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Ecological conditions, phytochemical composition and antioxidant activity of three Mediterranean halophytes in Egypt

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Abstract : Halophytes that can live in a wide range of saline soils have significant economic value and can be employed for environmental restoration and medicinal applications. The soil characteristics, secondary metabolites, and antioxidant potency of three Mediterranean halophytes (*Halocnemum strobilaceum* (Pall.) M.Bieb., *Mesembryanthemum crystallinum* L., and *M. nodiflorum* L.) from Egypt were determined in this study. The findings revealed that the examined halophytes favored soil with a coarse-sandy texture, a slightly alkaline pH, a high salt content, and low organic matter and macronutrient levels. *M. nodiflorum* had the highest concentration of total phenols (44.42 mg GAE g^{-1} dry extract), alkaloids (9.48 mg g^{-1} dry extract), and tannins (26.3 mg TAE g^{-1} dry extract), while *M. crystallinum* had the highest concentration of flavonoids (11.45 mg CE g^{-1} dry extract) and saponins (19.17 mg g^{-1}). *M. nodiflorum* exhibited the highest scavenging activity against DPPH (IC50= 2.8 mg/ml) and ABTS (55.1% inhibition). The current study's findings suggest that the investigated halophytes are possibilities for green use as food or feed supplements, or biological applications.

Keywords: antimicrobial, antioxidant, halophytes, secondary metabolites.

1.Introduction

The need for food, fodder, pharmaceuticals, and raw resources has expanded with the world's population, and major cultivated lands are predicted to yield 50% more in the future **[1]**. Drought and salt cause agricultural coastal regions in dry countries to shrink by 1% to 2% annually **[2]**. Furthermore, climatic shift patterns in dry areas have a noteworthy impact on terrestrial ecosystems, agricultural lands, and soil properties by altering soil salinity **[3]**. As a result, salinity is often regarded as the most critical environmental factor affecting plant growth and agricultural output.

Halophytes are salt-tolerant plants that grow in saline areas all over the world. Hydro- or xero-halophytes are exceptionally salt-tolerant plants that frequently complete their life cycles in high salt concentrations **[4]**. Halophytes undergo a variety of morphological, anatomical, and biochemical changes in response to salinity tolerance, which are linked to the synthesis and accumulation of a wide range of metabolites **[5]**. Halophytes have a powerful radical oxygen scavenging system that mitigates the harmful effects of reactive oxygen species **[6]**. Despite their high salt content, halophytes can be grown and harvested as raw ingredients for food, feed, and medicines. Halophytes play a vital role in preserving the coastal ecosystem and ensuring ecological stability along the Mediterranean coastal strip of the Nilotic region. The current study focused on three major halophyte species: Halocnemum strobilaceum, Mesembryanthemum crystallinum, and M. nodiflorum.

The goals of this study were to (i) assess the habitat features where the studied halophytes grow; and (ii) identify the secondary metabolites of these halophytes.

2. Materials and methods

2.1. Study area

Based on dominance in the study area, three halophytes, namely *Halocnemum strobilaceum*, *Mesembryanthemum crystallinum*, and *M. nodiflorum* were collected from five populations each along the Deltaic Mediterranean coast of Egypt **(Figure 1)**. The identification of these halophytes was carried out by the last author, according to Boulos **[7].**

Fig (1): Map of Egypt shows the study area and sampling sites (Modified after Abdelaal et al., 2024).

2.2. Soil analysis

From each halophyte canopy, bulk soil samples were collected at 0–50 cm depth, air-dried, sieved, and stored. Physical (texture, WHC and porosity) and chemical (pH, EC, OC, Cl-, HCO_3, SO_4, TN, TP, Na, K, Ca and Mg) properties of soils were estimated according to AOAC [8].

2.3. Phytochemical analysis

The aerial-dried powdered samples of three halophytes were combined with methanol to determine secondary metabolites. Total phenolic [9], flavonoid [10], tannins [11], alkaloids [12], and saponins [13] were estimated in the three investigated halophytes.

2.4. Biological activity

Two methods were used to test the antioxidant scavenging activity of the methanolic extracts of the selected halophytes: the DPPH colorimetric assay [14] and ABTS+ [15].

2.5. Statistical analysis

The means ± standard errors were used to present the study's findings. For significance at $P \leq 0.05$, a one-way ANOVA was performed.

3. Results

3.1. Habitat features

Table (1) shows that there were no significant changes at $P<0.05$ seen in the soil data of the three halophyte habitats represented, with the exception of clay fraction, water-holding capacity, and potassium. Three halophytes have environments with coarse-sandy soil that contains minimal clay. The soil of H. strobilaceum and *M. nodiflorum* had the highest porosity and water-holding capacity, at 40.64% and 41.82%, respectively, whereas the soil of *M. crystallinum* had the lowest porosity and WHC, at 35.2% and 22.96%, respectively. All of the halophytes under study had weakly alkaline or alkaline soil pHs (pHs between 7.7 and 7.9). The EC results showed a range of values from 175.72 to 220.2 µmhos/cm, indicating varying and high saline levels. The highest concentrations of organic carbon (0.99%) and $CaCO_3$ (4.96%) were found in the soil of *H. strobilaceum*. Moreover, the greatest amounts of magnesium (24.98 mg/100g), potassium (17.32 mg/100g), and bicarbonates (0.21%) were found. The soil of *M. nodiflorum* has high levels of chlorides, sulfates, TN, TP, sodium, and calcium.

3.2. Phytochemical content

The results revealed that the levels of total phenols, alkaloids, and saponins among the three halophytes in the methanolic extracts varied substantially ($p<0.05$) apart from flavonoids and tannins **(Figure 2)**. *M. nodiflorum* had the highest observed concentrations of total phenols (44.42 mg GAE g^{-1}), alkaloids (9.48 mg g^{-1}), and tannins (26.3 mg TAE g^{-1}). However, *M. crystallinum* had the highest concentration of saponins (19.17 mg g^{-1}) and flavonoids (11.45 mg CE g^{-1}). *H. strobilaceum*, on the other hand, had the lowest amounts of flavonoids (8.80 mg CE g^{-1}), alkaloids (3.93 mg g^{-1}), tannins (6.07 mg TAE g^{-1}), and saponins (2.86 mg g^{-1}).

3.3. Antioxidant potency of three halophytes

Figure 3 shows the antioxidant scavenging activity of methanolic extracts of three halophytes as measured by the reduction of both DPPH and ABTS levels. *M. nodiflorum's* methanolic extract had the highest antioxidant potential (DPPH IC50 = 2.8 mg extract/ml), followed by *M. crystallinum's* (IC50 = 3.5 mg extract/ml), and *H. strobilaceum's* (IC50 = 5.1 mg extract/ml) lowest activity. Likewise, *M. nodiflorum* extract was the most successful in inhibiting ABTS+, scavenging 55.1% of ABTS+; it was followed by *M. crystallinum* (45.85%) and *H. strobilaceum* (30.45%).

Table (1). Soil properties (mean values± standard errors) support target halophytes' habitats.

Soil factors	*H. strobilaceum*	*M. crystallinum*	*M. nodiflorum*
Physical properties			
Sand (%)	96.4±0.51	96.92±0.48	95.36±1.18
Silt (%)	2.8±0.41	2.54±0.46	3.42±1.16
*Clay (%)	0.8±0.28	0.54±0.01	1.02±0.15
Porosity (%)	40.64±1.83	35.2±2.04	40.01±1.77
*WHC (%)	37.78±1.19	22.96±2.08	41.82±1.26
Chemical properties			
pH	7.7±0.27	7.9±0.12	7.9±0.29
EC (µmhos/cm)	220.2±33.0	194.95±31.88	175.72±26.19
$CaCO_3$ (%)	4.96±0.60	3.68±0.48	4.14±0.61
OC (%)	0.99±0.25	0.78±0.05	0.83±0.22
Cl^- (%)	0.25±0.06	0.13±0.07	0.38±0.11
SO_4 (%)	0.40±0.19	0.18±0.03	0.41±0.14
HCO_3 (%)	0.16±0.02	0.26±0.14	0.15±0.0
TN (mg/100g dry soil)	18.92±4.41	10.86±2.55	25.13±4.45
TP (mg $100g^{-1}$ dry soil)	11.21±2.52	6.65±1.45	16.99±8.42
Na^+ (mg $100g^{-1}$ dry soil)	26.0±4.62	30.11±7.10	37.15±10.37
*K^+ (mg $100g^{-1}$ dry soil)	6.40±1.76	17.32±3.0	6.84±2.11
Ca^{++} (mg $100g^{-1}$ dry soil)	19.85±5.35	11.72±4.68	23.84±4.51
Mg^{++} (mg $100g^{-1}$ dry soil)	11.50±2.44	24.98±6.72	14.43±4.45

* indicates significance at $p<0.05$. WHC: water holding capacity, EC: electric conductivity, OC: organic carbon, TN: total nitrogen, TP: total phosphorous.

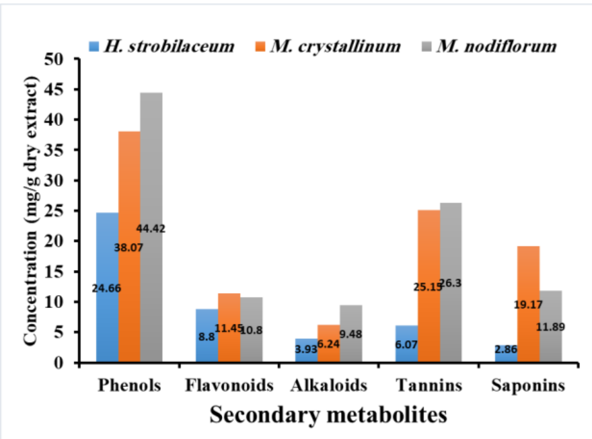

Fig (2). Secondary metabolites (mean values± SE) of three studied halophytes.

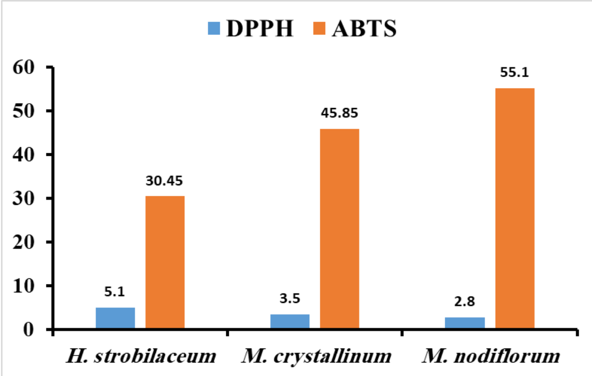

Fig 3. The antioxidant scavenging activity (mean values± SE) of the studied halophytes.

4. Discussion

Researchers ought to explore substitutes for traditional farming practices in order to tackle worldwide issues such increasing population, depletion of freshwater resources, and shrinkage of arable area [16]. Among the options are halophytes, which don't need arable soils or fresh water. These halophytes are hydrated with seawater and can grow on salt-degraded, non-arable soils. Moreover, these plants have the potential to produce large amounts of active chemicals that function as human defense mechanisms and essential nutrients. It is imperative to address the soil conditions that facilitate the growth and persistence of alternative halophytes in order to enhance their production and biomass for economic applications. A group of tests called a soil analysis looks at the availability and condition of nutrients for plant growth [17]. Because soil regulates the flow and availability of nutrients, air, and water for plants, it is the most significant environmental factor in supporting secondary metabolites [18].

The soil conditions of the three halophytes match the knowledge of halophyte habitats on the Mediterranean coast of Egypt [19-20]. The current study's coarse sandy texture, alkaline range, low water holding capacity, low fertility (low organic matter, TN, TP, K+, Ca2+, and Mg2+), and high salinity were characteristics of the coastal soil supporting the halophytes under investigation. These results were in keeping with other studies [21-22, 16] that showed a range of soil types with little organic matter, an acidic to alkaline pH range, and high salinity in the Mediterranean region. Low fertility and a lack of organic matter in coastal salt marshes are attributed by [23] to a number of factors, including erosive processes, restricted nutrient retention and mineral bioavailability, irregular or sparse rainfall, and long, hot summers. Salinity gradients, soil moisture, and biological interactions were found to be significant determinants of plant abundance and distribution in these conditions [24].

In reaction to abiotic stimuli, such as salt, halophytes synthesize secondary metabolites such as phenolics, tannins, alkaloids, terpenoids, and so forth through chemo-defense mechanisms [25]. These metabolites are crucial to human health because they carry out several biological processes and act as antimicrobials and anticancer agents. The investigated halophytes' secondary metabolite concentrations varied significantly depending on the species and extraction solvents used. In the current study, all of the secondary metabolites in the halophytes under consideration were highly scavenged by the methanol solvent. Because of its high polarity and solubility, methanol is a useful solvent for bioactive compounds that have antioxidant qualities and polyphenolics [26-27].

Based on total phenols, alkaloids, and tannins, the halophytes under analysis might be ordered as follows, according to the present results: *M. crystallinum* > *M. nodiflorum* > *H. strobilaceum*, whereas *M. nodiflorum* > *M. crystallinum* > *H. strobilaceum* for flavonoids and saponins. The impacts of genetics, age, the environment, and soil nutrients may have an impact on the bioactive metabolites and their bioactivity among the halophytes under study. According to earlier studies, plants have more polyphenols as the salinity rises [28,16, 26]. Compared to methanolic extracts of *Arthrocnemum macrostachyum*, *Atriplex halimus*, *Limbarda crithmoides*, and *Tamarix nilotica* collected from Egypt, the total phenol content of the halophytes investigated in this study was lower [16]. The reasons for this diversity include the extraction procedure, solvent type, plant organs, and biogeography [29-30].

5. Conclusion

The results of this investigation suggested that three halophytes' methanolic extracts could be possible sources of secondary metabolites. Based on their antioxidant activity and content of secondary metabolites, the halophytes under study were ranked as follows: *H. strobilaceum* > *M. nodiflorum* > *M. crystallinum*. In particular, *M. nodiflorum* high antioxidant activity, which attributed to the combined effect of flavonoids and total phenols.

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