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Habitat Preference, Phytochemical Constituents and Biological Potency of Four Egyptian Mediterranean Halophytes

Mohamed Abdelaal[1 *], Eman A. Moustafa[1,2], Abeer Y. Alattar[1], Ghada A. El-Sherbeny[1], Ibrahim A. Mashaly[1], and Aya A. Yahia[1]

[1]Botany Department, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt

[2]Botany Department, Faculty of Science, University of Benghazi, Libya

ABSTRACT

In the current study, the soil characteristics, secondary metabolites, and biological activity (antioxidant, antibacterial, and anticancer activities) of four Mediterranean halophytes (*Atriplex halimus*, *Arthrocaulon macrostachyum*, *Limbarda crithmoides*, and *Tamarix nilotica*) from Egypt were determined. The results showed that the studied halophytes favored soil with a coarse-sandy texture, slightly alkaline, and highly saline, with low contents of organic matter and macronutrients. *A. macrostachyum* showed the highest concentration of total phenols (181.75 mg GAE g^{-1} dry extract) and flavonoids (13.90 mg CE g^{-1} dry extract), while *T. nilotica* had the highest concentration of alkaloids (6.43 mg g^{-1} dry extract). Lower contents of soil sulfates, phosphorous, and calcium could induce a greater accumulation of total phenols and flavonoids in these halophytes. The extract of *A. macrostachyum* exhibited the highest scavenging activity against DPPH (IC_{50}= 0.26 mg/ml) and ABTS (71.16% inhibition). The methanolic extracts of four halophytes exerted a pronounced effect against both *Bacillus subtilis* and *Staphylococcus aureus*, while extracts of *A. macrostachyum* and *T. nilotica* released an antibacterial effect against *Escherichia coli* and *Pseudomonas aeruginosa*. Moreover, *A. macrostachyum* extract exhibited moderate cytotoxicity against liver hepatocellular carcinoma (HePG2), mammary gland carcinoma (MCF-7), and prostate cancer (PC3). The findings of the current study recommend that the studied halophytes are candidates for green use as food or feed supplements or in various biological applications against antibiotic-resistant bacteria and human cancer cells.

Keywords: Anticancer, Antimicrobial, Antioxidant, Halophytes, Secondary metabolites.

INTRODUCTION

The demand for food, fodder, medicines, and raw materials has increased due to the progressive rise in world population, and it is predicted that the major cultivated lands will need to yield 50% more in the future (Godfray *et al*., 2010). Agricultural coastal areas in arid and semi-arid countries are annually decreasing by 1% to 2% due to salinity and drought (Patterson *et al.*, 2013). Moreover, the climate change patterns in arid lands are noticeably influencing terrestrial ecosystems, agricultural lands, and soil properties by impacting soil salinity (Corwin, 2021). Accordingly, salinity is recognized as the main environmental factor for plant growth and agricultural productivity. Approximately 10% of the earth's surface comprises saline and salt-affected areas that are broadly distributed worldwide and support the growth of a wide group of halophytes (O'leary and Glenn, 1994). Therefore, scientists should look for unconventional plants that can survive or stand in highly saline-soils. This goal could be achieved by restoring and rehabilitating salt-affected regions for agriculture by using halophytes that respond to salt stress factors in a short time (Abdellaoui *et al.,* 2023).

Halophytes are salt-tolerant plants that have the remarkable ability to survive in extreme saline conditions. These plants are found all over the world and play a crucial role in maintaining the ecological balance of saline environments. A study conducted by Joshi et al. in 2015 shed light on the life cycles of halophytes, particularly their ability to complete their life cycles in high salt concentrations In response to salinity tolerance, halophytes display a variety of morphological, anatomical, and biochemical adaptations that are connected to the synthesis and accumulation of a wide range of metabolites (Hasanuzzaman *et al.*, 2019). In addition, halophytes exhibit an efficient radical oxygen scavenging system that can neutralize the harmful effects of reactive-oxygen species (Stanković *et al.*, 2023). Halophytes can be developed and reaped as raw constituents for food, feed, and medications despite having a great salt content. Also, they are distinguished by the presence of bioactive compounds (polyphenols and terpenes), which may have therapeutic applications and be used as food additives (Hasanuzzaman *et al.*, 2019).

Egypt encompasses six biogeographical sectors, namely: Libo Nubian, Nilotic, Marioutico Arishian, Sinaico Arabian, Elbanian, and Suezian (Abdelaal *et al.*, 2020). The salt-affected lands are almost evenly distributed among the six biogeographical sectors. As a biodiversity hotspot, the Mediterranean coastal region of Egypt is in both the Nilotic and Marioutico-Arishian sectors. The saline areas in Egypt are distinguished into different habitats, including coastal salt marshes, wetlands, and lakes (Zahran and Willis, 2009). Along the Mediterranean coastal strip of the Nilotic sector (the Nile Delta), halophytes play a crucial role in protecting the coastal ecosystem and ensuring ecological stability. Among dominant halophytes, four species were selected in the current study, namely:

* *Corresponding author e-mail:* mohamed_eco@mans.edu.eg

Atriplex halimus L., *Arthrocaulon macrostachyum* (Moric.) Piirainen & G.Kadereit, *Limbarda crithmoides* (L.) Dumort. and *Tamarix nilotica* (Ehrenb.) Bunge. The abundance of these halophytes varies depending on their salt-tolerance capacity, distance from the Mediterranean Sea, and human threats. Therefore, the objectives of this study were to (i) evaluate the physicochemical properties of the soil in which the studied halophytes are growing; detect the primary type of secondary metabolites produced by these halophytes; (iii) establish the correlation between soil characteristics and secondary metabolites. (iv) assess the biological activities, including antioxidant, antibacterial, and anticancer activities, of these halophytes.

MATERIALS AND METHODS

Study area

Egypt is distinguished by its sole geographical location between Africa and Asia and coastlines on the Mediterranean Sea and the Red Sea (Zahran and Willis, 2009). Egypt's Mediterranean coastal land extends eastward from Sallum to Rafah, with an average width of 15–20 km, and is subdivided into three subsectors: western (Marioutic subsector), middle (Deltaic subsector), and east (Arishian subsector). The Deltaic subsector (the study area) extends for ca. 180 km from Port-Said to Alexandria with a width of ca. 12 km and receives ca. 100–200 mm of annual rainfall (Figure 1). Despite the importance of the Mediterranean coastal region as a major natural resource, it is threatened by serious issues such as urbanization, mining and quarrying, tourism activities, climate change, etc.

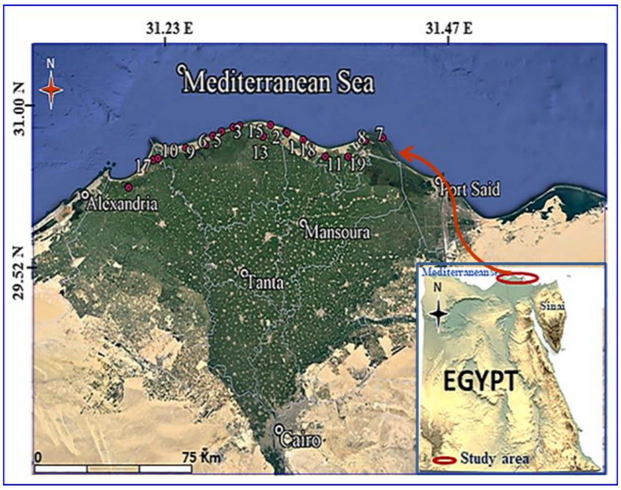

Figure (1): Map of Egypt shows the study area and sampling sites along the middle Mediterranean coast (Google Earth, accessed April 2023).

Plant materials collections

Based on their dominance in the study area, four halophytes, namely *Atriplex halimus*, *Arthrocaulon macrostachyum*, *Limbarda crithmoides*, and *Tamarix nilotica* were collected from five populations/sites each and selected for the current study (Photo-plate 1). The identification of these halophytes was carried out by the first author, according to Boulos (2009), Täckholm (1974) and POWO (2022). Voucher specimens from each halophyte plant were kept in the herbarium of Mansoura University. The floristic features of the selected halophytes are displayed in Table (1). *Atriplex halimus L*., is a nitrophilous, halophytic perennial-shrub that can survive under harsh conditions (Walker *et al*., 2014). In the Mediterranean zone, *A. halimus* offers livestock feed or silage (Khattab, 2007). *Arthrocaulon macrostachyum* (synonymous: *Arthrocnemum macrostachyum*) is a perennial halophytic shrub native to the Mediterranean coastal territory. The value of *A. macrostachyum* is owing to its nutritional, health benefits and phytoremediation potential (ElNaker *et al.*, 2020). *Limbarda crithmoides* (synonymous *Inula crithmoides* L.) is a perennial succulent halophyte belnging to family Asteraceae. *L. crithmoides* is a salt-tolerant plant with antioxidant and biological activity that is widely used in traditional medicine (Bucchini *et al.*, 2013; Jallali *et al.*, 2020). *Tamarix nilotica* (Family Tamaricaceae) is a perennial evergreen halophyte growing naturally in Mediterranean salt marshes. The leaves and young branches of *T. nilotica* are used to treat spleen edema and mixed with ginger to treat uterine infections (Bakr *et al*., 2013). During April-May 2022, the aerial parts of selected halophytes were collected, washed with dist-illed water, air-dried at room temperature until full dryness, then ground, and finally kept in polyethylene bags.

Soil sampling and physicochemical analysis

From each halophyte canopy, bulk soil samples were collected at 0-30 cm depth. The samples were air-dried, sieved, and stored. Soil texture, porosity, and water-holding capacity were evaluated by Piper's procedures (Piper, 1966). Organic carbon was determined using the Walkley-Black method (Jackson, 2005). An electric pH meter was used to determine the soil pH in 1:5 (w/v) aqueous solutions. Electric conductivity (EC) was measured by the conductivity meter (Apera AI209-T, Apera). Chlorides, sulfates, bicarbonates, total phosphorus (TP), and total nitrogen (TN) were estimated according to Burt's manual (Burt, 2004). Na^+ and K^+ were valued using a flame photometer (Han-chen FP-6431, UK), while Ca^{2+} and Mg^{+2} were analyzed by an atomic absorption spectrophotometer (Perkin-Elmer, USA).

Preparation of plant extracts

To prepare plant extracts, the aerial-dried powdered samples of four halophytes (10 g each) were macerated in methanol to extract the secondary metabolites. The samples were placed in a water-bath shaker and preserved at a temperature of 40°C for 4 hrs at 200 rpm. Afterward, the extracts were filtered using Whatman filter paper No. 1, evaporated under vacuum conditions at 40°C, and then stored at 5°C.

Phytochemical investigation

Total phenolic was quantified by the Folin-Ciocalteu assay (Wolfe *et al.*, 2003) and reported as mg gallic acid (GAE)/g dry extract. The flavonoid content (in mg catechin equivalent (CE)/g dry extract) was estimated by using the aluminum chloride colorimetric assay (Zhishen *et al.*, 1999). For tannins, the vanillin-hydrochloride technique was applied (Burlingame, 2000) and conveyed as mg tannic acid equivalent (TAE)/g dry extract. Alkaloids content (mg g^{-1} dry extract) was esti-

Photo-plate (1): Morphology of the studied halophytes: (A) *Atriplex halimus;* (B) *Limbarda crithmoides*; (C) *Arthrocaulon macrostachyum;* and (D) *Tamarix nilotica*.

Table (1): List of the investigated halophytes and their floristic features

Voucher number	Species	Synonymous	Family	Life span and form	Chorotype	Habitat-type
MANS2022106	*Atriplex halimus* L.	*Chenopodium halimus* Thunb.	Amaranthaceae	P, Ph	ME+SA-SI	Salt marshes and lakes
MANS2022107	*Arthrocaulon macrostachyum* (Moric.) Piirainen & G.Kadereit	*Arthrocnemum macrostachyum* (Moric.) K.Koch		P, Ch	ME+SA-SI	Salt marshes and lakes
MANS2022206	*Limbarda crithmoides* (L.) Dumort.	*Inula crithmoides* L.	Asteraceae	P, Ch	ME+ER-SR+SA-SI	Salt marshes and saline drainage canals
MANS2022306	*Tamarix nilotica* (Ehrenb.) Bunge	*Tamarix arabica* Bunge	Tamaricaceae	P, Ph	SA-SI+S-Z	Dry salt marshes and saline sand mounds

The codes for life span and life forms are as follows: P, perennial; Ph, phanerophytes; Ch, chamaephytes; chorotypes include: ME, Mediterranean; SA-SI, Saharo-Sindian; ER-SR, Euro-Siberian and S-Z, Sudano-Zambezian.

imated by using an ammonium hydroxide solution (Harborne, 1998). Saponins content (mg g^{-1} dry extract) was estimated by consecutive solvent extractions (Obadoni and Ochuko, 2002). The details were covered in our previous work (Mahdi *et al.*, 2023).

Biological potency

Antioxidant scavenging activity

The antioxidant scavenging activity of the methanolic extracts of the selected halophytes was tested by two methods: the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) colorimetric assay (Kitts *et al.*, 2000) and the 2, 2′-azinobis- (3- ethylbenzothiazoline-6- sulfonic acid) ($ABTS^+$) (Re *et al.*, 1999). From the plotted graph corresponding to the inhibition percentage of DPPH against extract concentrations, the extract concentration offering 50% inhibitory (IC50) was computed. The DPPH-IC_{50} is inversely prop-ortional to the antioxidant ability of the tested samples (Parejo *et al.*, 2000). Regarding the ABTS, the anti-oxidant potential of each extract was evaluated based on its ability to inhibit the ABTS. Ascorbic acid was considered a reference antioxidant.

Antibacterial activity

The methanolic extracts of the selected halophytes were tested against four bacterial strains: two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. In this test, the agar-well diffusion technique was applied (Prabuseenivasan *et al.*, 2006). Spreading a certain amount of inoculum across the agar surface, then, using a sterile cork-borer, eight mm-diameter holes were aseptically created, and 40 µL of tested extracts were added at the desired concentrations. The agar plates were then incubated under appropriate conditions based on the inoculum. Each inhibitory zone's diameter was measured in millimeters, and the average results were considered. Cefoperazone and clarithromycin anti-biotics were used as positive controls.

Anticancer potential

Three human tumor cell lines: HePG2 (liver hepatocellular carcinoma), MCF-7 (mammary gland carcinoma), and PC3 (prostate cancer) were used in the current study. The colorimetric assay of MTT (3- [4, 5-dimethylthiazol-2-yl] -2, 5-diphenyl- 2H-tetrazolium bromide) reduction from yellow to purple was used to test the anticancer activity of selected halophytes (Bondock *et al.*, 2012). With a density of 5.0 x 10^3 cells/well and 100 µl of RPMI- 1640 medium at 37°C, the cells were seeded in 96-well plates for 48 h under 5% CO_2. After incubation, cells were treated with various concentrations of the methanolic extracts of target halophytes and incubated for 24 hrs MTT solution (20 µl) was added and allowed to stand for 4 hrs. Then, the medium was removed from the plates, and 100 µl of DMSO was poured into each well. To estimate the reduction in cell growth, the absorbance (at 570 nm) of each well was measured. The IC_{50} values for each extract were calculated. For comparison, doxorubicin was considered as a reference anticancer drug. All compounds were prepared or solubilized in dimethyl sulfoxide (DMSO, 10 mM stock). The antitumor potency of each extract was evaluated depending on the IC_{50} inhibitory concentration (µg) as follows: 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak), and greater than 100 (non-cytotoxic) (El-Zayat *et al.*, 2021).

Statistical analysis

The results of this study were expressed as means± standard errors. The one-way ANOVA followed by the Kruskal-Wallis analysis was applied to test the significance at $p \leq 0.05$. The correlation between soil factors and secondary metabolites of four halophytes was tested using Canonical Correspondence Analysis (CC-A) and Pearson's correlation. All statistical analysis was carried out by the XLSTAT program (2016) and package "metan" in R software version 4.2.3.

RESULTS

Soil physicochemical properties supporting the target halophytes

The soil data of the represented habitats of the four halophytes showed no significant variations at $p \leq 0.05$ except for total phosphorus (TP) and calcium (Table 2). The soil texture in the habitats of four halophytes is coarse-sandy with little clay content. Porosity and water holding capacity were the highest in the soil of *L. crithmoides* (42.86% and 43.93%, respectively), and the lowest in the soil of *T. nilotica* (37.34% and 36.48%, respectively). The soil pH, collected from all studied halophytes was weakly alkaline or alkaline ranging from pH7.4 to 8.3. Electrical conductivity (EC) displayed values between 0.50 and 0.94 mmhos/cm, indicating high and variable salinity levels. The soil of *L. crithmoides* had high levels of calcium carbonates, organic carbon, sulfates, total phosphorus, and calcium. Furthermore, the highest concentrations of bicarbonates (0.21%) and total nitrogen (29.89 mg/100g dry soil) were recorded in the soil of *A. halimus*. The soil of *A. macrostachyum* had the highest content of Cl^- (0.53%), Na^+ (147.28 mg/100g dry soil) and K^+ (19.82 mg/100g dry soil).

Phytochemical compounds content

Except for saponins, the results showed that total phenols, flavonoids, tannins, and alkaloids in methanolic extracts significantly varied ($p \leq 0.05$) among four halophytes (Figure 2). The highest contents of total phenols (181.75 mg GAE g^{-1} dry extract) and flavonoids (13.90 mg CE g-1 dry extract) were observed in *A. macrostachyum*. *T. nilotica* attained the highest value of alkaloids (6.43 mg g-1 dry extract). Furthermore, *A. halimus* attained the highest concentrations of tannins (12.41 mg TAE g^{-1} dry extract) and saponins (2.39 mg g^{-1} dry extract). In contrast, *L. crithmoides* attained the lowest contents of total phenols (127.82 mg GAE g^{-1} dry extract), flavonoids (7.28 mg CE g^{-1} dry extract), tannins (4.45 mg TAE g-1 dry extract), and saponins (1.46 mg g^{-1} dry extract), while *A. halimus* exhibited the lowest content of alkaloids (3.43 mg g^{-1} dry extract).

Correlation between soil characters and secondary metabolites of four halophytes

The correlation between soil features and the secondary metabolites of four halophytes is illustrated through CCA and Pearson-correlation diagrams (Figure 3). Total phenols and flavonoids content showed a negative significant correlation with sulfates, total phosphorus, and calcium; and tannins concentration is positively correlated with bicarbonates, total nitrogen, and potassium. Regarding the studied halophytes, *A. halimus* showed a positive correlation with bicarbonates, total nitrogen, and pH; *T. nilotica* displayed a positive correlation with sulfates, sand, and pH; *L. crithmoides* was closely related to sodium, magnesium, total phosphorous, and calcium; and *A. macrostachyum* showed a positive correlation with water–holding capacity, organic carbon, chlorides, and electric conductivity (EC).

Antioxidant scavenging activity

The results of antioxidant scavenging activity, based on the reduction of both DPPH and ABTS by methanolic extracts of four halophytes, are demonstrated in Table (3). The methanolic extract of *A. macrostachyum* expressed the highest antioxidant potential (DPPH IC_{50}= 0.26 mg extract/ml), followed by *T. nilotica* (IC_{50}= 0.30 mg extract/ml) and *A. halimus* (IC_{50}= 0.38 mg extract/ml). Meanwhile, *L. crithmoides* displayed the lowest antioxidant activity. Similarly, regarding the $ABTS^+$ inhibition, *A. macrostachyum* extract was the top effective one as it scavenged 71.10% $ABTS^+$, followed by *T. nilotica* (60.90%), *A. halimus* (55.81%), and *L. crithmoides* (40.16%).

Antibacterial activity

The inhibitory effect of methanolic extracts of studied halophytes on four pathogenic bacterial isolates is shown in Table 4. Regarding the Gram-positive isolates, the extract of *A. macrostachyum*, followed by *T. nilotica*, *A. halimus*, and *L. crithmoides*, showed an inhibitory effect against both *S. aureus* and *B. subtilis*. On the other hand, the extracts of both *A. macrostachyum* and *T. nilotica* were active against *E. coli* (inhibition zones of 14.60 and 10.50 mm, respectively) and *P. aeruginosa* (inhibition zones of 16.35 and 12.30 mm, respectively). No inhibitory effect was detected against *E. coli* and *P. aeruginosa* by methanolic extracts of *A. halimus* and *L. crithmoide.*

Anticancer potential

The cytotoxic effects of the methanolic extracts of the studied halophytes against three human tumor cell lines: HePG2, MCF-7 and PC3 are illustrated in Figure 4. According to the potency scale, the results displayed that, *A. macrostachyum* exhibited moderate activities against three human tumor cells, HePG2, MCF-7, and PC3, with IC_{50} values of 32.0± 3.01, 42.67± 2.78, and 49.20± 4.61 µg ml^{-1}, respectively, while the extract of *T. nilotica* showed moderate potency against both HeP-G-2 (IC_{50}= 38.33± 3.93) and MCF-7 (IC_{50}= 39.0± 3.23) and a weak potency against PC3 (IC_{50}= 65.05± 1.69). On the other hand, the extracts of *A. halimus* and *L. crithmoides* showed relatively weak cytotoxic potency against the three tumor cell lines.

Table (2): Comparative analysis of soil physiochemical characteristics attributes of A. macrostachyum, *A. halimus*, *L. crithmoides*, and *T. nilotica* in Mediterranean ecosystems.

Soil characteristics	Soil sample collected from halophytes' habitats			
	A. macrostachyum	***A. halimus***	***L. crithmoides***	***T. nilotica***
Physical properties				
Soil texture				
Sand (%)	95.12±0.85[a]	95.9±2.88[a]	96.00±0.63[a]	96.66±1.43[a]
Silt (%)	3.62±0.47[a]	3.30±0.55[a]	2.53±0.51[a]	2.48±0.04[a]
Clay (%)	1.25±0.32[a]	0.80±0.14[a]	1.47±0.26[a]	0.66±0.09[a]
Porosity (%)	41.99±1.21[a]	42.36±0.86[a]	42.86±0.44[a]	37.34±1.40[a]
WHC (%)	43.27±2.12[a]	38.13±3.05[a]	43.93±1.39[a]	36.48±2.34[a]
Chemical properties				
pH	7.5±0.08[a]	8.3±0.09[a]	8.1±0.03[a]	7.4±0.38[a]
EC (mmhos/cm)	0.94±0.26[a]	0.50±0.07[ab]	0.54±0.19[ab]	0.70±0.04[b]
$CaCO_3$ (%)	5.07±0.43[a]	4.07±0.78[a]	5.79±0.52[a]	3.10±0.45[a]
OC (%)	1.04±0.17[a]	1.20±0.12[a]	1.26±0.04[a]	0.55±0.11[a]
Cl^- (%)	0.53±0.12[a]	0.34±0.04[ab]	0.36±0.09[ab]	0.42±0.06[b]
SO_4 (%)	0.12±0.05[a]	0.47±0.16[a]	0.70±0.11[a]	0.18±0.09[a]
HCO_3 (%)	0.14±0.00[a]	0.21±0.02[a]	0.15±0.00[a]	0.17±0.01[a]
TN (mg/100g dry soil)	21.47±4.61[a]	25.89±3.44[a]	29.63±1.26[a]	24.58±3.45[a]
TP (mg $100g^{-1}$ dry soil)	7.27±0.70[a]	15.05±2.73[ab]	26.97±3.76[ab]	10.46±2.86[b]
Na^+ (mg $100g^{-1}$ dry soil)	147.28±3.90[a]	114.10±1.21[ab]	106.14±1.09[ab]	122.76±1.45[b]
K^+ (mg $100g^{-1}$ dry soil)	19.82±1.02[a]	18.52±0.72[a]	13.16±0.45[a]	16.32±0.90[a]
Ca^{++} (mg $100g^{-1}$ dry soil)	28.18±2.94[a]	42.20±0.81[ab]	50.49±4.83[ab]	34.11±1.83[b]
Mg^{++} (mg $100g^{-1}$ dry soil)	21.94±1.91[a]	18.87±1.57[a]	19.00±1.53[a]	32.81±3.97[a]

WHC: water holding capacity, EC: electric-conductivity, OC: organic-carbon, TN: total nitrogen, and TP: total phosphorous.
Different lowercase letters in each row specify significant differences among studied halophytes ($p \leq 0.05$).

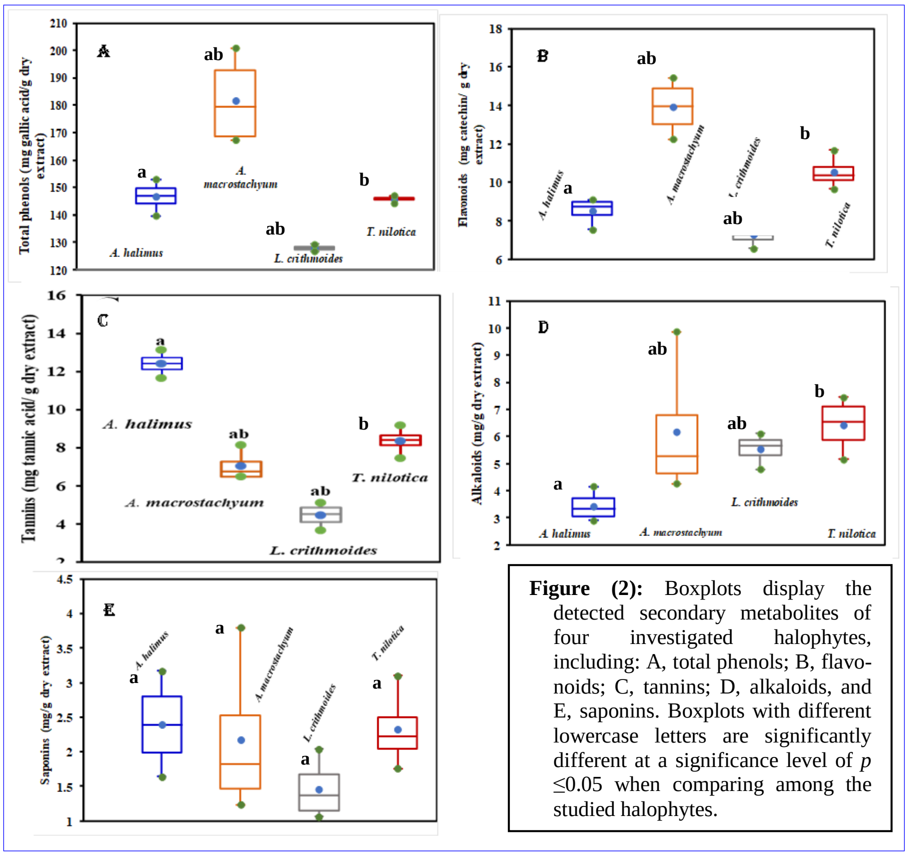

Figure (2): Boxplots display the detected secondary metabolites of four investigated halophytes, including: A, total phenols; B, flavonoids; C, tannins; D, alkaloids, and E, saponins. Boxplots with different lowercase letters are significantly different at a significance level of *p* ≤0.05 when comparing among the studied halophytes.

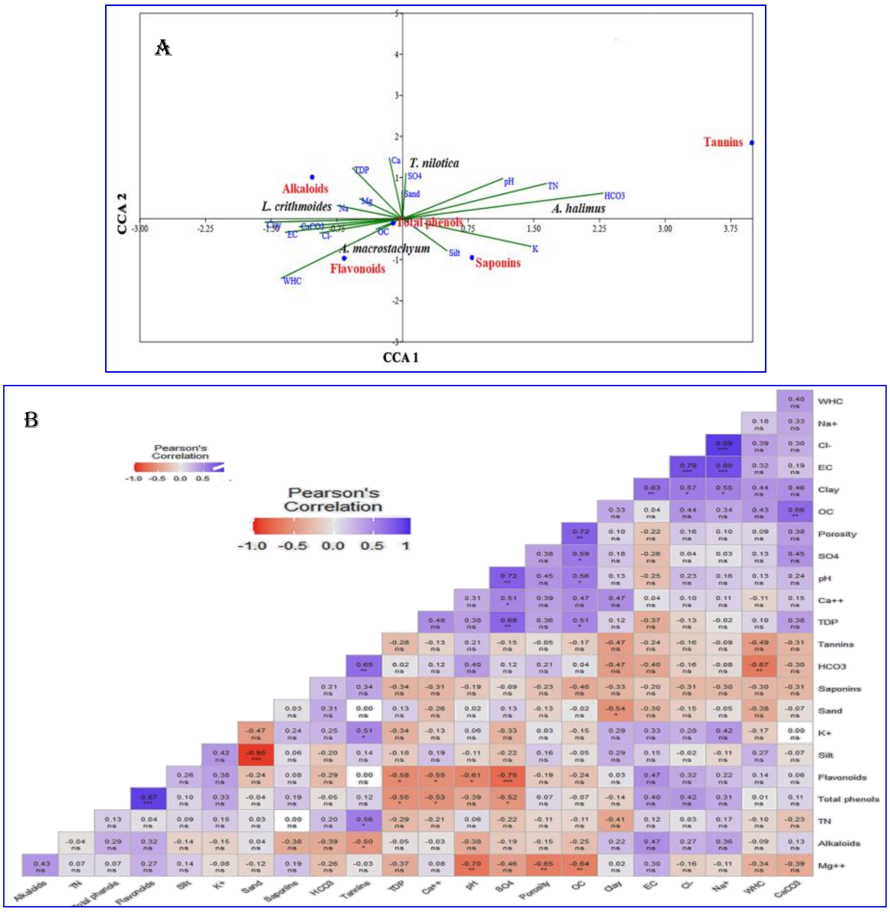

Figure (3): Correlation between soil physicochemical parameters and secondary metabolites in the studied halophytes: (A) Canonical Correspondence Analysis (CCA); and (B) Pearson's correlation matrix with the *p*-value. Eigenvalues of 65.86% for CCA 1 and 15.95% for CCA 2. Soil parameters abbreviations include: TN, total nitrogen; TDP, total phosphorous; OC, organic carbon; WHC, water holding capacity; and EC, electric conductivity.

Table (3): The antioxidant scavenging activity of the studied halophytes as compared with ascorbic acid as positive control. Data are in mean values± standard errors.

Studied Halophyte	DPPH	ABTS
	IC_{50} (mg extract/ml)	% inhibition
A. halimus	0.38 ±0.011[ab]	55.81 ±0.90 [ab]
A. macrostachyum	0.26 ±0.00 [ab]	71.10 ±1.50 [ab]
L. crithmoides	1.12 ±0.024[b]	40.16 ±1.10 [a]
T. nilotica	0.30 ±0.00 [ab]	60.90 ±2.05 [ab]
Ascorbic acid[†]	0.02 ±0.00 [a]	89.50 ±0.68 [b]

Means with different lowercase letters per column are significant different at level $p \leq 0.05$. [†], Positive control.

Table (4): Antibacterial activity of methanolic extracts of the investigated halophytes against human pathogen including Gram-positive, *Staphylococcus aureus* and *Bacillus sutilius;* and Gram-negative, *Escherichia coli* and *Pseudomonas areginosa*. Data are in mean values± standard errors.

Studied Halophyte	Diameter of inhibition zone (mm)			
	Human pathogens			
	Gram-positive		Gram-negative isolates	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Positive control (Standard antibiotics)				
Clarithromycin	20.0±0.50[b]	25.6±0.33[b]	-	-
Cefoperazone	-	-	28.0±0.88[b]	34.66±0.80[b]
Negative control (DAMSO)	-	-	-	-
A. halimus	11.0±0.50[ab]	14.0±0.57[ab]	-	-
A. macrostachyum	18.0±0.57[ab]	20.0±0.33[ab]	14.60±1.76[ab]	16.35±2.02[ab]
L. crithmoides	8.90±0.08[a]	12.0±0.57[a]	-	-
T. nilotica	15.10±1.00[ab]	15.0±0.58[ab]	10.50±0.88[a]	12.30±0.88[a]

-, negative result; means with different lowercase letters, per column, are significant different at level $p \leq 0.05$. DMSO, dimethyl sulfoxide.

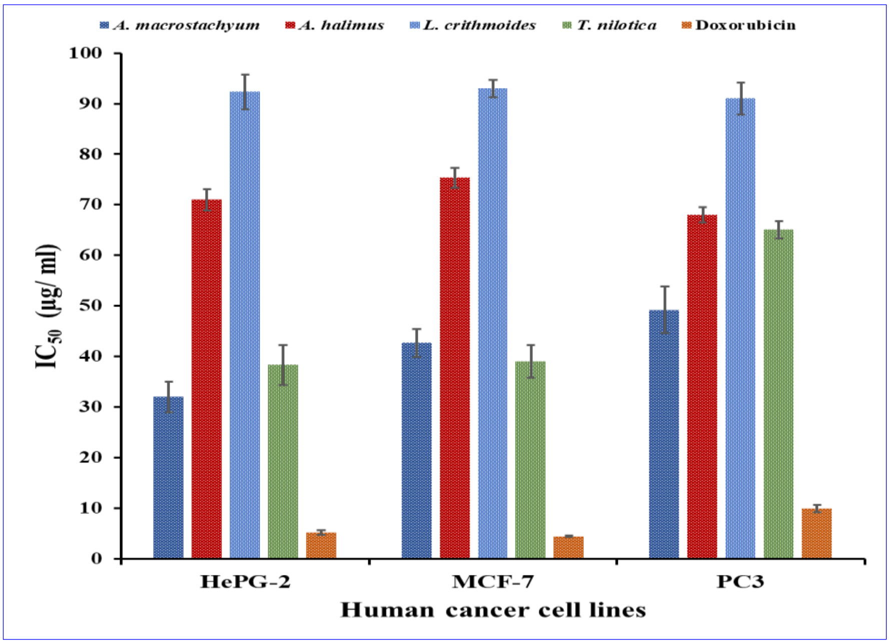

Figure (4): Cytotoxicity of halophyte extracts on different cancer cell lines, .HePG2, liver hepatocellular carcinoma; MCF-7, mammary gland carcinoma; and PC3, prostate cancer. The data expressed as IC_{50}. Data is shown as mean ± standard error (SE), n=3.

DISCUSSION

To face the global challenges due to the growing human population, scarcity of fresh-water, and decline of arable land, scientists should search for alternative plants to conventional crops. Among these alternatives plants are halophytes, which do not need fresh-water or arable soils. These halophytes can grow on non-arable, salt-degraded soils and be watered with seawater. Moreover, these plants can produce large amounts of active chemicals that are not just defense mechanisms but also vital for humans.

To optimize the yield and biomass of alternative halophytes for economic use, it is important to address the soil features that support their growth and persistence. The soil analysis is a set of analyses that refer to the nutrient status and availability for plant growth (Cardoso *et al.,* 2013). Among environmental factors, soil is the most crucial variable in stimulating secondary metabolites, as it regulates the movement and availability of water, air, and nutrients in plants (Chaouqi *et al.,* 2023). The soil conditions of the four investigated halophytes are in the range of what has been informed about the habitats of halophytes on the Mediterranean coast of Egypt (Serag, 1999; Zahran and Willis, 2009). In the current study, the coastal soil supporting the studied halophytes was featured by its coarse sandy texture, alkaline range, low water holding capacity, low fertility (low organic matter, TN, TP, K^+, $Ca^{2+,}$ and Mg^{2+}), and high salinity. These findings were in agreement with reports that displayed that, the Mediterranean region hosts different types of soils with low amounts of organic matter, an acidic to alkaline range, and high salinity (Abdelaal *et al.,* 2018; Ciccarelli, 2015; El-Sherbeny *et al.,* 2021). Specifically, such low fertility and deficiency of organic matter along the coastal salt marshes habitat may be attributed to limited nutrient retention capacity and minerals bioavailability, degradation and erosive processes, scarce or irregular rainfall, and long, hot summers (Sardans and Peñuelas, 2013). In such habitats, salinity gradients, soil moisture, and biological interactions were recognized as the key factors influencing plant distribution and abundance (Caravaca *et al.,* 2005).

Under abiotic stresses, including salinity, as a chemo-defense mechanism, halophytes trigger the synthesis of a variety of secondary metabolites such as phenolics, tannins, alkaloids, terpenoids, etc. (Agrawal and Konno, 2009). Moreover, these metabolites play a vital role for humans as antimicrobials, anticancer agents, and other biological activities. The concentration of secondary metabolites in the studied halophytes greatly varied depending on species and extraction solvents. In the current study, the methanol solvent scavenged high levels of all secondary metabolites in the studied halophytes. Methanol is a good solvent for most polyphenolics and bioactive compounds with strong antioxidant capacity due to its good solubility and polarity (Galanakis *et al.,* 2013; Mahdi *et al.,* 2023; Oalđe Pavlović *et al.,* 2021; Bakr *et al.,* 2013). The current results disclose that the analyzed halophytes can be ranked according to total phenols and flavonoids as follows: *A. macrostachyum*> *T. nilotica* > *A. halimus* > *L. crithmoides*, while for alkaloids, *T. nilotica*> *A. macrostachyum* > *L. crithmoides* > *A. halimus*. The sequence of both tannins and saponins regarding the studied halophytes is *A. halimus* > *T. nilotica* > *A. macrostachyum* > *L. crithmoides*. The variation in the bioactive metabolites and their bioactivity among the studied halophytes could be attributed to the effects of environmental factors, genetics, age, and nutrients in the soil. Previous research found that the rise in salinity increases the content of polyphenols in plants (Bartwal *et al.,* 2013; El-Sherbeny *et al.,* 2021). The total phenols of *A. macrostachyum* from the current study showed greater phenols content than ethanol extract of *A. macrostachyum* collected from the United Arab Emirates (45.6 mg GAE g^{-1} dry extract) (Jitan *et al.,* 2018), hexane extract of *A. macrostachyum* from Portugal (39 mg GAE g^{-1} dry extract) (Rodrigues *et al.,* 2014), and different solvents of *A. macrostachyum* collected from Algeria (Chekroun-Bechlaghem *et al.,* 2019), but lower flavonoids than the Portuguese type (Barreira *et al.,* 2017). This variation is attributed to biogeography, plant organs, solvent type, and extraction process (Chekroun-Bechlaghem *et al.,* 2019; ElNaker *et al.,* 2020). According to Zengin et al. (2018), *A. macrostachyum* has different phenolic acids such as gallic acid, rosmarinic acid, caffeic acid, etc. On the other hand, the methanolic extract of *A. halimus* showed more total phenols, tannins, alkaloids, and saponins than the Algerian species (Benhammou *et al.,* 2009). In addition, the total phenols and flavonoids reported for *T. nilotica* in this study were greater than the previous study estimating total phenols and flavonoids in *T. nilotica* (Bakr *et al.,* 2013). Previous literature reported that *L. crithmoides* contains significant levels of secondary metabolites such as phenolic acids, flavonoids, terpenoids, and essential oils (Adorisio *et al.,* 2020; El-Sherbeny *et al.,* 2021).

Concerning soil physicochemical features associated with phytochemical components of the studied halophytes, lower concentrations of soil sulfates, total phosphorous and calcium induce a greater accumulation of total phenols and flavonoids in the studied halophytes. This finding was consistent with many authors (Lea *et al.,* 2007; Martins-Noguerol *et al.,* 2023; Stewart *et al.,* 2001), who revealed the negative correlations between phenols and flavonoids yields and soil nutrients. Moreover, tannins content is triggered by increasing soil bicarbonates, total nitrogen and potassium. Based on carbon nutrient-balance, the total phenols should rise in nutrient poor soils and decrease in nutrient-rich soils, which was in agreement with our results (Martins-Noguerol *et al.,* 2023). Therefore, nutrient limitation (particularly organic matter, nitrogen, and phosphorous) and coarse-textured saline soils in the current coastal study area induce a high yield of total phenols and flavonoids in the studied halophytes. This finding has been well documented by

Lea *et al.* (2007) and Stewart *et al.* (2001). As a result, the depletion of nutrients such as nitrogen, phosphorus, and organic matter could be an intriguing and cost-effective strategy for halophytes to produce higher levels of polyphenols. Alteration of macronutrient levels has already been recommended as a mode for modifying the levels of these desirable compounds and improving plant quality (Lillo *et al.,* 2008). Similar results have been reported for *Cakile maritima*, whereby, with increasing salinity in arid conditions, the polyphenols and antioxidant activity increase (Ksouri *et al.,* 2007). However, in response to salinization, the phenol accumulation in halophytes is varied, depending on species genotype, organ-specific factor, ontogenetic state, and duration and intensity of salinization (Pungin *et al.,* 2023).

Currently, safe antioxidant supplements of natural origin are required not just for their use in stopping the oxidative deterioration of food products but also for inhibiting oxidative damage at a cellular level (Salehi *et al.,* 2018). In terms of the extract's antioxidant power based on the neutralization of synthetic DPPH and $ABTS^+$ radicals, the antioxidant potentials of methanolic extracts of four halophytes were sequenced as follows: *A. macrostachyum*> *T. nilotica*> *A. halimus*> *L. crithmoides*. In fact, several studies that examined the relationship between antioxidant activity and bioactive constituents in halophytes, medicinal plants, and fruits addressed the positive correlation among phenols, flavonoids, and *in-vitro* antioxidant activity (Ebrahimzadeh and Bahramian, 2009; Mahdi *et al.,* 2023; Mohammed *et al.,* 2021). Consequently, the variation in antioxidant capacity among four halophytes might be related to either the difference in their phenolic content, flavonoids, or the presence of specific metabolites unique to this plant. Although halophytes, *T. nilotica* and *A. halimus*, contain almost the same amount of phenols, *T. nilotica* showed higher antioxidant activity. In this case, the differences were perhaps due to the amount of flavonoids, and alkaloids, which were higher in *T. nilotica*, or the presence of unique compounds in *T. nilotica* extract. On the other hand, under abiotic stress, including saline conditions and nutrient deficiency in coastal areas, reactive oxygen species (ROS) are produced, which are toxic to the cell (Miller *et al.,* 2010). Hence, the application of high levels of NaCl to *Thymus vulgaris* and *Glaux maritima* cultures triggers the production of phenols and flavonoids, which improve the antioxidant potential (Bistgani *et al.,* 2019; Pungin *et al.,* 2023). The IC_{50} scavenging activity of *A. macrostachyum* was noticeably higher than the one reported earlier for the same species by other authors (Lopes *et al.,* 2016; Rodrigues *et al.,* 2014). A similar observation was recorded for *T. nilotica* leaves collected from Sudan, with significant antioxidant activity (Hassan *et al.,* 2014). As agreed with our results, the higher scavenging activity of *A. macrostachyum* recommends its use as a potent source of antioxidants (Chekroun-Bechlaghem *et al.,* 2019; Custódio *et al.,* 2012; Zengin *et al.,* 2018). As compared with *L. crithmoides* collected from South Portugal, the Egyptian species contained higher flavonoids and lower levels of total phenols (Lopes *et al.,* 2016).

Antibiotic misuse, combined with antibiotic-resistant bacteria and a lack of new medication development, has compelled the search for natural antimicrobials solely or in combination with antibiotics. In the current study, the methanolic extracts of *A. macrostachyum* and *T. nilotica* showed broad antibacterial spectra against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, while the extracts of *A. halimus* and *L. crithmoides* exhibited an inhibition effect against only *S. aureus* and *B. subtilis*. Previous similar findings (Ahmed *et al.,* 2022; Al-Saleh *et al.,* 1997) have elaborated on the antibacterial activity of *A. macrostachyum* against *B. subtilis*, *E. coli*, and *P. aeruginosa*. However, the study of Ferreira *et al.* (2022) addressed the importance of plant parts and solvents during the antimicrobial investigation of halophytes. The alcoholic extract of *T. nilotica* leaves exhibited significant antioxidant, antiviral, and antitumor activity (Abdelgawad, 2017). A similar finding was also reported by Rahman *et al.* (2011), who revealed the antibacterial potency of *A. halimus* against *S. aureus*. On the other hand, due to its phenolics and flavonoids-rich solvents, *L. crithmoides* greatly contributed to antibacterial and antifungal activities as well as cytotoxicity effects (Adorisio *et al.,* 2020).

Based on the MTT assay and cell viability, the *in-vitro* cytotoxic potency of four halophyte extracts against three human cancer cells (HePG2, MCF-7, and PC3) was evaluated. The methanolic extracts of *A. macrostachyum* and *T. nilotica* displayed moderate cytotoxicity against both HepG2 and MCF-7, and only *A. macrostachyum* against PC3. *A. halimus* and *L. crithmoides* showed weak cytotoxicity against the three cell lines. The difference in cytotoxicity among the four halophytes might be attributed to their phenolic content and bioactive compound specificity. The present study confirms the selective cytotoxicity of the studied halophytes towards the three human tumor cells. In this context, selective cytotoxicity might be released from an interaction between active metabolites unique to each plant extract and specific cancer associated receptors or molecules in cancer cells that cause the death of cancer cells (Harada *et al.,* 1997; Hassan *et al.,* 2014). The cytotoxicity of *A. macrostachyum* against HepG2, and Hela (human cervical adenocarcinoma) was also previously reported (ElNaker *et al.,* 2020; Rodrigues *et al.,* 2014). Bakr *et al.* (2013) documented the cytotoxicity of *T. nilotica* against Huh-7 (liver carcinoma) and A549 (lung carcinoma). The ethanolic extract of *A. halimus* leaves exhibited a significant reduction against Hepg2, MCF-7, and A549 (Al-Senosy *et al.,* 2018), while *L. crithmoides* aerial parts showed an anticancer potency against OCI-AML3 (acute leukemia) (Adorisio *et al.,* 2020).

CONCLUSION

The results of the present study provide evidence that the methanolic extracts of four halophytes have the potential to serve as valuable sources of secondary metabolites with strong antioxidant, antibacterial, and antitumor properties. These halophytes were investigated for their content of secondary metabolites and biological potency, and can be ranked accordingly as follows: *A. macrostachyum*> *T. nilotica*> *A. halimus*> *L. crithmoides*. Among these halophytes, *A. macrostachyum* exhibited the highest biological potency, which can be attributed to its antioxidant activity. This high biological potency may be attributed to the combined contribution of total phenols and flavonoids. This is the first study addressed the linkage between 18 soil physicochemical parametes and secondary metabolites yield in these halophytes. In particular, lower levels of soil sulfates and macro-nutrients (TP and Ca^{+2}) under saline conditions could promote a greater production of phenols and flavonoids.

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تفضيل الموطن والمكونات الكيميائية النباتية والفعالية البيولوجية لأربع نباتات ملحية مصرية في منطقة البحر الأبيض المتوسط

محمد عبدالعال[1]*،ايمان مصطفى[1,2]، عبير يوسف العطار[1]، غادة الشربينى[1]، إبراهيم مشالى[1]، أيه يحيى[1]

[1]قسم النبات، كلية العلوم، جامعة المنصورة، المنصورة 35516 ، مصر

[2]قسم النبات، كلية العلوم، جامعة بنغازى، ليبيا

الملخص العربـــــي

في الدراسة الحالية، تم تحديد خصائص التربة، والنواتج الثانوية، والنشاط البيولوجي (مضادات للأكسدة، ومضادات للبكتيريا، ومضادات للسرطان) لأربعة نباتات ملحية في البحر المتوسط *Atriplex halimus*، *Arthrocaulon macrostachyum*، *Limbarda crithmoides*، *Tamarix nilotica*) من مصر. أظهرت النتائج أن النباتات الملحية المدروسة تفضل التربة ذات القوام الرملي الخشن، القلوية قليلا، وعالية الملوحة، مع محتوى منخفض من المواد العضوية والمغذيات الكبرى. أظهر *A. macrostachyum* أعلى تركيز للفينولات الكلية (181.75 مجم) والفلافونيدات (13.90 مجم) ، في حين أن *T. nilotica* كان لديه أعلى مستوى من القلويدات (6.43 مجم). يمكن أن تؤدي المستويات المنخفضة من كبريتات التربة والفوسفات والكالسيوم إلى تراكم أكبر للفينولات والفلافونيدات في هذه النباتات الملحية. أظهر مستخلص *A. macrostachyum* أعلى نشاط مضاد للاكسده. كان للمستخلصات الميثانولية للنباتات الملحية تأثير واضح ضد كل من *Bacillus subtilis* و*Staphylococcus aureus*، في حين اظهرت مستخلصات *A. macrostachyum* و*T. nilotica* تأثيرًا مضادًا للبكتيريا ضد *Escherichia coli* و *Pseudomonas aeruginosa.* علاوة على ذلك، أظهر *A. macrostachyum* سمية خلوية معتدلة ضد سرطان الكبد (HePG2)، وسرطان الغدة الثديية (MCF-7)، وسرطان البروستاتا (PC3). اثبتت النتائج الحالية بأن النباتات الملحية موضوع الدراسة مؤهله للإستخدام كمكملات غذائية/أو أعلاف أو في تطبيقات بيولوجية مختلفة ضد البكتيريا المقاومة للمضادات الحيوية والخلايا السرطانية البشرية و ذلك بعد إجراء المزيد من الدراسات.