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# Antioxidant capacity of some edible and wound healing plants in Oman

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#### Abstract

An ethnobotanical review of the uses of plants in Oman led us to investigate some edible and wound-healing herbs for antioxidant activity, using in vitro DPPH and phosphomolybdenum assay methods. Of the 19 plants investigated, the aqueous ethanol extracts of Becium dhofarense, Pulicaria crispa, Allophylus rubifolius, Olea europaea, Acacia senegal, Pluchea arabica, Anogeissus dhofarica, Moringa peregrina, Cordia perrottettii, Ficus lutea and Acalypha indica showed the best inhibition of DPPH radical at 89–93%, after 15 min of incubation at a test concentration of 50  $\mu$ g/ml. The lowest IC<sub>50</sub>values of 4.45 and 7.11  $\mu$ g/ml were observed for the ethanol extracts of A. dhofarica and A. rubifolius. The highest total antioxidant capacity as gallic acid equivalents of 1790, 913, 814, 893 mg/g of ethanol extracts were obtained for P. crispa, O. europaea, M. peregrina and Caralluma quadrangula in the phosphomolybdenum assay. A. dhofarica an endemic plant, is active against Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. The uses of the plants are rationalised on the basis of their antioxidant capacity.

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Keywords: Antioxidant; DPPH; Wound healing; IC<sub>50</sub> values; Total phenolics; Gallic acid equivalents; Phosphomolybdenum method; Oman

### 1. Introduction

Reactive oxygen species are involved in a number of degenerative diseases such as artherosclerosis, cancer, cirrhosis and diabetes [\(Aboutwerat et al., 2003; Arouma,](#page-5-0) [1998; Baynes, 1991; Dreher & Junod, 1996](#page-5-0)), and also in wound healing [\(Bodeker & Hughes, 1998](#page-5-0)). Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH·) or super- $\alpha$ oxide radical (O<sub>2</sub>) scavengers ([Govindarajan, Vijayakumar,](#page-5-0) [& Pushpangadan, 2005; Robards, Prenzler, Tucker, Swai](#page-5-0)[tsitang, & Glover, 1999\)](#page-5-0). They are also strong chelators of metal ions ([Rice-Evans, Miller, Bolwell, Bramley, &](#page-5-0) [Pridham, 1995\)](#page-5-0). Thus, a practical way to control these diseases is to increase the dietary intake of fruits and vegetables, many of which are rich sources of antioxidants ([Demo, Petrakis, Kefalas, & Boskou, 1998; Proteggente](#page-5-0) [et al., 2002; Sun, Chu, Wu, & Liu, 2002](#page-5-0)).

The inhabitants of rural communities in the Arabian Peninsula traditionally use herbs to cure or suppress a variety of diseases and also as food and spices. The majority of lesser-known edible herbs of the Arabian Peninsula belong to the Amaranthaceae, Anacardiaceae, Araceae, Asclepiadaceae, Boraginaceae, Chenopodiaceae, Cruciferaceae, Capparaceae, Hydnoraceae, Iridaceae, Moraceae, Mimosaceae, Portulacaceae, Rhamnaceae and Sapindaceae families ([Heywood, 1997; Miller, Morris, & Stuart, 1988\)](#page-5-0). Very little information has been published on the antioxidant properties of the lesser-known edible vegetables and wound-healing plants of the Arabian countries [\(Elegami,](#page-5-0) [Almagboul, Omer, & El Tohami, 2001; Ljubuncic et al.,](#page-5-0) [2005](#page-5-0)), although several reports on the traditional uses of medicinal plants of Israel, Saudi Arabia, Oman, Yemen

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and the Socotra archipelago [\(Azaizeh, Fulder, Khalil, &](#page-5-0) [Said, 2003; Miller et al., 1988; Rahman, Mossa, Al-Said,](#page-5-0) [& Al-Yahya, 2004; Said, Khalil, Fulder, & Azaizeh,](#page-5-0) [2002\)](#page-5-0) are available. The Sultanate of Oman is one of the countries in the Arabian Peninsula. The Dhofar region in the extreme south west of Oman, lies within the monsoon belt and has wetlands and desert ecological regions with diverse plants, some of which are regionally endemic, edible or used to treat wounds ([Ghazanfar, 1994; Miller](#page-5-0) [et al., 1988\)](#page-5-0). The edible herbs are cooked or eaten raw and some are added to sauces, stews or tea, as fresh or dry leaves. The leaves of Acalypha indica, for instance, used to be sold in the market as a leafy green vegetable, steamed or eaten raw with a squeeze of lime. The young leaves of Moringa peregrina are also eaten as a vegetable. The tubers of Dorstenia foetida and Remusatia vivipara are cooked and eaten. The entire Caralluma quadrangula plant is edible; in some communities, the juice expressed from its stem is added to fresh milk as a general tonic.

The traditional ways of using herbs to heal wounds are remarkably similar; either as a poultice or expressed juice from fresh plants [\(Miller et al., 1988](#page-5-0)). The aqueous extracts of some herbs are used to clean and disinfect wounds. The leaves of some plants are flamed and used to dress injured skin, to stimulate healing and to ward off infection. The leaves of Allophylus rubifolius and Anogeissus dhofarica, for example, are applied as a paste around infected wounds [\(Miller et al., 1988\)](#page-5-0). The leaves of Withania qaraitica are crushed and mixed with castor oil to treat carbuncles, or crushed with garlic and applied as a poultice to burns or scorpion stings. The dried leaves of A. indica or the bark of Acacia senegal are made into a poultice to treat bedsores and wounds. The leaves of Becium dhofarense are crushed, mixed with water and used as shampoo, or extracted with warm water and used as skin lotion to treat sores and spots of measles; the leaves are also rubbed on skin to suppress allergies to stings by biting flies during the monsoon season. The juice from crushed leaves of B. dhofarense or Cordia perrottettii is applied to the eyes to soothe itching or treat eye injury; the juice from the bark of  $M$ . peregrina is used as a disinfectant and to speed up wound healing; the juice of A. indica is added to oil or lime and used to treat a variety of skin disorders and the juice expressed from the leaves of Pluchea arabica is applied to sores with or without onion. It is a painful but efficacious method of stimulating rapid wound healing. The latex of *Aloe dhufar*ensis is painted over sores and wounds of circumcision, to speed up healing and to prevent infection.

It thus appears appropriate to examine the polar extracts of the plants for antioxidant and antimicrobial properties.

This investigation was initiated to identify wound-healing plants and lesser-known edible plants in Oman that have high antioxidant capacity. The plants were extracted with 20% aqueous ethanol after a preliminary extraction with chloroform, and the extracts were screened for antioxidant activities using two in vitro assay models, the DPPH radical scavenging assay [\(Chen, Wang, Rosen, & Ho, 1999](#page-5-0)) and the phosphomolybdenum method [\(Prieto, Pineda, &](#page-5-0) [Aguilar, 1999](#page-5-0)). The total phenolics in the extracts were estimated according to the Folin–Ciocalteu method in alkaline medium, using gallic acid as a standard. Wound healing plants were further screened for antimicrobial properties, using the agar-dilution-streak antimicrobial assay ([Mit](#page-5-0)[scher et al., 1972](#page-5-0)).

## 2. Materials and methods

## 2.1. Chemicals

All the chemicals used were of analytical grade. 1,1- Diphenyl-2-picryl hydrazyl radical (DPPH- ), gallic acid, n-propyl gallate, tert-butylhydroxyanisole (BHA), tertbutylhydroquinone (TBHA) and  $\alpha$ -tocopherol were purchased from Sigma–Aldrich. Optical densities were recorded on a Cary 50 Conc UV–Visible spectrophotometer (Varian, North Carolina) and the solvents used for extraction were re-distilled. Wound-healing herbs were screened for antimicrobial activities against Escherichia coli ATCC 9637, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213 and Candida albicans ATCC 10231.

#### 2.2. Preparation of plant materials

Plant collections were made in October 2001 and September 2002 under the supervision of Dr. Annette Patzelt, Department of Biological Sciences, Sultan Qaboos University and Dr. Shahina A. Ghazanfar, a plant taxonomist from the Royal Botanic Gardens, Kew, Richmond, UK. The voucher specimens of plants were deposited in the Herbarium in the Department of Biology, Sultan Qaboos University. All plant materials, except those of Aloe sp., and the fruits or tubers of plants, were dried in a well-ventilated hot room at 50  $\degree$ C for two weeks and powdered in a local mill. One hundred grams of each pulverized plant material was initially extracted by maceration with 1.6 l of chloroform for two weeks and extracted further with the same quantity of 20% aqueous ethanol for another two weeks. The respective extracts were concentrated in vacuo (Rotavapor, Buchi, Switzerland) and the residues from the chloroform and ethanol extracts were weighed and stored in sealed vials in a freezer until tested.

## 2.3. DPPH scavenging activity

The antioxidant activity of the plant extract was estimated using a slight modification of the DPPH radical scavenging protocol reported by [Chen et al. \(1999\)](#page-5-0). For a typical reaction, 2 ml of 100  $\mu$ M DPPH· solution in ethanol was mixed with  $2 \text{ ml of } 100 \text{ µg/ml of plant extract.}$  The effective test concentrations of DPPH<sup>-</sup> and the extract were therefore 50  $\mu$ M and 50  $\mu$ g/ml, respectively. The reaction mixture was incubated in the dark for 15 min and thereaf-

ter the optical density was recorded at 517 nm against the blank. For the control, 2 ml of DPPH<sup>-</sup> solution in ethanol was mixed with 2 ml of ethanol and the optical density of the solution was recorded after 15 min. The assay was carried out in triplicate. The decrease in optical density of DPPH- on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) (see [Table 2\)](#page-4-0) of DPPH radical.

Percentage inhibition  $(\%IP) = [(A_{t=0} - A_{t=15})]/(A_{t=15}) \times 100$ 

where  $A_{t=15}$ : absorbance of the test sample after 15 min;  $A_{t=0}$ : absorbance of the control after 15 min.

Extracts whose %IP values were above 70% at 50  $\mu$ g/ml were serially diluted to give concentrations between 0 and 30  $\mu$ g/ml. From a plot of concentration against %IP, a linear regression analysis was performed to determine the  $IC_{50}$  value for each plant extract (see [Table 3](#page-4-0)) and for some commercial antioxidants, gallic acid, n-propyl gallate, tertbutylhydroxylanisole and tert-butylhydroquinone, using SPSS version 3.0 software (SPSS Inc. Chicago, IL).

#### 2.4. Determination of total phenolics

The total phenolics in extracts were determined according to Folin–Ciocalteu procedure [\(Singleton & Rossi,](#page-5-0) [1995](#page-5-0)). Four hundred microlitres of sample (two replicates) were taken in test tubes; 1.0 ml of Folin–Ciocalteu reagent (diluted 10-fold with distilled water) and 0.8 ml of 7.5% sodium carbonate were added. The tubes were mixed and allowed to stand for 30 min and the absorption at 765 nm was measured against a blank, which contained  $400 \mu l$  of ethanol in place of sample. The total phenolic content was expressed as gallic acid equivalents in mg/g of ethanol extract [\(Table 2\)](#page-4-0). Correlation studies between total phenolic contents of the extract and activities in the DPPH and phosphomolybdenum assays were performed using SPSS version 10.0 software.

## 2.5. Evaluation of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of the plant extracts was evaluated by the method of [Prieto et al. \(1999\).](#page-5-0) An aliquot of 0.3 ml of the sample solution (two replicates) was mixed with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The effective concentration of the sample was  $50 \mu g/ml$  in the reaction mixture. For the blank, 0.3 ml ethanol was mixed with 2.7 ml of the reagent. The absorbance of the test sample was measured at 695 nm. Gallic acid, tert-butyl hydroxyanisole, tert-butylhydroquinone, *n*-propyl gallate and  $\alpha$ -tocopherol were used as standards. The antioxidant activity was expressed for the samples as gallic acid equivalents (mg/g of ethanol extract).

## 2.6. Antimicrobial activity

Antimicrobial assay was performed following the agardilution-streak method ([Mitscher et al., 1972](#page-5-0)) on tryptone soy-agar plates. The test sample (20 mg) was dissolved in 0.2 ml of dimethyl sulfoxide. An aliquot of the solution (0.1 ml) or its suspension was transferred into a Petridish and mixed with 20 ml of sterile agar to test extract at 1000  $\mu$ g/ml. The remaining 0.1 ml solution was diluted with an equal volume of dimethyl sulfoxide and 0.1 ml of the resulting solution was mixed with 20 ml sterile agar in a Petridish to test at  $500 \mu g/ml$ . Lower test concentrations were obtained by serial 2-fold dilutions. The saline suspension of organisms was separately used to streak tryptone soy-agar plates containing test samples, negative and positive controls with gentamycin and ketoconazole as standards. The concentration of test sample that inhibited the growth of each organism after incubation at  $37^{\circ}$ C for 24 h was recorded.

## 3. Results and discussion

[Table 1](#page-3-0) lists some edible and wound-healing herbs used by the Dhofaris in the Sultanate of Oman and the major phytocompounds previously isolated from the plant genus or species. In order to achieve complete and quick extraction of the polar fractions, the interaction between nonpolar and polar compound in plant materials, the so-called 'matrix effect' [\(Van Beek, 1999](#page-5-0)), was broken up by a preliminary extraction with chloroform. Out of the 19 plants screened, the aqueous ethanol extracts of B. dhofarense, P. crispa, A. rubifolius, O. europaea, A. senegal, P. arabica, A. dhofarica, M. peregrina, C. perrottettii, F. lutea, A. indica and P. arabica showed very good DPPH scavenging activities with %IP values  $>70\%$  at an effective test concentration of 50  $\mu$ g/ml.

Hydrogen and electron transfer from antioxidant analytes to DPPH- and Mo(VI) complex occur in the DPPH and phosphomolybdenum assay methods. The transfers occur at different redox potentials in the two assays and also depend on the structure of the antioxidant. Several flavonoids and polyphenols have been isolated from plant extracts with potent DPPH scavenging activities [\(Lee](#page-5-0) [et al., 1998\)](#page-5-0), whereas the phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, a-tocopherol, and carotenoids [\(Prieto et al.,](#page-5-0) [1999](#page-5-0)). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols, and aromatic amines have the ability to donate hydrogen and electrons and can thus be detected by the two assay models. In general, the aqueous ethanol extracts showed better antioxidant activities than the chloroform extracts in DPPH assay. Aqueous alcohol is considered the best solvent for extracting phenolic compounds from plant materials ([Antolovich, Prenzler,](#page-5-0) [Robards, & Ryan, 2000; Negi, Jayaprakasha, & Jena,](#page-5-0) [2003](#page-5-0)). The total amount of phenolics in the extracts and their quality as antioxidants varied widely in the plants

<span id="page-3-0"></span>



investigated. The edible parts of Ziziphus hajarensis, Caralluma flava, D. foetida, C. quadragula and R. vivipara showed lower levels of total phenolics as gallic acid equivalents in the range of 15–35 mg/g of ethanol extracts and also weaker antioxidant activities in the DPPH assay [\(Table 2](#page-4-0)), whereas, wound-healing plants such as B. dhofarensis, P. crispa, A. dhofarica and F. lutea, and edible plants such as A. rubifolis, M. peregrina and A. indica, which are also traditionally used to heal wounds, are more potent in the DPPH assay and contain higher amounts of total phenolics, which ranged from 72 to 453 mg/g of EtOH extracts (as gallic acid equivalents) ([Table 2\)](#page-4-0). It is known that the Folin–Ciocalteu method gives different responses to different phenolic compounds, depending on chemical structures ([Satue-Gracia, Heinonen, & Frankel,](#page-5-0) [1997\)](#page-5-0). Nevertheless, a good correlation (correlation coefficient,  $r = 0.812$ ) was observed between  $1/IC_{50}$  and the total phenolics of the wound-healing plants investigated.  $1/IC_{50}$ is directly proportional to the antioxidant activity in the DPPH scavenging assay (see [Table 3\)](#page-4-0).

High radical-scavenging capacity could be responsible for wound healing [\(Martin, 1996\)](#page-5-0) and ophthalmic uses of plants, through inhibition of reactive oxygen species, which are often radicals. Reactive oxygen species are associated

with oxidative damage of protein ([Davies, 1993\)](#page-5-0) and eye diseases like cataracts [\(Altomare et al., 1995](#page-5-0)). Wound-healing processes could include cell proliferation, suppression of inflammation and contraction of the collagen tissue [\(Houghton, Hylands, Mensah, Hensel, & Deters, 2005\)](#page-5-0), and could be delayed by reactive oxygen species ([Bodeker](#page-5-0) [& Hughes, 1998\)](#page-5-0) or microbial infection. When tested for antimicrobial properties against E. coli, P. aeruginosa, S. aureus and C. albicans, the extracts of the majority of wound-healing plants were inactive. However, the aqueous ethanol extracts of P. arabica inhibited the growth of C. albicans at  $250 \mu g/ml$ ; A. dhufarensis inhibited all four organisms at  $1000 \mu g/ml$  and A. dhofarica was active against S. aureus at  $250 \mu g/ml$  and against P. aeruginosa and C. albicans at 500 µg/ml. A. dhofarica, A. rubifolius and M. peregrina had the lowest  $IC_{50}$  values of 4.46, 7.11 and 7.61  $\mu$ g/ml, respectively.

M. peregrina, A. rubifolius, O. europaea, C. perrottettii and A. indica could have some non-nutritional benefits, as a result of their high radical-scavenging capacity. They are regularly consumed by the older generation in Oman, particularly in the countryside. Of special interests are the Caralluma plants. Several succulent asclepiadaceae plants are sold in Oman as food, tonic or vitality herbs. Somewhat

<span id="page-4-0"></span>Table 2 Antioxidant activities and total phenolics of plant extracts

Plant name	Parts <sup>a</sup>	DPPH assay $(\%IP)^b$	Phosphomolybdate assay as gallic acid equivalents $(mg/g \text{ of EtOH extract})^c$	Total phenolics as gallic acid equivalents $(mg/g \text{ of ethanol extract})^c$
Becium dhofarense Sebald	Sh	$93.1 \pm 0.7$	$510 + 8.1$	$137 + 5.9$
Pulicaria crispa (Forsskal) Benth	W	$92.2 \pm 0.3$	$1790 \pm 7.2$	$96.6 \pm 0.6$
Allophylus rubifolius (Hoscht. Ex A. Rich)	L	$89.8 \pm 0.9$	$448 \pm 1.8$	$146 \pm 3.5$
Olea europaea L.	Sh/F	$89.8 \pm 0.1$	$913 \pm 55.6$	$144 \pm 4.9$
Acacia senegal (L.) Willd. (AS)	B	$89.2 \pm 0.7$	$472 \pm 13.9$	$75.5 \pm 0.1$
Anogeissus dhofarica A.J. Scott	T	$89.1 \pm 0.5$	$551 + 5.4$	$406 \pm 24.8$
Moringa peregrina (Forssk.) Fiori	B	$87.8 \pm 0.9$	$814 \pm 16.1$	$454 \pm 16.3$
Cordia perrottettii Wright	B	$85.4 \pm 1.0$	$646 \pm 9.4$	$145 \pm 10.7$
Ficus lutea Vahl	W	$84.6 \pm 0.4$	$335 \pm 43.5$	$224 \pm 9.1$
Acalypha indica L.	W	$81.6 \pm 0.4$	$279 \pm 7.6$	$72.4 \pm 4.9$
Pluchea arabica (Boiss.) Qaiser & Lack	W	$77.0 \pm 2.1$	$343 \pm 12.1$	$76.9 \pm 0.6$
Withania garaitica Miller & Biagi	W	$60.1 \pm 0.4$	$250 \pm 14.4$	$67.8 \pm 2.2$
Aloe dhufarensis Lavranos	W	$58.7 \pm 0.4$	$229 \pm 0.2$	$430 \pm 5.4$
Ziziphus hajarensis L.	W	$44.9 \pm 0.8$	$309 \pm 17.0$	$35.4 \pm 2.7$
Capparis cartilaginea Decaisne	L	$41.0^{b} \pm 0.1$	$168 + 2.3$	$38.3^{b} \pm 0.1$
	S	$36.9^{\circ} \pm 0.9$	$184 \pm 24.2$	$27.1^{\circ} \pm 1.6$
Caralluma flava N.E. Brown	W	$31.5 \pm 1.0$	$335 \pm 0.5$	$23.6 \pm 0.5$
Dorstenia foetida	T	$21.1 \pm 1.1$	$269 \pm 18.8$	$26.5 \pm 1.6$
Caralluma quadrangula (Forssk.) N.E. Brown	W	$14.5 \pm 1.4$	$899 \pm 29.2$	$18.7 \pm 0.4$
Remusatia vivipara Schott	T	$8.4 \pm 1.5$	$161 \pm 4.9$	$14.9 \pm 1.6$
Gallic acid		$94.5 \pm 0.0$		
$n$ -Propyl gallate		78. $6 \pm 1.6$	$711 \pm 17.0$	$801 \pm 3.9$
tert-Butylhydroxyanisole		$69.5 \pm 1.3$	$2500 \pm 10.8$	$353 \pm 23.2$
tert-Butylhydroquinone		$68.9 \pm 0.5$	$1900 \pm 68.2$	$517 \pm 82.5$
α-Tocopherol		$94.9 \pm 0.1$	$998 \pm 9.2$	$140 \pm 7.0$

<sup>a</sup> Part tested: B, bark; L, leaf; S, stem; Sh, shoot; T, tuber; W, whole plant. b Part tested: mean of triplicate assays,  $\pm$  SE.

 $\degree$  Part tested: mean of duplicate assays,  $\pm$  SE.

## Table 3





<sup>a</sup> Means  $\pm$  SD ( $n = 10$ ).

surprising is that *C. quadrangula*, a member of the succulent asclepiads, lacks antioxidant activity in the DPPH assay. However, C. quadrangula and C. flava demonstrated very strong antioxidant activity in the phosphomolydenum assay (see Table 2). Further analysis of the data revealed a poor correlation (correlation coefficient,  $r = 0.287$ ) between total phenolic contents of extract and antioxidant activity in the phosphomolybdenum assay model. From a literature survey [\(Abdel-Sattar, Al-Yahya, Nakamura, &](#page-5-0) [Hattori, 2001; Al-Yahya, Abdel-Sattar, & Guittet, 2000](#page-5-0)) and our preliminary phytochemical investigation of the aqueous ethanol extracts of C. quadrangula, the most abundant constituents of Caralluma plants are not phenolics but pregnane glycosides.

In conclusion, our results suggest that the extracts of some edible or wound-healing plants investigated have radical-scavenging capacity. The re-introduction of the leaves of A. rubifolius and A. indica into the regular diet is acceptable and could be a relevant cultural practice in the control of diseases in which free radicals are involved. As far as we know, this is also the first study concerning the free radicalscavenging capacity and antimicrobial activity of the lesserknown plants that are endemic to Oman. Of the plants investigated for wound healing, A. dhofarica seems to be the most promising because it combines antioxidant properties with antimicrobial activities.

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