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# Utilization of Halophyte Species as New Sources of Bioactive Substances

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**Abstract:** Halophytes grow in a wide variety of saline habitats, from coastal regions, salt marshes and mudflats to inland deserts. In these saline areas, unfavorable environmental conditions increased production of reactive oxygen species (ROS) in plants, leading to cellular damage, metabolic disorders, and senescence processes. Halophytes are known for their ability to overcome and quench these toxic ROS, since they are equipped with a powerful antioxidant system. Among them, phenolics have an important effect on oxidative stability and microbial safety. Additionally to their role as antioxidant, these compounds exhibit a wide spectrum of preventive medicinal properties. In this context, several works studied a large flora of Tunisian halophytic species well known for their utilization in traditional medicine and investigated their biochemical composition and biological activities in order to reveal their capacities. For instance, some halophytic species exhibit a higher content of polyphenol between 40 (*Suaeda fruticosa*) and 200 mg EAG.g$^{-1}$MS (*Tamarix gallica*). Moreover, these halophytes showed a higher activity in each antioxidant system with an $IC_{50}$ values more interesting than positive control BHT (scavenging ability against DPPH, ABTS and β-carotene bleaching) and BHA (lipid peroxidation inhibition). In addition, some halophytic plant extracts showed appreciable antibacterial properties against pathogen strains and a highest anti-inflammatory activity to inhibit RAW 264.7 macrophages stimulated by (LPS).

**Key Words:** Antioxidant compounds, Bioactive substances, Biological activities, Halophytes

## 1. Introduction

In Tunisia, as in several Mediterranean countries, drought and salinity constitute the two major constraints responsible for the limitation of crop productivity and the deterioration of vegetation cover. Several spontaneous species (Extremophile plants: halophytes, xerophytes and xero-halophytes) have been facing a strong pressure of selection in these stressing biotopes and have therefore acquired the necessary features, allowing them to successfully cope with salinity, water deficit stress, extreme temperatures and excessive luminosity. It is well known that these extreme conditions generate an oxidant stress resulting in the large accumulation of harmful reactive oxygen species (ROS), which cause lipid membrane peroxidation and protein and DNA deterioration (Ksouri *et al*., 2010). Under these unfavourable conditions, tolerant plants often develop a powerful antioxidant system implying several components: (i) the increase of osmolyte biosynthesis (or compatible compounds such as betains, proline, sugars, polyols) involved in the osmotolerance and in particular in the protection of the cellular structures against the toxic effects of salt, (ii) the biosynthesis of bioactive substances like polyphenols, vitamins (C and E) and carotenoids, which constitute the principal category of compounds with high antioxidant capacity, and (iii) the activation of enzymatic systems and antioxidant substances, involved in the ROS scavenging (glutathione, ascorbate, catalases, peroxidases, superoxide dismutases) (Jaleel *et al*., 2009). The study of plant response to these constraints (in their biotope or under laboratory conditions) allows not only the elucidation of the mechanisms enabling them to survive under these permanent stressing conditions, but may also have economic implications in the field of the biotechnology applied to health and agriculture, such as the development of products with industrial applications. For example, polyphenols have a great importance considering their multiple virtues and utilizations in the food, cosmetic, pharmaceutical, and medicinal industries (Macheix *et al*., 2005). These natural antioxidants are strongly recommended to replace synthetic antioxidants (potentially carcinogenic) currently used in therapy and food. Indeed, they present a large spectrum of physiological activities (antimicrobial, anti-allergic, anti-carcinogens) and preventive action against cardiovascular diseases (Balasundram *et al*., 2006). In this context, Tunisian extremophile species, well known for their ethno-pharmacological uses in traditional medicine and culinary, was making them good candidates for industrial application.

In order to characterize and valorize local halophytes as new sources of bioactive substances, the aim of this study was: (i) to evaluate the phenolic content and composition of some halophytic species, (ii) to investigate antioxidant activities using different tests, (iii) to estimate antimicrobial capacities against human pathogen strains, and (iv) anti-inflammatory activity of lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages.

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## 2. Materials and methods

### 2.1. Plant sampling and preparation for extract

Halophyte species (*Tamarix gallica* L., *Limoniastrum monopetalum* L., *Limoniastrum guyonianum* L., *Suaeda fruticosa* Forssk., and *Mesembryanthemum edule* L.) were harvested at full bloom in several Tunisian salt localities (May 2007). The harvested shoots were rinsed with distilled water, left at room temperature for 5 days in the dark, oven-dried for 1 h at 60°C, and grinded to fine powder. Extracts were obtained by magnetic stirring of 2.5 g dry powder in 25 ml pure methanol for 30 min. Extracts were kept for 24 h at 4°C, filtered through a Whatman no. 4 filter paper, and evaporated under vacuum. Then, they were stored at 4°C until analysis (Falleh *et al*., 2008).

### 2.2. Determination of total polyphenol content

Total polyphenols quantification was determined, as described by Dewanto *et al*. (2002). An aliquot of 125 µl of diluted extract were added to 500 µl of distilled water and 125 µl of the Folin-Ciocalteu reagent. The mixture was shaken, before adding 1250 µl $Na_2CO_3$ (7%), adjusting with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation for 90 min at 23°C in the dark, the absorbance versus prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total phenolic content of organs was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid, ranging from 0 to 400 µg/ml. All samples were analyzed in triplicate.

### 2.3. Assessment of antioxidant activities

The most common tests used for the determination of antioxidant activities of plant extracts such as DPPH test, Fe-reducing power, and β-carotene linoleate system were assessed according to Ksouri *et al*. (2009). All samples were analyzed in triplicate.

### 2.4. Determination of antibacterial activity

The antibacterial activity of shoot extracts was assessed by the agar disk diffusion assay (Bagamboula *et al*., 2003) against five human pathogenic bacteria: Gram-positive cocci including *Staphylococcus epidermidis* (Collection Institute Pasteur 106510), *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (NCIMB 8166), and Gram-negative bacteria including *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853). All samples were analyzed in triplicate.

### 2.5. Measurement of anti-inflammatory activity by nitrite quantification

Exponentially growing cells were plated in 24-well microplates (BD Falcon) at a density of $2\times10^5$ cells per well in 400 µl of culture medium and were allowed to adhere overnight. Cells were then treated or not with positive control N (G)-nitro-l-arginine methyl ester (L-NAME), or increasing concentrations of extracts dissolved in the appropriate solvent, and incubated at 37°C, 5% $CO_2$ for 24 h. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cells were then stimulated with 100 µg/ml lipopolysaccharide (LPS). After 24 h, cell-free supernatants were collected and stored at −80°C until NO determination using the Griess reaction (Green *et al*., 1990) with minor modifications. All samples were analyzed in triplicate.

### 2.6. Analysis of individual phenolic compounds

The identification of phenolic compounds was achieved according to Ksouri *et al*. (2009).

## 3. Results and Discussion

Results showed that shoot methanolic extracts of all halophytes exhibit an important polyphenol contents, ranged from 38 (*Suaeda fruticosa*) to 190 mg GAE/g DW (*Tamarix gallica*) (**Table 1**) as compared to glycophyte medicinal plants such as *Mentha pulegium* (Bourgou *et al*., 2008) or *Nigella sativa* (Karray-Bouraoui *et al*., 2010). Moreover, these species exhibited higher antioxidant activities for all antioxidant tests as compared to the positive control (BHT or BHA). For example, the antioxidant activity against DPPH radical was significantly higher in shoot methanolic extracts of all halophytes except *S. fruticosa*, $IC_{50}$ values were ranged

**Table 1. Total polyphenol content and antiradical activity against DPPH radical of studied halophytes.** Data concerning *Mentha pulegium*, *Nigella sativa* and BHT are respectively from Karray-Bouraoui *et al*. (2010); Bourgou *et al*. (2008); and Ksouri *et al*. (2009).

| Species | Polyphenol content (mgGAE.g/ DW) | DPPH test ($IC_{50}$ in µg/ml) |
|---|---|---|
| *T. gallica* | 190 | 1 |
| *L. monopetalum* | 40 | 2 |
| *L. guyonianum* | 99 | 1 |
| *S. fruticosa* | 38 | 20 |
| *M. edule* | 71 | 10 |
| BHT | - | 11.5 |
| *Mentha pulegium* | 20 | 44 |
| *Nigella sativa* | 10 | 280 |

**Table 2. Assessment of Fe-reducing power and β-carotene bleaching activity of the studied halophytes, BHT and vitamin C.**

| Species | Fe-reducing power ($EC_{50}$ in µg/ml) | β-carotene bleaching test ($IC_{50}$ in µg/ml) |
|---|---|---|
| *T. gallica* | 45.2 | 54.7 |
| *L. monopetalum* | 137 | 110 |
| *L. guyonianum* | 142 | 37 |
| *S. fruticosa* | 365 | 30 |
| *M. edule* | 0.2 | 130 |
| BHT | - | 75 |
| Vit C | 37 | - |

from 1 to 10 µg/ml, than BHT ($IC_{50}$=11.5 µg/ml), hence indicating a notably superior efficiency of halophytes. In reality, several authors have reported a positive and significant relationship between the antioxidant components including phenol acids, flavonoids and tannins, respectively with the Fe-reducing power and DPPH radical scavenging capacity (Trabelsi *et al*., 2010). Therefore, the high content of total phenols in all methanolic extracts might explain the strong antioxidant properties of these halophytes.

Concerning the other antioxidant tests (Fe-reducing power and β-carotene bleaching activity), $IC_{50}$ values were considerably higher in *M. edule* as compared to vitamin C for iron-reducing activity (**Table 2**). In addition, *T. gallica*, *L. guyonianum*, and *S. fruticosa* displayed the highest activity to inhibit β-carotene bleaching as compared to BHT. In fact, these antioxidant activities depend on phenolic nature, structure and synergistic interactions (Djeridane *et al*., 2006).

Moreover, *T. gallica* extracts showed appreciable antibacterial activities against Human pathogen strains. For instance, the highest activity was recorded against *Micrococcus luteus* and the poorest activity was observed against *Escherichia coli* (**Table 3**). These results suggest that methanolic extracts of *T. gallica* were more efficient to inhibit bacterial growth, probably in relation to their active molecules. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition (Rodriguez Vaquero *et al*., 2007).

Ksouri *et al*. (2009) showed after RP-HPLC analysis that *T. gallica* phenolic fingerprint was composed of seven phenolic acids (gallic, sinapic, chlorogenic, syringic, vanillic, *p*-coumaric, and trans-cinnamic acids) and six flavonoids ((+)-catechin, isoquercetin, quercetin, apigenin, amentoflavone, and flavone). These data established that the antioxidant and antimicrobial activities of *T. gallica* shoots could be attributed to their polyphenol compounds. Recent studies showed that (+)-catechin, and syringic, ferulic, vanillic, and *p*-coumaric acids gave high positive correlation with DPPH and ABTS

**Table 3. Antibacterial activity of *Tamarix gallica* extracts against pathogen human bacteria.**

| Bacteria | *Tamarix gallica* (100mg/ml) | Control (+) gentamycin (10UI) |
|---|---|---|
| *S. aureus, ATCC25923* | 10.00 ± 0.00 | 22 |
| *S. epidermidis, CIP106510* | 11.00 ± 0.00 | 30 |
| *Micrococcus luteus, NCIMB 8166* | 15.00 ± 0.00 | 26 |
| *E. coli, ATCC 35218* | 8.00 ± 0.00 | 27 |
| *P. aeruginosa, ATCC 27853* | 10.33 ± 0.66 | 16 |

No antimicrobial activity (□), inhibition zone <1 mm. Weak antimicrobial activity (w), inhibition zone = 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (++), inhibition zone 4–5 mm. High antimicrobial activity (+++), inhibition zone 6–9 mm. Strong antimicrobial activity (++++), inhibition zone >9 mm. Standard deviation ± 0.5 mm. For all bacteria, the inhibition zone of the control (+) gentamycin (10 UI) was higher than 9 mm (++++). The diameter of disc was 6 mm. SD: standard deviation.

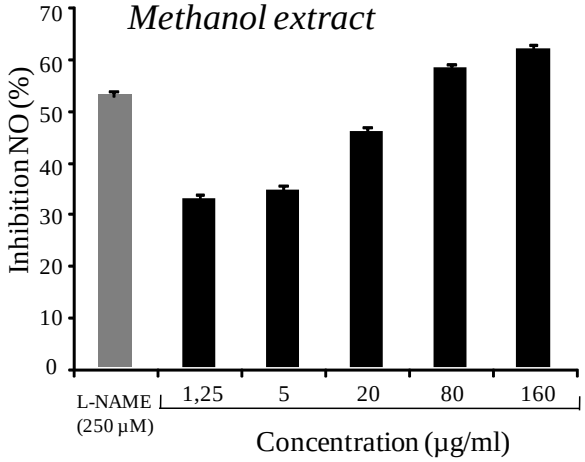


**Fig. 1. Effect of methanol extracts from *Suaeda fruticosa* on NO overproduction in LPS-stimulated RAW 264.7 macrophages.**

scavenging activities and reducing power. Moreover, gallic acid and catechin were closely correlated to metal chelating activity and inhibition of lipid peroxidation, respectively (Tsai *et al*., 2007). In addition, catechin, quercetin and gallic acid were reported for their high antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* (Rodriguez Vaquero *et al*., 2007). On the other hand, the edible medicinal halophyte, *Suaeda fruticosa*, showed a higher anti-inflammatory activity, inhibiting NO release, by 66.4% at 160 µg/ml in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages (**Fig. 1**) as compared to the positive control L-NAME which inhibit NO release by 58.2%. The elevated anti-inflammatory activity of methanolic extract could be due, in part, to the presence of phenolic compounds.

Besides, methanol extract of *Saussurea costus* was frequently used in Korean traditional prescriptions for inflammatory diseases, exhibited more than 50% of inhibition on tumor necrosis factor (TNF)-alpha production in

LPS-stimulated RAW 264.7 cells (Cho *et al*., 1999).

## 4. Conclusion

In conclusion, our results showed that the high biological capacity of halophytes were ascribed to the exceptionally high content in polyphenols and to the quality of its phenolic acids and flavonoids identified by RP-HPLC analysis. As a whole, these findings may confirm the interesting potential of these halophytes as valuable source of natural bioactive molecules.


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