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Potential Antioxidant Effects of Common Omani Ethnobotanical Plants

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ABSTRACT: Phytonutrients (e.g., phenolic compounds and flavonoids) are secondary plant metabolites that play an important role in the defense against pathogens and protection from oxidative injury because of their potential ability to neutralize reactive oxygen species. The present study aimed to determine the antioxidant contents, scavenging activity, and toxicity of aqueous extracts of common Omani plants. The total phenolic content (TPC), total flavonoid content (TFC), scavenging activity against hydrogen peroxide (H_2O_2), and brine shrimp lethality of the aqueous extracts of commonly used Omani ethnobotanical plants were evaluated. The samples exhibited a wide range of the investigated parameters. TPC ranged from 0.52 to 65.14 mg gallic acid equivalent/g dry solid, whereas TFC ranged from 0.07 to 37.14 mg catechin equivalent/g dry solid. Moreover, the scavenging activity ranged from 6.9% to 91.9%. Among 18 plant species that were examined, *Pteropyrum scoparium*, *Moringa peregrina*, *Dodonaea viscosa*, *Rhus aucheri*, *Acridocarpus orientalis*, and *Prosopis cineraria* showed high values in almost all parameters. At exposure levels of 1 to 1,000 μg/mL, the lethality test using four plants with the highest TPC values and scavenging activity (*M. peregrina*, *P. scoparium*, *R. aucheri*, and *P. cineraria*) revealed that they may be safe for consumption as food or medicine. In general, the study demonstrated that some Omani plant species may be potential sources of phenolic compounds and flavonoids. Thus, these plant species should be propagated to be used in the food and nutraceutical industries. Moreover, they can be consumed to combat chronic oxidative stress-mediated diseases.

Keywords: antioxidant, flavonoids, phytonutrient, polyphenols

INTRODUCTION

Plants are the most valuable resources on Earth, providing oxygen, food, and medicine to animals and humans. They possess bioactive chemicals that have likely contributed to ethnobotanical uses in traditional treatment practices before modern medicine (Aburjai et al., 2007). The use of indigenous medicinal plants in developing countries was estimated to account for more than 80% of healthcare needs (Hostettmann et al., 2000). There are many natural products manufactured as clinical agents, which have been isolated from plants and are still in use today. For example, atropine and hyoscine are obtained from the Solanaceae family (i.e., potato family), digoxin is obtained from the leaves of wooly foxglove (*Digitalis lanata*), codeine and morphine are obtained from the opium poppy (*Papaver somniferum*), latex is obtained from the rubber plant (*Hevea brasiliensis*), and quinine is obtained from quina bark (*Cinchona officinalis*) (Phillipson, 2001).

Out of more than 1,400 plant species identified, 260 were documented as being used indigenously in Oman (Ghazanfar and Al-Al-Sabahi, 1993). The utilization of these plants can be considered as a key example of Omani culture and heritage. According to Ghazanfar and Al-Al-Sabahi (1993), because of changes in social patterns and the modernization of Oman, the recipes and practices associated with the indigenous use of these plant species would be lost if they are not preserved and the plants are not described in detail for future generations. Indeed, there are a limited number of studies on the active compounds and uses of some Omani plants. Marwah et al. (2007) examined the antioxidant activity of 19 Omani plants and found that 11 of them, including wild olive

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(*Olea europaea*), Dhofari basil (*Becium dhofarense*), gum Arabic tree (*Acacia senegal*), false fleabane (*Pulicaria crispa*), and lowveld false rhus (*Allophylus rubifolius*), had high antioxidant activity. Moreover, the Dhofari buttontree (*Anogeissus dhofarica*), which is endemic to Oman, was described to have potent activity against infectious agents, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* (Marwah et al., 2007).

Secondary metabolites (e.g., flavonoids and phenolics) are compounds produced by plants and other organisms that help them to adapt and interact with their environment. These compounds often work as antioxidants that protect cells from harm caused by some metabolites or endogenous chemical substances such as reactive oxygen species (ROS) (Velazhahan et al., 2024).

Several studies have emphasized that the potential health benefits of plant-derived secondary metabolites are due to their antioxidant properties. For example, recent studies have discussed and indicated the therapeutic ability of phenolic compounds and flavonoids and their role in combating oxidative stress (Varesi et al., 2022).

Antioxidants play a vital role in protecting against oxidative cell damage, which plays a role in the development of various diseases such as cancer, heart disease, Alzheimer's disease, diabetes, and chronic inflammation (Dinstel et al., 2013). They exist at low concentrations in the cell and prevent or delay oxidative damage by neutralizing free radicals in the body (Rajendran et al., 2014). Plant antioxidants are good for the health. Thus, they have been used in the food industry. For example, antioxidants are used in lipids and lipid-containing foods to reduce rancidity, delay the production of toxic byproducts due to the oxidation process, maintain the nutritional quality, and increase the shelf life of food products (Maisuthisakul et al., 2007).

Naturally, antioxidants, such as flavonoids, phenolics, curcuminoids, tannins, coumarins, xanthones, terpenoids, and lignans, are found in different plant parts (e.g., fruits, leaves, and seeds) (Jeong et al., 2004). At present, many synthetic antioxidants, including butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and butylated hydroxyanisole (BHA) are manufactured and used in the food industry because they are more cost-effective and efficient than natural ones (Duh and Yen, 1997). However, the use of synthetic antioxidants as food additives has raised some concerns related to their potential health problems and toxicity. TBHQ has been banned in some European countries and Japan (Shahidi, 1997). Ito et al. (1982) presented evidence that BHA and BHT may be carcinogenic. Alternatively, phenolics and flavonoid compounds have been identified as natural antioxidants that may reduce oxidative damage in the human body. They have phenolic and hydroxyl groups as good hydrogen donors and can chelate metal ions (Wong et al., 2006).

There is a lack of research identifying the potential biological and health aspects of edible and/or medicinal wild Omani plants. Therefore, understanding the potential antioxidant value and usability of such plants is important to develop effective interventions to prevent oxidative stress-mediated diseases.

The present study aimed to describe the antioxidant contents of selected Omani plant species by measuring the total phenolic content (TPC) and total flavonoid content (TFC) of assayed samples (Table 1). Moreover, it aimed to investigate the scavenging activity of assayed plant extracts against hydrogen peroxide (H_2O_2) as an oxidizing agent and to screen the toxicity of aqueous ex-

Table 1. Common Omani ethnobotanical plants included in the study

Scientific name	Acronym	Type of plant	Local name	Local use
Acridocarpus orientalis	AO	Shrub	Qafas	Medicine
Caralluma penicillata	CP	Herb	Ddaja	Medicine
Dodonaea viscosa	DV	Shrub	Shahs	Food and medicine
Euphorbia larica	EL	Shrub	Isbiq	Medicine
Fagonia indica	FI	Herb	Shukaa	Medicine
Ficus cordata	FC	Tree	L'thab	Food and medicine
Juniperus excelsa	JE	Tree	Al'alan	Medicine
Lavandula subnuda	LS	Herb	Sumar	Medicine
Moringa peregrina	MP	Tree	Shua	Food and medicine
Olea europaea	OE	Tree	Itm	Food and medicine
Oxalis corniculata	OC	Herb	Hommath	Food and medicine
Plocama aucheri	PA	Herb	Mkuroman	Medicine
Portulaca oleracea	PO	Herb	Farfenaa	Food and medicine
Prosopis cineraria	PC	Tree	Ghaf	Food
Pteropyrum scoparium	PS	Herb	Sidaf	Food and medicine
Rhus aucheri	RA	Shrub	Ka'tf	Medicine
Teucrium mascatense	TM	Herb	Ja'ada	Medicine
Teucrium stocksianum	TS	Herb	Ja'ada	Medicine

tracts of selected plant species using a simple brine shrimp lethality assay.

MATERIALS AND METHODS

Collection of plant samples

Fresh plants were collected from the wild areas of Al Rustaq, Nakhal, and Al Jabal Al Akhdar (northern parts of Oman). Dr. Amina Al Farsi [Botanist, Department of Biology, Sultan Qaboos University (SQU)] and Abdul Rahman Al Hinai [Botanist, Oman Botanic Garden (OBG), Ministry of Heritage and Tourism] identified and/or confirmed the species identification of the collected plants. Voucher specimens of plants were deposited at the herbarium of SQU and OBG. The sampled parts were leaves. A minimum of three samples for each plant species was obtained from three individual herbs, shrubs, or trees. The plants were then transported to the Department of Food Science and Nutrition laboratory (College of Agricultural and Marine Sciences, SQU) and stored at −20°C (Foster cold room) until processing.

Chemicals

The chemical reagents used in the analysis included sodium nitrite ($NaNO_2$) and aluminum chloride ($AlCl_3$) (Sigma), Folin-Ciocalteu's phenol reagent and buffer (pH 7.00±0.02) (BDH Chemicals Ltd.), sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3) (Merck), and H_2O_2 (27.5%, Gainland Chemical Company). All solutions were prepared with 18.2 MΩ cm^{-1} deionized water (ELGA).

Drying of plant samples

The leaves were cleaned with tap water and cut into small pieces, placed in a 100 mL glass and/or plastic containers, and kept in a low-temperature (−40°C) freezer (Dairei Europe Ltd.) for 24 h. The frozen samples were lyophilized using freeze-dryers (Benchtop Freeze Dry System, LABCONCO). The dried samples were stored in a desiccator before being ground into powder using an electric grinder (PHILIPS). Then, the powdered plant samples were kept in airtight glass containers and stored in an ultra-low freezer (−60°C) for later use in extract preparation.

Preparation of extracts

The extraction procedures were modified according to the assay performed. The extraction was prepared from an even mixture (i.e., pooling) of three samples of each plant. Approximately 0.5 g of plant powder was added to a conical flask with 100 mL of deionized water and stirred for 2 h at room temperature. The flasks were wrapped and covered with aluminum foil to avoid light exposure. Then, the mixture was centrifuged at a relative centrifugal force of 4,427 g for 20 min using centrifuges (Harrier 18/80 Centrifuge, MSE; and Avanti J-25I Centrifuge, Beckman Instruments Inc.). Finally, the supernatant was filtered through Whatman No.1 filter paper, and the filtrate was stored in an ultra-low freezer (−60°C) until assay analysis.

Determination of the total phenolic content

The TPC in the aqueous extracts of powdered plant samples was determined spectrophotometrically using Folin-Ciocalteu's reagent in accordance with the methods of Singleton and Rossi (1965). In this method, an aliquot of the extract (50 − 1,700 μL) was placed in a test tube and mixed with 250 μL of Folin-Ciocalteu's reagent and 750 μL of Na_2CO_3 (1.9 M). The total volume of the mixture was made up to 5,000 μL by adding deionized water, and the mixture was vortexed for 5 to 15 s. Then, the mixture was incubated in the dark for 2 h, and the absorbance of samples was measured using an ultraviolet-visible spectrophotometer (Thermo Fisher Scientific) at 765 nm against a blank solution containing all assay reagents, except the plant extract. The calibration curve of the standard solution was prepared using gallic acid with a concentration range of 0.000 to 0.007 mg/mL. TPC was calculated in mg gallic acid equivalent (GAE)/g dry solids of plant samples from the calibration curve equation. The analysis was performed in triplicate.

Determination of the total flavonoid content

The TFC of freeze-dried powdered samples was estimated according to the method of Zhishen et al. (1999). The extract solution (1 − 7 mL) was poured into a test tube and then added with 4 mL of deionized water. Next, 0.3 mL of 5% (w/v) $NaNO_2$ was added to the test tube, followed by the addition of 0.3 mL of 10% (w/v) $AlCl_3$. After 5 min, 2 mL of 1 M NaOH was added to the mixture, diluted with 2.4 mL of deionized water, and mixed using a vortex mixer (Nickel Electro Ltd.). Then, the absorbance of the colored mixture was measured at 510 nm against a blank solution using an ultraviolet-visible spectrophotometer. A calibration curve was prepared using the standard solutions of catechin with a concentration range of 0.00 to 0.10 mg/mL. The results were expressed as mg catechin equivalent (CE)/g dry solids, and all determinations were performed in triplicate.

Determination of H_2O_2 scavenging activity

The ability of plant extracts to scavenge H_2O_2 was determined following the procedure described elsewhere (Ruch et al., 1989). Using an analytical balance (Benchmark Scientific), the extraction from the plant sample was prepared by adding 0.01 g of powder in 50 mL of deionized water and then filtered through a Whatman

No.541 filter paper (GE Healthcare Life Sciences). Immediately, 4.5 mL of plant extract [200 μg/mL, except *Moringa peregrina* (MP), *Pteropyrum scoparium* (PS), *Rhus aucheri* (RA), and *Prosopis cineraria* (PC) with only 50 μg/mL because their absorbance readings were not stable at 200 μg/mL] was mixed with 0.5 mL of the 40 mM H_2O_2 solution [prepared in phosphate-buffered saline (PBS), pH 7.0]. Then, the mixture was kept in the dark for 10 min to allow the reaction to take place. The absorbance of the solution was determined spectrophotometrically at 230 nm (UV-3100PC, VWR) against a blank solution containing plant extracts in PBS without H_2O_2. The analysis was conducted in triplicate. The scavenging activity of plant extracts against H_2O_2 was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

The scavenging activity was expressed as a percentage (%) of the remaining H_2O_2 concentration after the 10-min incubation time. The H_2O_2 concentrations were determined spectrophotometrically from a calibration curve.

Brine shrimp lethality test

The brine shrimp lethality test was performed in accordance with the method of Meyer et al. (1982). Artificial seawater (salinity of 25‰) was prepared by adding 25 g of sea salt (Tetra Co.) per 1 L of distilled water with continuous aeration for at least 24 h. Approximately 0.2 to 0.4 g of cysts of brine shrimp (*Artemia salina*) (INVE Aquaculture) was placed in 1 L of seawater with continuous aeration under a light source for synchronized hatching. After 24 h, the newly hatched nauplii were collected into a glass beaker containing freshly prepared and well-aerated seawater. The aqueous extracts of plant species with the highest scavenging activity against H_2O_2 and TPC were selected to evaluate their toxicity on brine shrimp. These included MP, PS, RA, and PC. The exposure was conducted using four concentrations (1, 10, 100, and 1,000 μg/mL) plus a control (seawater only). The concentrations were prepared by serial dilution of the stock aqueous extract (5,000 μg/mL), which was prepared for TPC and TFC analyses. Ten milliliters of each concentration was added into a small Petri dish (5 cm diameter), and 10 shrimps were transferred into each dish and incubated at room temperature (24°C – 25°C) for 24 h. After 24 h, all shrimps (alive and dead) were counted. The survival percentage of brine shrimp was calculated for each concentration of plant extract. For each selected plant species, the exposure was performed in triplicate. The experimental protocol used in this study followed the guidelines established by the Sultan Qaboos University Animal Ethics Committee (ethical approval No. SQU/AEC/2019-20/08).

Statistical analysis

Minitab software for Windows (version 17, Minitab Inc.) and SigmaStat software for Windows (version 4, Systat) were used to analyze and visualize the data. One-way analysis of variance (ANOVA) was used to compare the TPC, TFC, and scavenging activities of plant species against H_2O_2. Multiple post hoc comparisons were performed using the Tukey test to determine significant differences between species. All data were reported as mean±standard error of the mean, and the level of statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The phytochemical constituents have a wide spectrum of medicinal and nutritional uses because of the presence of phenolic compounds and flavonoids. They are potential contributing factors to the antioxidant activity of plant extracts. Dietary antioxidants are important for overall health and preventing certain diseases (Alvarado et al., 2006; Jiménez et al., 2008; Wootton-Beard and Ryan, 2011). Consequently, this study assessed the TPC and TFC of aqueous extracts from some locally used Omani plants. The antioxidant activity of extracts was also quantified and evaluated for their potential to scavenge H_2O_2, a nonradical ROS.

Total phenolic content

Fig. 1 shows the TPC expressed as GAE per gram of dry solid or lyophilized plant leaves. On average, the TPC values ranged from 0.52±0.06 mg GAE/g dry solid to 65.14±2.41 mg GAE/g dry solid. The TPC analysis showed that the aqueous extract of PS leaves had the highest phenolic compounds among the analyzed plant species. By contrast, *Portulaca oleracea* (PO) had the lowest TPC. Relatively considerable amounts of TPC were also observed in MP, *Dodonaea viscosa* (DV), and RA (53.02, 52.83, and 47.02 mg GAE/g dry solid, respectively). A statistically significant difference was observed between wild plant species in terms of TPC ($F_{17,36}=1.92$, $P<0.01$).

Phenolic compounds are one of the largest groups of secondary compounds that contain the phenol group. They play potential roles as protective agents in plants and humans through their diet. In this study, the TPC varied widely among the studied plants (0.52 – 65.14 mg GAE/g dry solid). Noticeably, PS had a high TPC compared with other plants. This species, locally known as Sidaf, is considered as a regional endemic plant to Oman and the United Arab Emirates (Patzelt, 2014). Previous studies reported that the aqueous extract of PS contained a high TPC (289.80 mg GAE/g) (Shah et al., 2016).

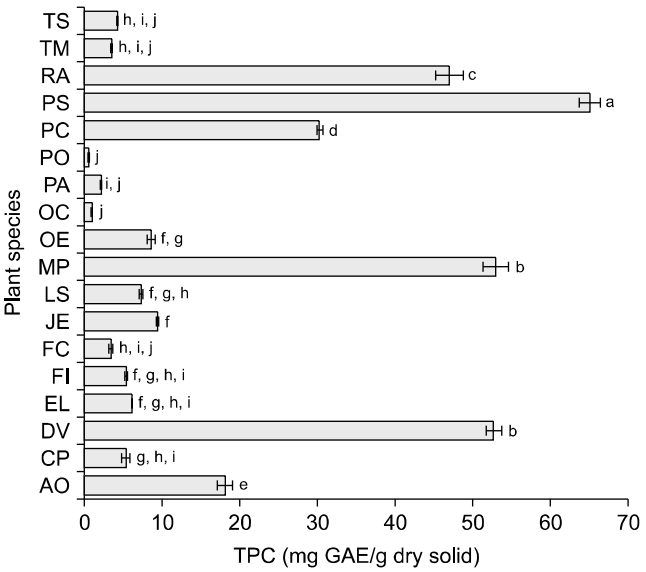

Fig. 1. Total phenolic content [TPC; mg gallic acid equivalent (GAE)/g dry solid] of the aqueous extract of wild plant species collected from Oman. The values are presented as mean± standard error of samples (n=3) for each plant species. Based on analysis of variance followed by post hoc multiple comparisons (Tukey's test), bars sharing the same letters (a–j) are not statistically different TPC (P>0.05). Refer to Table 1 for the names of plant species.

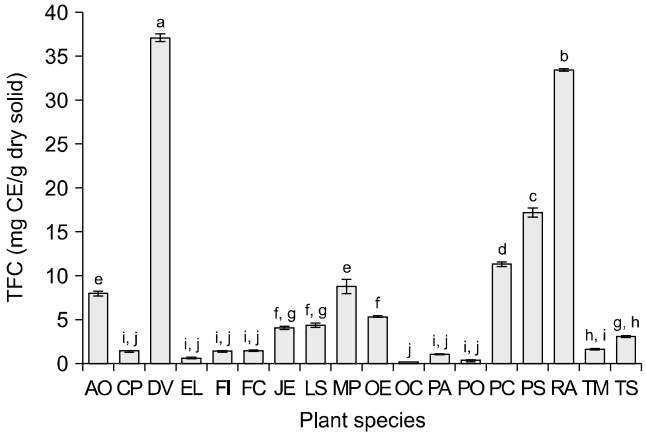

Fig. 2. Total flavonoid content [TFC; mg catechin equivalent (CE)/g dry solid] of the aqueous extract of wild plant species collected from Oman. The values are presented as mean±SEM of samples (n=3) for each plant species. Based on analysis of variance followed by post hoc multiple comparisons (Tukey test), bars sharing the same letters (a–j) are not statistically different TFC (P>0.05). Refer to Table 1 for the names of plant species.

Similarly, the present study found that MP, DV, RA, and PC contained high TPC. MP has been domestically used in folk medicine to treat paralysis and skin rashes by rubbing the leaf extract over the skin (Ghazanfar and Al-Al-Sabahi, 1993). The leaves of DV are used to treat itching, aches, swelling, and bone fracture (Teffo et al., 2010) and rash and fever (Hussain et al., 2013). Akhtar et al. (2018) reported a lower TPC (38.20 mg GAE/g) in the aqueous extract of DV relative to the value (52.83 mg GAE/g) we determined for the same species. RA is an endemic plant that is found in northern Oman only (Patzelt, 2014). Aside from being consumed, the leaves of RA are also used as an antiseptic and treatment for different diseases (Ghazanfar, 1994). In a previous study, the aqueous extract of RA was found to contain 126.4 mg GAE/g of TPC (Singh et al., 2016), which is about two times higher than that reported in the present study (55.76 mg GAE/g). PC is widely distributed in Saudi Arabia and the eastern part of India (Pickering and Patzelt, 2010). It has been utilized for different medical purposes, mainly as a painkiller (Pickering and Patzelt, 2010).

Total flavonoid content

The TFC of the investigated plants was expressed as CE per g dry solid of freeze-dried plant leaf samples (Fig. 2). The TFC ranged from 0.07±0.03 mg CE/g dry solid for *Oxalis corniculata* (OC) to 37.14±0.76 mg CE/g dry solid for DV. Generally, most plants exhibited low TFC (i.e., −TFC <9 mg CE/g). One-way ANOVA revealed a significant difference in TFC ($F_{17,36}$=1.92, P<0.01), and post hoc multiple comparisons confirmed that DV, RA, PS, and PC have significantly higher TFC than the rest of the plant species.

We found that the TFC and TPC exhibited a parallel trend (i.e., when the TPC was higher for certain plant species, the TFC was also higher for the same species). Generally, TPC is quantitatively higher than TFC that has already been documented by previous investigations (Shan et al., 2005; Wojdyło et al., 2007; Roby et al., 2013). Furthermore, flavonoids are considered to be a subclass of phenolic compounds.

Being the plant species with the highest TFC (37.14 mg CE/g dry solid), DV is known to contain abundant content of flavonoids and their derivatives (Sachdev and Kulshreshtha, 1984; Getie et al., 2002; Mothana et al., 2010). Indeed, Akhtar et al. (2018) and Al-Oraimi and Hossain (2016) determined a high content of flavonoids in the aqueous extract of DV. Similarly, a high TFC was also observed in the aqueous extract of RA and PS (Fig. 2), demonstrating the availability of flavonoids in the water-based extract of such plants and supporting the earlier findings of Singh et al. (2016), particularly for RA (11.70 mg CE/g) and PS (5.70 mg CE/g), which are notably lower than our findings. On the other hand, the lowest TFC was observed in OC (0.07 mg CE/g), which is a plant that is distributed around the world and contains medicinal and nutritional benefits (Gunasegaran, 1992; Achola et al., 1995; Taranalli et al., 2004).

Scavenging activity against H_2O_2

The scavenging activity of the aqueous extract of wild plants against H_2O_2 is shown in Fig. 3. Here, the activity refers to the % of H_2O_2 concentration eliminated from the medium in the presence of the plant extract. It

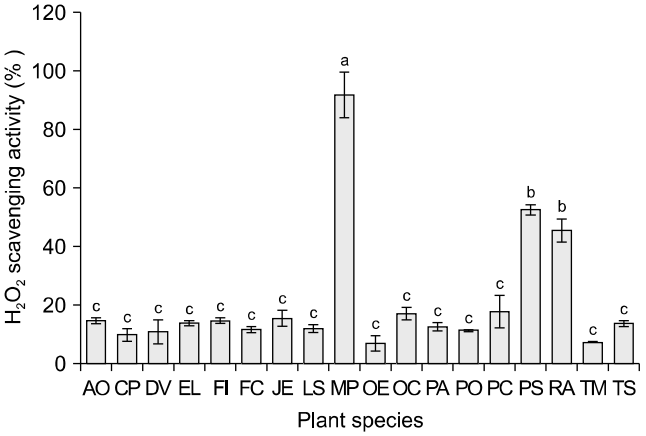

Fig. 3. Scavenging activity of the aqueous extract of wild plant species. The values are presented as mean±SEM of samples (n=3) for each plant species. Based on analysis of variance followed by post hoc multiple comparisons (Tukey test), bars sharing the same letters (a–c) are not statistically different in scavenging activity (P>0.05). Refer to Table 1 for the names of plant species.

ranged from 6.9%±4.5% to 91.9%±13.7% with statistically significant differences observed between the selected plant species ($F_{17,36}$=1.92, P<0.05). MP had the highest scavenging activity (91.9%) relative to all species. PS and RA had similar H_2O_2 scavenging activities (52.4% and 45.5%, respectively) compared with other plant species. Conversely, the aqueous extract of *Olea europaea* (OE) had the lowest scavenging value (Fig. 3).

H_2O_2 is a preconstituent in the formation of hydroxyl radicals, which can induce oxidative stress and initiate cytotoxicity. Therefore, substances that can eliminate H_2O_2 will help in protecting the living system (Van Wijk et al., 2008). The scavenging activity of MP was already reported using another free radical scavenging assay [2,2-diphenyl-1-picrylhydrazyl (DPPH) method] (Marwah et al., 2007). The high scavenging activity of MP against H_2O_2 and other oxidative stress-related species could be attributed to its rich TPC (Fig. 1). Moreover, the results of gas chromatography-mass spectrometry analysis provided evidence that MP leaves contain a wide range of bioactive phytoconstituents, including tannins, glycosides, alkaloids, flavonoids, and steroids (Al-Owaisi et al., 2014). These bioactive phytochemicals can neutralize free radicals through their hydrogen-donating ability (Waheed et al., 2014). In addition, PS and RA had high potential scavenging activity against H_2O_2 (Fig. 3). Singh et al. (2016) found a high DPPH reduction of 91% in water extracts of RA. The high TPC in aqueous and organic plant extracts could strongly contribute to neutralizing nonradical ROS, including H_2O_2. Polyphenolic compounds have been reported to play important roles in scavenging free radicals (Singleton et al., 1999; Wong et al., 2006; Mustafa et al., 2010; Al Nasiri et al., 2023).

Interestingly, the scavenging activity against H_2O_2 in the TPC-rich aqueous extracts of *Acridocarpus orientalis* (AO) and DV was low, contradicting our initial predictions. Although the Folin-Ciocalteu method provides a crude estimation of the phenolic compounds present in a sample, it is not specific to polyphenols only because other compounds would react with the reagent and hence provide higher TPC (Prior et al., 2005). In addition, the response of phenolic compounds to Folin-Ciocalteu's reagent depends on the number of functional phenolic groups that they possess (Singleton et al., 1999). The significant antioxidant capacity shown by certain plants with low TPC and TFC may suggest that their antioxidant activity is not always restricted to TPC. In fact, other secondary metabolites would also play a significant role in the antioxidant activity (Javanmardi et al., 2003).

Because of its limited availability in its aqueous fraction due to the hydrophobic nature of essential oils and other various constituents of secondary metabolites (Al Oraimi et al., 2012), the low scavenging activity of *Teucrium mascatense* (TM) (Fig. 3) is likely ascribed to its low TPC (Fig. 1). TM is endemic to northern Oman and has been used to treat epilepsy, diabetes, colic, fever, and stomach pain (Patzelt, 2014). Similarly, the low scavenging activity of *Caralluma penicillata* (CP) against H_2O_2 is likely due to its low TPC (2.81 mg GAE/g). Abdel-Sattar et al. (2001) and Abdul-Aziz Al-Yahya et al. (2000) confirmed that the most abundant constituents of *Caralluma* species (e.g., CP) are not phenolic compounds, but likely pregnane glycosides. In addition, to flavonoids and phenolic compounds, methyl linoleate, phenolic glycosides, triterpenes, and vitamin E are among the other bioactive substances found in OE (Benavente-Garcıa et al., 2000; Marwah et al., 2007).

Effects of the extraction solvent on the antioxidant capacity

The level of phenolic compounds in plant extracts primarily depends on the solvents used for extraction. In this study, we utilized water for preparing plant extracts rather than organic solvents because water is a convenient medium for human consumption and for the preparation of plant materials. In addition, it is cheap, simple, and environmentally friendly and leaves limited chemical residues, particularly during food processing and manufacturing. For the brine shrimp lethality test, aqueous extracts are far simpler to use than organic extracts. Goli et al. (2005) demonstrated that water was the most effective solvent for extracting phenolic compounds besides methanol.

Relative to our study, it is important to note that the yield of phenolic compounds and flavonoids has been reported to be higher in organic solvents. The preparation of PS extracts in ethanol and 30% aqueous glycerol resulted in higher TPC (261.40 and 453.40 mg GAE/g, re-

spectively) (Shah et al., 2016) compared with that in our study (Fig. 1). Al-Owaisi et al. (2014) indicated that MP has high TPC in organic solvents (chloroform, ethyl acetate, and methanol) with a range of 75.53 to 94.56 mg GAE/g. Marwah et al. (2007) demonstrated that MP contains a higher TPC (454 mg GAE/g) in ethanol extract than in aqueous extract (53.02 mg GAE/g) (Fig. 1). In a previous study, organic solvent extraction resulted in higher TFC for OC [up to 6.92 mg rutin equivalent (RE)/g extract] (Khan and Zehra, 2013). Ahmed et al. (2012) showed a wide range of TFC from the solvent fractions of methanol and water (0.05 – 0.36 mg RE/g dry solid), with water fractions containing the lowest TFC. Similarly, we found low TFC in the aqueous extracts of the investigated plant species (Fig. 2). This is likely because of the higher solubility of phytochemicals in organic solvents, which could be influenced by the polarity of the extraction solvents themselves (Zhao et al., 2006; Reddy et al., 2016). Therefore, the TPC and TFC may have been partially eliminated due to their limited solubility in water. Particularly for TFC, the solubility of flavonoids in water depends on their moieties. For example, although it is predicted that hydroxyl groups and sugars increase the solubility of flavonoids in water, modified isopentyl and methyl ethers make flavonoids more hydrophobic (i.e., lipophilic) (Agostini-Costa et al., 2012).

Regardless of the extraction solvents, the TPC for some plants agrees very well as it was the case for PC (37.78 mg GAE/g in the present study compared with 37.26 mg GAE/g by methanol) (Qasim et al., 2017). On the other hand, some plants expressed low TPC in organic- and water-based extracts. The low TPC of PO was already reported following extraction with three different solvents: methanol, ethanol, and water [2.95, 1.72, and 1.41 mg GAE/g fresh weight (Lim and Quah, 2007) and 3.60, 2.77, and 1.43 mg GAE/g (Uddin et al., 2012)]. Moreover, DV showed low TPC in various organic solvents ranging between 0.028 mg GAE/g and 0.208 mg GAE/g (Riaz et al., 2012). The reactivity of plant extracts against reactive metabolites or chemical species also appears to be extraction solvent dependent. For example, the methanol extract of MP exhibited the highest scavenging activity among all organic solvent extracts examined by the DPPH and H_2O_2 free reactive species scavenging methods (Al-Owaisi et al., 2014). Although the aqueous extracts of OE generally resulted in a low scavenging activity against H_2O_2 (Fig. 3), Marwah et al. (2007) showed that the ethanol extract of OE had a substantial scavenging activity (89.8%) against DPPH radicals. Furthermore, the minimal scavenging activity of the water-based extracts of *Euphorbia larica* and TM (Fig. 3) is plausibly explained by the dominant availability of TPC and TFC in their organic fractions (Jassbi, 2006; Noori et al., 2009; Al Oraimi et al., 2012).

Brine shrimp toxicity of plant extracts

The aqueous plant extracts of MP, PC, PS, and RA were found to be nontoxic to brine shrimps (Fig. 4). There was a slight decrease in the survival percentage in the highest tested concentration (1,000 μg/mL), but there was no statistically significant difference in the presence of the tested plant aqueous extracts ($F_{4,15}$=3.06, P>0.05). The brine shrimp lethality test is a simple and rapid bioassay for screening plant extracts to determine their cytotoxicity and to check for the presence of bioactive phytocompounds (Meyer et al., 1982; Karchesy et al., 2016). The percentage of surviving brine shrimp was almost 100% for all exposed concentrations (1, 10, 100, and 1,000 μg/mL) of the water extracts of MP, PS, RA, and PC (Fig. 4). These findings indicate that these plants with the highest TPC and scavenging activity may not be associated with toxic responses at the cellular level and exposed doses and are likely safe for consumption as medicine and food.

It is important to note that other species of *Moringa* (e.g., *Moringa oleifera*) showed high toxicity toward brine shrimp in organic solvents of leaf, bark, and fruit with lethal concentration (LC_{50}) values ranging from 0.43 μg/mL to 1.18 μg/mL (Urmi et al., 2012). However, Nkya et al. (2014) reported that the flower extracts of *M. oleifera* are nontoxic to brine shrimp nauplii. The difference could be related to the extraction methods, wherein the compounds that can have toxic effects on brine shrimp did not dissolve in aqueous solutions. Moreover, using organic solvents might be toxic to these crustaceans.

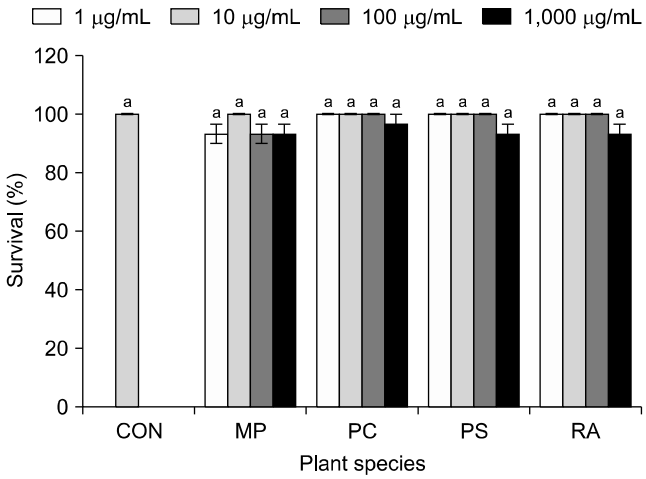

Fig. 4. Toxicity of the aqueous extract of four plant species: *Moringa peregrina* (MP), *Prosopis cineraria* (PC), *Pteropyrum scoparium* (PS), and *Rhus aucheri* (RA). The values are presented as mean±SEM of samples (n=3) for each plant species. CON is the control (artificial seawater without extract). Based on analysis of variance followed by post hoc multiple comparisons (Tukey test), bars sharing the same letters (a) are not statistically different in survival % (P>0.05).

Little is known about the cytotoxicity, if any, of the investigated plants. Thus, our data fill the gap of knowledge on the toxicity of the aqueous extracts of PC, RA, and PS. The leaves of these species can be consumed as food, and one of them is a medicinal plant (Table 1) and is unlikely to be toxic as demonstrated (Fig. 4). Furthermore, Robertson et al. (2012) found no toxic effects in mice when they were fed with the leaf and stem bark extracts of PC.

The investigation of the antioxidant properties of aqueous extracts from 18 native plant species showed a wide range of TPC, TFC, and scavenging activity against H_2O_2. Based on their aqueous leaf extracts, some Omani plant species, including AO, DV, MP, PC, PS, and RA, might be considered as potential sources of phenolic compounds and flavonoids. Aqueous extracts tend to contain lower concentrations of phenolic compounds and flavonoids, likely because of their lower solubility in water compared with organic solvents. The scavenging activity against H_2O_2 of plant extracts corresponded closely to their TPC and TFC, with higher activities observed for plant extracts having high TPC. The brine shrimp lethality test of four plants with the highest TPC and scavenging activity revealed that they may be safe for consumption as food and/or medicine. Plant species rich in phenolic compounds and flavonoids could be utilized in the food and nutraceutical industries. Moreover, they should be incorporated into the diet to fight against chronic diseases caused by oxidative stress.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: MAS. Data collection, analysis and interpretation: MAS. Drafting and writing the article: MAS. Critical revision of the article: HAR, MW. Final approval of the article: HAR, MW. Overall responsibility: MAS, HAR, MW.

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