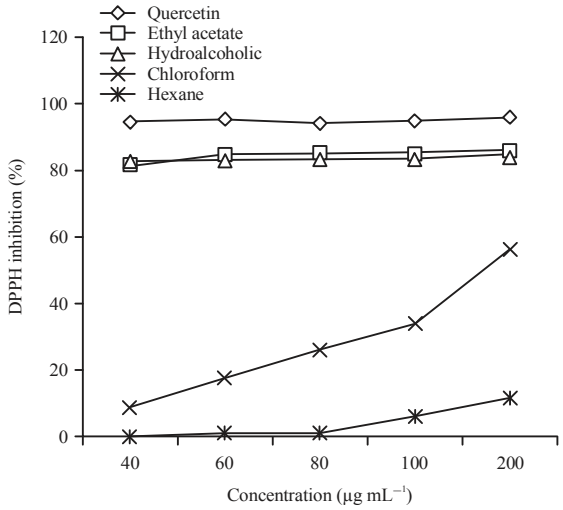


Fig. 1: DPPH scavenging activity of different organic extract from stem bark of *M. dhofarensis*

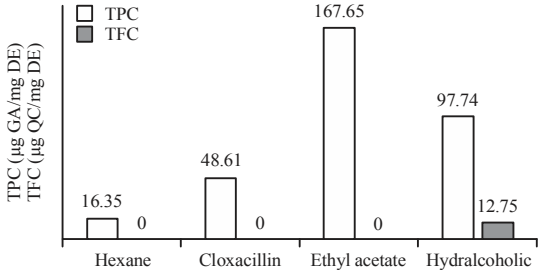


Fig. 2: Total phenolic content (TPC) and total flavonoid content (TFC) of different extracts from bark of *M. dhofarensis*
GA: Gallic acid, QC: Quercetin, DE: Dry extract

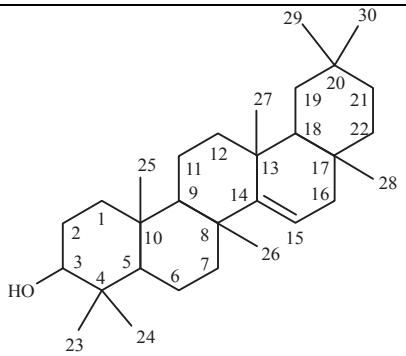
Antioxidant property of *M. dhofarensis* is similar to that of other species belonging to this genus. For instance, Gupta *et al.*[17] showed that the methanol extract of *M. emarginata* has significant, dose-dependent antioxidant activity against free radicals. Furthermore, the antioxidant activity observed in their study was also found to correlate with total amount of phenolic and flavonoid contents[17]. Another study by Shabbir *et al.*[18] showed that the therapeutic potential of *M. royleanus* leaves, in particular, methanol extract, ethyl acetate and n-butanol fraction against free-radical associated damages may be attributed to the high concentration of phenolic, flavonoid, tannins and terpenoids in this species.

Cytotoxic activity: The LC_{50} values of different extracts of *M. dhofarensis* against brine shrimp larvae are shown in Table 3. As can be seen from the table hexane extract possessed the highest cytotoxic activity against the test

Table 3: LC_{50} of different organic extracts from stem barks of *M. dhofarensis* (n= 10)

Extracts	LC_{50} (µg mL^{-1})
Hexane	5.15
Chloroform	47.08
Ethyl acetate	896.95
Hydroalcoholic	206.44

Table 4: 1H and 13C-NMR data of taraxerol



Taraxerol

Carbon No.	δ 13C ($CDCl_3$)	Multiplicity	δ 1H($CDCl_3$)
1	46.82	CH_2	1.92 and 0.94 (2H, m obs)
2	27.23	CH_2	0.79 and 0.94(2H, m, obs)
3	79.03	CH	3.20(1H,dd, J = 4.2 & 10.8 Hz)
4	38.77		
5	55.71	CH	0.71 (1H, dd, J = 1.8&11.5 Hz)
6	18.37	CH_2	1.37 and 1.52(2H, m, obs)
7	23.53	CH_2	1.87 and 0.82(2H, m, obs)
8	41.71		
9	47.63	CH	1.52 (1H, dd, obs)
10	32.45		
11	26.15	CH_2	1.09 and 0.80(2H, m, obs)
12	26.93	CH_2	1.97 and 1.74 (2H, m obs)
13	39.79		
14	145.19		
15	121.72	CH	5.16(1H,t,J= 3.66 Hz)
16	34.73	CH_2	1.49 and 1.3(2H, m, obs)
17	36.54		
18	47.22	CH	1.63(1H, obs)
19	37.13	CH_2	1.57 and 1.18 (2H, m, obs)
20	31.08		
21	32.64	CH_2	1.48 and 1.31(2H, m, obs)
22	38.58	CH_2	1.60 and 0.93 (2H, m, obs)
23	33.34	CH_3	0.85 (3H, s)
24	16.80	CH_3	0.97 (3H, s)
25	15.58	CH_3	0.93(3H, s)
26	25.99	CH_3	0.95 (3H, s)
27	23.69	CH_3	1.12 (3H, s)
28	15.49	CH_3	0.76 (3H, s)
29	28.40	CH_3	0.81(3H, s)
30	28.09	CH_3	0.85(3H, s)

organism (LC_{50} = 5.15 µg mL^{-1}) while ethyl acetate extract was the least active (LC_{50}>200 ppm, the cut-off concentration in cytotoxicity assay using BST). The order of cytotoxic activity was hexane>chloroform>hydroalcoholic>ethyl acetate extract. This result is comparable to other finding on *Maytenus* species such as a Brazilian plant *Maytenus salicifolia*[19].

Taraxerol: A quick chromatographic analysis of the hexane fraction afforded a taraxerol as a major component. However, this molecule was found to be inactive against ovarian (MCAS) and breast (MDA MB231) cancer cell lines. Observed *in vitro* IC_{50} values against these cell lines were 432.99 and 1097 µg mL^{-1}, respectively[15]. The structure of the isolated compound was elucidated using 1D-&2D-NMR data (Table 4) together with its high resolution ESIMS spectrum. All NMR data of the isolated compound matched well with those of taraxerol[20]. The isolated compound is thus, a triterpenoid known as taraxerol (Table 4).

CONCLUSION

Hexane, chloroform, ethylacetate and hydroalcoholic extracts of *M. dhofarensis* possess strong radical scavenging activity. The extracts also showed excellent cytotoxic activity against brine shrimp larvae with hexane extract exhibiting the highest activity. Taraxerol isolated from hexane was found inactive against ovarian (MCAS) and breast (MDA MB231) cancer cell lines. Furthermore, all extracts showed only mild activity against strains of tested gram +ve and gram -ve bacteria. Further investigations on extracts prepared from this species to isolate the bioactive metabolites will be worthwhile.

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