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## MEDICINAL CHEMISTRY | SHORT COMMUNICATION (TF)

# Ethanol/water extracts from halophyte species *Arthrocnemum macrostachyum* and *Tetraena qatarensis*

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**Abstract:** Microwave-assisted extraction using various concentrations of ethanol in water (25%, 50%, 75% v/v) was carried out using biomass from halophytes; *Arthrocnemum macrostachyum* and *Tetraena qatarensis*. Total phenolic content (TPC; expressed as mg Gallic acid equivalent; GAE) and antioxidant activity using half-maximal inhibitory concentration of DPPH-free radical (IC<sub>50</sub>; expressed as µg/mL) was highest in extracts from 75% ethanol in both plant species; TPC = 45.6 ± 1.0 and 54.4 ± 0.8 mg GAE/g extract; IC<sub>50</sub> = 62.7 ± 0.4 and 67.9 ± 4.8 µg/mL for *A. macrostachyum* and *T. qatarensis*, respectively. UV-VIS spectral analysis and metabolome profile analysis obtained using UHPLC-Q-ToF-MS suggest the 50% and 75% ethanol extracts are similar in *A. macrostachyum*, whereas the 25% and 50% ethanol extracts are similar in *T. qatarensis*. Increasing the concentration of ethanol results in phytoextracts with greater chemical complexity.

**Subjects:** Environment & Agriculture; Botany; Biochemistry

**Keywords:** antioxidant; IC<sub>50</sub>; MAE; metabolomics; TPC

### ABOUT THE AUTHORS

Arid desert plants are extraordinary because they exist and survive in an ecosystem known for its harsh environment (e.g. high temperatures, drought, and poor soils). The diversity of secondary metabolites produced by desert plants enables their survival and supports the establishment of their ecological range in the desert. The Lina F. Yousef (LFY) research group ([www.lfy-group.com](http://www.lfy-group.com)) focuses on studying soils and plants in arid environments. One arm focuses on regeneration practices via farming of desert plants as crops. The second arm focuses on the extraction and identification of biologically active metabolites from desert plants. The study reported highlights the chemical complexity of plant extracts from two desert halophytes native to the UAE. Future work will focus on fractionating extracts to identify biologically active metabolites exhibiting antitumor and antimicrobial activity.

### PUBLIC INTEREST STATEMENT

Conventional crops used as a source of food, fuel and medicine require fresh water and sweet (arable) soils to grow properly. However, there is a global shortage of fresh water supplies and arable land to meet the demands of the growing human population. Scientists are now looking at alternative plants that do not require fresh water or arable soils to grow. Alternative candidates include salt tolerant plants, collectively known as halophytes. These plants can use seawater to grow and they can be cultivated on non-arable, salt-degraded soils. Scientists also discovered that halophytes produce large amounts of chemicals such as antioxidants that are beneficial to human health. For this reason, scientists around the world are studying the diversity of halophytes and the chemicals that can be extracted from them for use in health applications. This study looks at the antioxidant content and diversity of chemicals that can be extracted from two different halophytes that normally on salt-impacted, non-arable soil.

## 1. Introduction

There is increasing commercial interest in establishing halophyte plantations for the purpose of extracting high value phytochemicals, such as antioxidants, for application in food, health and medicine (Ksouri et al., 2012; Lopes et al., 2016). Halophytes are defined as salt tolerant plants that can grow and reproduce in environments that contain salt concentrations exceeding 200 mM (Flowers, Galal, & Bromham, 2010). Normally, exposure of plants to high salinities induces oxidative stress caused by the production of reactive oxygen species (ROS) (Bose, Rodrigo-Moreno, & Shabala, 2014; Lopes et al., 2016). Halophytes in contrast can withstand high salinities because they produce high amounts of low molecular-weight compounds that scavenge-free radicals (Bose et al., 2014; Jithesh, Prashanth, Sivaprakash, & Parida, 2006; Ksouri et al., 2009). These include glutathione, carotenoids, vitamins (C and E) as well as phenolic compounds such as flavonoids and phenolic acids (Bose et al., 2014). Plant phenolics in particular are known to play an important role in the inhibition of human diseases associated with free radical reactions (Ambigaipalan, 2015; Schemeth et al., 2015), and many studies are dedicated to extracting and quantifying the phenolic content of plant extracts (e.g. Cai, Luo, Sun, & Corke, 2004; Dai & Mumper, 2010; Dudonné, Vitrac, Coutière, Woillez, & Mérillon, 2009).

In this study, we evaluated the total phenolic content (TPC), antioxidant capacity and metabolomics profile of extracts from two perennial halophytes; *Arthrocnemum macrostachyum* (Moric.) K. Koch and *Tetraena qatarensis* Beier and Thulin. The two plant species are adapted to grow on saline soils, but each has its own geographic range. *A. macrostachyum* is a coastal shrub belonging to the Chenopodiaceae family and is widespread across coastal zones in the Mediterranean basin, Middle East and Asia (Navarro-Torre et al., 2017; Redondo-Gómez, Mateos-Naranjo, & Andrades-Moreno, 2010). *T. qatarensis* (previously *Zygophyllum qatarense*) is an inland shrub belonging to the Zygophyllaceae family and is mainly distributed in arid regions of Africa and Asia (Beier, Chase, & Thulin, 2003). The limited information available in literature on phytochemical profiles obtained from these plants motivated the initiation of this study, particularly because these two halophytes have the potential to be utilized as food and/or medicinal crops in non-conventional halophyte based agricultural systems (Ksouri et al., 2012; Lopes et al., 2016). Specifically, cultivation of halophytes such as *A. macrostachyum* and *T. qatarensis* on salinized and salt impacted farms could be a mechanism to reestablish vegetation, and the biomass can be harvested as sources of antioxidants and other active phytochemicals that have high commercial value (Ladeiro 2012; Al-Yamani et al. 2013).

The chemical profile and extraction of phenolics from any plant material will depend on the choice of solvent used (Dai & Mumper, 2010). Generally, higher phenolic content and antioxidant activity are obtained using aqueous organic solvent mixtures of ethanol, methanol, ethyl acetate or acetone (Peschel et al. 2006). In this study we investigate how increasing the concentration of ethanol in the extraction solvent affects yields, TPC, antioxidant capacity and metabolomics profiles of *A. macrostachyum* and *T. qatarensis* extracts. Future studies will focus on the purification and characterization of phytochemical constituents in the two halophyte extracts for downstream applications in food and health.

## 2. Results and discussion

The halophytes used in this study were collected from the United Arab Emirates (Böer & Sargeant 1998), a country characterized by arid climatic conditions and poor soil conditions with high salinity being a dominant factor restricting plant growth (Abdelfattah & Shahid 2007). A few studies evaluated the phenolic content and antioxidant capacity of extracts from *A. macrostachyum* (Rodrigues et al., 2014), and none are available on *T. qatarensis*. In this study, we chose to use increasing concentrations of ethanol in water (25%, 50%, and 75% v/v) as the extraction solvent because it is safe for human consumption and extracts are suitable for downstream applications in health (Dai & Mumper, 2010). Microwave Assisted Extraction (MAE) was chosen as the extraction method because it is simple and time efficient

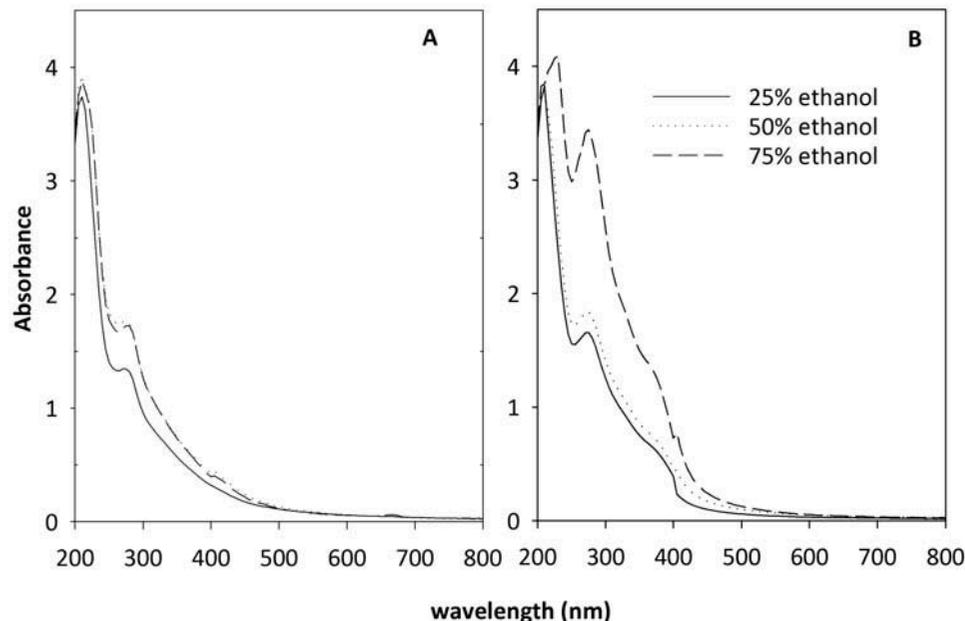
compared to conventional extraction methods such as Soxhlet extraction (Proestos & Komaitis 2008). The extract yields (reported as mg dry extract/g dry biomass), TPC (reported as mg of Gallic acid equivalent (GAE)) and antioxidant capacity (IC<sub>50</sub> of DPPH radical; expressed as µg/mL) from the various ethanol extractions are shown in Table 1. We observed that increasing the concentration of ethanol in extraction solvent resulted in smaller amounts of extract yields, but the derived extracts contained higher TPC and resulted in lower IC<sub>50</sub> values compared to extracts derived using 25% ethanol (Table 1). The TPC of *A. macrostachyum* extracts from this study (Table 1) are in range of what has been reported for hexane extracts obtained from plants collected from Southern Portugal (39 mg GAE/g dry extract) (Rodrigues et al., 2014). We could not find any reports on TPC from *T. qatarensis* in literature. Generally, the TPC of extracts in both *A. macrostachyum* and *T. qatarensis* is low compared to reported TPC of aqueous extracts from a few industrially important plants such as oak (*Quercus robur*), pine (*Pinus maritime*) and cinnamon (*Cinamomum zeylanicum*) which ranged from 300–400 mg GAE/g (Dudonné et al., 2009). We also found a positive correlation ( $r^2 = 0.76$ ) between TPC and IC<sub>50</sub> of DPPH radical in this study (Supplementary Figure 1), suggesting a role for phenolics as antioxidants/radical scavengers. The results are in agreement with other studies that reported significant relationships between measured antioxidant properties and TPC from many plant extracts (Cai et al., 2004; Dudonné et al., 2009; Piluzza & Bullitta, 2011).

UV-VIS spectral analysis provides qualitative information about the constituents of crude extracts and can also demonstrate differences in chemical composition between extracts (Arceusz et al., 2013; Cheok et al., 2012). The UV-VIS absorbance spectra (200–800nm) of halophyte extracts (1mg/mL) is shown in Figure 1. Several observations are made: (1) All extracts have broad absorbance in the 300 to 450 nm of the visible spectrum, and have sharp absorbance maxima in the UV spectrum (Figure 1). (2) Spectra of 50% and 75% ethanol extracts appear to be more similar in *A. macrostachyum* (Figure 1A) whereas the spectra of 25% and 50% extracts appear to be more similar in *T. qatarensis* (Figure 1B). (3) Absorbance peak maxima of 210 and 270 nm are observed in all *A. macrostachyum* extracts, but the 50% and 75% ethanol extracts have higher absorbance at 270 nm compared to 25% ethanol extract. (4) A shift in absorbance peak maxima is observed in *T. qatarensis* 75% ethanol extract compared to the 25% and 50% ethanol extracts (230 nm vs 210), and all extracts have another absorbance maxima at 270 nm (Figure 2B). The absorbance peak maxima in extracts could possibly correspond to the absorbance from phenolic compounds such as phenolic acids and flavonoids. The absorbance maxima of specific phenolic acids have been reported by others (Holser & Smernik, 2012; Robbins, 2003). Accordingly, the absorbance maxima of caffeic acid is 326 nm, vanillic acid is 294–320 nm, syringic acid at 276–328 nm, p-coumaric acid at 312–361 nm, ferulic acid at 312 nm and sinapic acid at 326 nm. The absorbance maxima in the UV range could correspond to flavonoids such as luteolin and quercetin, both of which are reported to exhibit absorbance maxima at 210 nm (Chen, Inbaraj, & Chen, 2012).

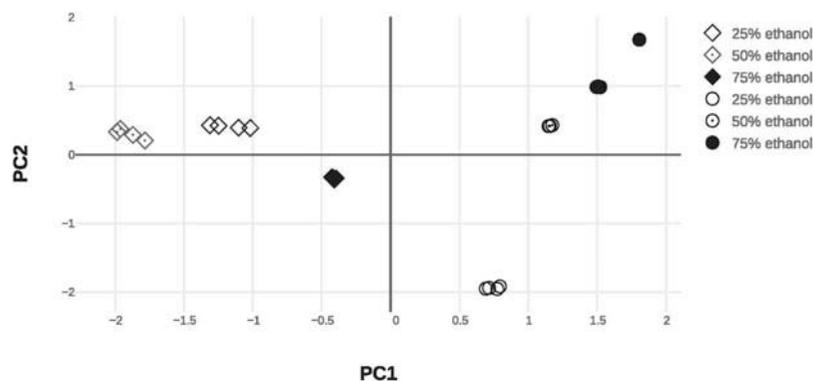
**Table 1.** Effect of varying the concentrations of ethanol in water on amount of extraction yields, total phenolic content (TPC) and half maximal inhibitory concentration (IC<sub>50</sub>) of crude extracts for DPPH-free radical from *Tetraena qatarensis* (*Tq*) and *Arthrocnemum macrostachyum* (*Am*). Values are means ± standard error (SE). Means with different letters are significantly ( $p < 0.05$ ) different from each other

Ethanol	Extraction Yield (mg/g dry biomass)		TPC (mg GAE/g dry extract)		IC <sub>50</sub> (µg/ml)	
	<i>Tq</i>	<i>Am</i>	<i>Tq</i>	<i>Am</i>	<i>Tq</i>	<i>Am</i>
25%	383.4 ± 10.2a	338.8 ± 6.5a	22.6 ± 0.2a	36.0 ± 0.2a	103.7 ± 1.0a	86.6 ± 0.5a
50%	337.5 ± 10.3a	266.9 ± 2.7b	30.0 ± 0.8b	42.3 ± 0.4b	95.8 ± 2.7a	71.6 ± 0.5b
75%	169.2 ± 11.1b	261.3 ± 12.1b	54.4 ± 0.8c	45.6 ± 1.0c	67.9 ± 4.8b	62.7 ± 0.4c

**Figure 1.** UV-VIS absorbance spectra in the range of 200-800 nm of halophyte extracts (1mg/mL) obtained using various concentrations of ethanol in water (A) *Arthrocnemum macrostachyum* (B) *Tetraena qatarensis*.



**Figure 2.** Graphical representation of the first two principal components PC 1 and PC 2 comparing extracts of *Tetraena qatarensis* (diamond shape) and *Arthrocnemum macrostachyum* (circles). Ethanol concentrations are distinguished by empty (25% ethanol), single dot (50% ethanol) and filled (75% ethanol) symbols. PCA was conducted without data scaling and a minimum explained variance of 98%.



To gain a global view of the chemical complexity of the extracts from both plants and the effect of ethanol on each, we analyzed the spectral profile of extracts using UV-VIS and global metabolomes of each extract using UHPLC-QToF-MS. The chemical complexity, based on the number of detected features showed a positive correlation with ethanol concentrations. The total number of features detected in the *T. qatarensis* extracts were 3595, 3736 and 4004 for the 25%, 50% and 75% ethanol extracts, respectively. The same pattern was observed with *A. macrostachyum* where the numbers of detected features were 3822, 4234 and 4561 for the 25%, 50% and 75% ethanol extracts, respectively. These results suggest that although increasing ethanol concentrations reduces the total yield, it leads to a greater chemical complexity in extracted compounds. PCA plots for all extracts showed strong clustering based on plant identity and ethanol concentrations (Figure 2). For example, all *T. qatarensis* extracts clustered separately from *A. macrostachyum* extracts. For *A. macrostachyum*, 50% and 75% ethanol extracts clustered more strongly than the 25% ethanol extract, suggesting that the composition of the former two extracts are more similar. In contrast in *T. qatarensis*, the 25% and 50% ethanol extracts clustered more strongly than the 75% ethanol extract (Figure 2). These observations corroborate our spectral profile analysis, which

showed similar patterns (Figure 1). Furthermore, considerable differences are observed when comparing metabolomics profiles between the two halophyte species (Figure 2), suggesting differences in chemical composition and the presence of metabolites unique to each plant. Future work will involve fractionating the extracts to identify specific compounds that have high market value.

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#### Competing Interest

The authors declare no competing interests.

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#### Supplemental material

Supplemental data for this article can be accessed [here](#).

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