Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **7(7),** 1-9, July **(2018)** *Res. J. Recent Sci.*

Antioxidant and antimicrobial activities of *Halocnemum strobilaceum* fractions and their related bioactive molecules identification by GC/MS and HPLC

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Available online at: www.isca.in, www.isca.me
Received 15th January 2018, revised 27th June 2018, accepted 1st July 2018

Abstract

Polar and apolarfractions of Halocnemum strobilaceum shoot extracts were evaluated for their phenolic contents and biological activities. HPLC and GC/MS methods have been implemented for phenolics and fatty acids content identification, respectively. Results showed that phenolic contents and antioxidant activities varied considerably according to the fraction nature. In fact, the apolar fraction contains two folds higher flavonoïd and tannin contents (1.87 and 0.83mg CE.g^{-1}DW, respectively) compared to the polar one. In contrast, total polyphenol content was superior in the polar fraction. The important phenolics content in chloroformic extract was correlated to the best antioxidant activity such as total antioxidant activity (5.54mg GAE.g^{-1}DW), antiradical activity against DPPH radical, β-carotene bleaching and Fe-reducing capacities with the lowest IC_{50} and EC_{50} values compared to the polar fraction. Furthermore, unlike the polar fraction, the chloroformic one was efficient against all pathogen strains mainly Salmonella typhimurium. The HPLC analysis showed that among twelve phenolic compounds detected only five were successfully identified (rutin hydrate, p-coumaric, O-coumaric, rosmarinic and 3,4-dimethoxybenzoic acids). The major fatty acids identified by GC/MS were palmitic, oleic and linolenic acids. The antioxidant and antimicrobial potency differences between the selected fractions was mainly attributed to different bioactive molecules contained in each fraction. Such characteristics would be of paramount importance for this halophyte valorization and use in cosmetic and pharmaceutical industries as a valuable source of antioxidant molecules.

Keywords: *Halocnemum strobilaceum*, fractionation, antioxidant activities, phenolic compounds, HPLC; GC/MS.

Introduction

In recent years, an increasing interest appeared for the identification of bioactive compounds from plants for fundamental research and especially for the production of more effective foods and remedies[1]. Several studies have confirmed that plants biological activities are stronger than synthetic antioxidants currently used in therapy and food industries. Furthermore, those latter are accused for their dangerous effect on human health[2].

In another hand, the development of the commercial application of bioactive molecules as anti-ageing and photo protective ingredients in cosmetic products, next to the growing demand for non-toxic antioxidants led to the expansion of investigations concerning relevant plant derived extracts[3]. Several varieties of natural antioxidants are used as food additives to minimize rancidity and to preserve food quality by slowing down oxidation and spoilage[4]. For those reasons, the identification such bioactive compounds among traditional medicinal plants such as halophytes living in extreme environments. Developing adaptive responses including the biosynthesis of several bioactive molecules as a new source of healthy products like functional foods, nutraceuticals or active principles, has gained a great attention during the last decade[5-7].

In Tunisia, a wide halophytic species range of various interests is available. For instance, *Halocnemum strobilaceum* L. is a succulent obligatory halophyte shrub (belonging to the Chenopodiaceae family) that grows in saline habitats from northern Africa and Mediterranean Europe to western Asia[8]. This plant is browsed occasionally by animals after drying up and it is considered as a source of potassium[9]. In addition, this specie has been used for the production of phenolic molecules mainly cafeic acid known for its antioxidant power and its anti-cancerous activity, as well as coumarin compound which is used in medicine for its anti-edematic effects[10,11].

Several studies showed that different solvent systems have been used for the extraction of polyphenols from plant material[12]. In fact, extraction yield is dependent on the solvent and the method of extraction[13]. For polyphenol extraction from plant matrix, water as well as the aqueous mixtures of organic solvents such as ethanol, methanol and acetone are frequently used[14]. Wang

and Helliwell reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea[9]. However, water was found to be a better solvent for extracting tea catechins compared to 80% methanol or 70% ethanol[15]. Moreover, a single extraction of polyphenols is not sufficient as compared to multiple extraction procedure or fractionation[13]. In fact, the phenolic compounds solubility is generally directed not only by the degree of phenols polymerization, but also by the solvent nature next to the phenols interaction with other plant constituents resulting in insoluble complex formation[16].

This work aimed to compare polar and apolar *H. strobilaceum* shoot extracts for their phenolic compounds including mainly flavonoids, tannins and total polyphenols), antioxidant and antimicrobial activities. HPLC and GC/MS methodologies were used for phenolic compounds identification and fatty acids composition determination in *Halocnemum strobilaceum* sample extract.

Materials and methods

The halophyte sampling, conservation and preparation for extraction: *H. strobilaceum* plant has been harvested from Sabkhet El Kalbia (Governorate of Kairouan), (300Km south Tunis, 35°49'N, 10°59'E, semi-arid bioclimate) in September 2009 during the vegetative stage. The harvested aerial parts were washed (using distilled water) and left at ambient temperature for a week in obscurity. Subsequently, they were dried for 1h at 60°C and grinded to fine powder. In fact, dry powder (2.5g) were added to 25ml of (chloroform/ methanol/ water) mixture (12/5/3). The extraction procedure was repeated twice. Both obtained fractions (aqueous and chloroformic) were separated after water addition and decantation. After solvent elimination by a rotary evaporator, dry residues were weighed and dissolved in methanol (100%). Finally, samples were stored at 4°C[5].

Colorimetric quantification of phenolics: Total phenolic contents: Following Singleton's method slightly modified by Dewanto et al.[17] was carried for the quantification of total phenolic using the Folin-Ciocalteu reagent. In fact, 0.125ml of suitable diluted samples was added to 0.125ml of the Folin-Ciocalteu reagent and 0.5 ml of distilled water. The preparation was mixed and allowed to settle for 6min. Then, 1.25ml of Na_2CO_3 solution (7%) was added. Finally, 1ml of deonized water was added to reach a final volume of 3ml. The obtained solution was then mixed thoroughly. After incubation for one hour and half at room temperature, the absorbance was determined at 760nm. Measurements were triplicates. Total phenolic content is expressed as mg of gallic acid equivalents (GAE).g^{-1} DW.

Total flavonoid content: Total flavonoids content were estimated as described by Dewanto et al.[17]. In fact, 0.075ml of $NaNO_2$ solution (5%) was added to 0.25ml of diluted samples, blended and left for 6min. Then, 0.15ml of a freshly prepared $AlCl_3$ solution (10%) was added. Then, 0.5ml of NaOH solution (1M) was added after an incubation of 5min. Finally, the appropriate volume of distilled water was added to adjust the final volume to 2.5ml. The obtained solution was thoroughly mixed and its absorbance was determined at 510nm. Total flavonoids content were expressed as mg (+)-catechin.g^{-1} DW (mg CE.g^{-1}DW), through the calibration curve of (+)-catechin.

Total condensed tannin assay: Total condensed tannin content were estimated according the modified vanillin assay[18]. Actually, 3ml of methanolic vanillin solution (4%) and 1.5ml of pure HCl were added to 0.05ml of suitably diluted samples. The mixture was incubated for 15min at ambient temperature. The quantity of total proanthocyanidins is expressed as mg catechin.g^{-1}DW (mg CE.g^{-1}DW). All samples absorbance were measured in triplicates at 500nm.

Assessment of antioxidant activities: Total antioxidant ability assessment: Prieto et al.[19] has described the analysis protocol: 1ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4 Mm ammonium molybdate) was added to 0.1ml of the plant fractions. The obtained mixtures were then incubated at 95°C for 90min. After the solution had cooled at the room temperature, samples optical densities were measured at 695nm. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE.g^{-1} DW).

DPPH radical–scavenging activity: The diphenylpicryl-hydrazine (DPPH) quenching ability of plant fractions was measured according to Hanato et al.[20] in triplicate. The DPPH radical–scavenging activity was expressed as IC_{50} (µg.ml^{-1}). A lower IC_{50} value corresponds to higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated according to the following equation:

$$\text{DPPH Scavenging effect} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Where: A_1 is the absorbance of the sample at 30min and A_0 is the absorbance of the control at 30min.

Determination of the iron reducing power: The method of Oyaizu was employed to evaluate the reducing power of *H. strobilaceum* shoot[21]. 1ml each fractions diluted at different concentrations was mixed with 2.5ml of 1% potassium ferricyanide $K_3Fe(CN)_6$ and 2.5ml of a 0.2M sodium phosphate buffer (pH = 6.6), then incubated for 20min in a water bath at 50°C. Then, 2.5ml of 10% trichloroacetic acid were added and the mixture was centrifuged for 10min at 650×*g*. The supernatant (2.5ml) was then mixed with an equal volume of distilled water and 0.5ml of 0.1% ferric chloride solution. The absorbance of the blue-green appearing color was measured at 700nm.

β-Carotene bleaching test (BCBT): Analysis were performed according to the method described by Koleva et al.[22]. A microtitre reader (Lab systems Multiskan MS, model EAR 400)

was used for absorbance determination at 470nm. Equation-2 was used to evaluate the antioxidant activity (AA) of the extracts in term of b-carotene blanching:

$$AA\ (\%) = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

Where: A_0 is the absorbance of the control at 0min, and A_1 is the absorbance of the sample at 120min. The results are expressed as IC_{50} values (µg/ml).

Antibacterial activities measurement: Agar disk diffusion assay was used to assess the antibacterial activity of the plant extract against some pathogenic bacteria: Gram-positive cocci such as *E.faecalis* (ATCC 29212), *S.aureus* (ATCC 25923) and Gram-negative bacteria such as *Salmonella typhimurium* (ATCC 1408), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218)[23]. Seed cultures were performed on Muller Hinton medium (37°C, 24h). Then, nutrient agar was inoculated by the bacterial strains. The experimental methodology proposed by R'bia et al. was considered[24]. Dishes were incubated at 37°C for 24h. The growth inhibition zone surrounding the discs were measured for the antibacterial activity assessment. According to CASFM 2005 guidelines, Gentamycin Standard discs (10UI) were considered as positive antibiotic controls. As for negative controls, discs containing100% of methanol (10µl) were considered.

Phenolic compounds identification using HPLC: The preparation of plant extract was realized according to the slightly modified method of Proestos et al.[25]. The obtained extract was injected to HPLC after a filtration through a 0.45 µm membrane filter. The separation of phenolics was realized with an Agilent apparatus, a diode array detector and a Prostar Pump. A C18 column was used for analysis. The solvents A (0.025% TFA in H_2O) and B(MeCN) composed the mobile phase. The sample injection was performed directly. 280 and 306nm were the used wavelengths for chromatograms determination.

Extraction protocol of fatty acids and their composition: The oil of *H. strobilaceum* shoot was obtained using a Soxhlet extractor (a weight of 35g of the sample and 200ml of hexane) during 8h. Extract oil was kept at -20°C. Fames analysis was performed as described by R'bia et al.[24].

Statistical analysis: Data were analyzed using the one-way analysis of variance according to the STATI-CF software. Difference significance was considered at $p < 0.05$.

Results and discussion

Phenolic compounds: Results showed that phenolic contents varied significantly between the two types of extracts (Table-1). In fact, the polarfraction showed the highest amount of polyphenols equal to 4.16 mg GAE.g^{-1}DW (3.5 fold higher than in the apolar one). In previous investigation, Megdiche-Ksouri et al. reported that the amount of polyphenols in *Frankenia thymifolia* shoot was higher in methanolic extract (14.18mg GAE.g^{-1}DW) than that in chloroformic one, indicating clearly the solvent effect on the extractability of phenolic compounds[26]. Similar results were as well observed in other studies which showed that solvent nature influence strongly the phenolic extraction in many species[27,28]. Independently of the solvent effect, total polyphenol contents in *Halocnemum strobilaceum* methanolic extract were higher than those of several species cited in the literature such as Sarrazin (3.13mg GAE.g^{-1}DW), Acacia (0.93mg GAE.g^{-1}DW)[29], *Thapsia garganica* (2.5mg GAE.g^{-1}DW), *Mesembryanthemum crystallinum* and *M. nodiflorum* (1.43 and 1.72 mg GAE.g^{-1}DW, respectively)[30,31].

Table-1: Phenolic content (total polyphenol, flavonoids and condensed tannin) of *Halocnemum strobilaceum*. Means (three replicates) followed by at least one same letter are not significantly different at P< 0.05.

Fractions	Polyphenol content (mg GAE/g DW)	Flavonoid content (mg CE/g DW)	Tannin content (mg CE/g DW)
Polar	4.6^a	0.78^b	0.48^b
Apolar	1.3^b	1.87^a	0.83^a

As for total phenolics, flavonoid and condensed tannin contents also varied significantly according to the solvent effect with a maximal amount in the chloroformic fraction (Table-1). In this context, Ozturk et al. found that flavonoid contents of *Rheum ribes* stems and roots chloroformic extracts (20.10 and 145.59µg PEs.mg^{-1} extract respectively) were higher than the methanolic one (13.66 and 16.23µg PEs.mg^{-1} extract respectively)[32]. This could be due to the low polarity of the chloroform (4.4) which confers to this fraction an important extracting power of low polar antioxidants such as flavonoids and tannins. In fact, the solvent nature used for extraction has a great effect on the solubility of the target compounds and the bioactivity of the resulting extract.

Therefore, it is difficult to develop a common extraction procedure suitable for the extraction of all plant phenols.

Assessment of antioxidant activities: Several methods are generally used for antioxidant activities determination. The chemical complexity of extracts, often a mixture of compounds with different functional groups, polarity and chemical behavior, could lead to scattered results, depending on the test employed[32]. Thus, an assay multiplication approach for the plant extract antioxidant efficiency evaluation would be useful. In this work, total antioxidant capacity, DPPH radical scavenging activity, β-carotene bleaching ability and ferric reducing power were used for the antioxidant activities determination.

Total antioxidant capacity: Results in Table-2 showed that both fractions present a significant difference in terms of their total antioxidant capacity (TAC). The obtained value was 1.3 fold higher in the apolar extract (5.54mg GAE.g^{-1}DW) in comparison with the polar fraction (4.17mg GAE.g^{-1}DW). These data indicated that this total antioxidant capacity is related to flavonoid than total phenolic contents. In fact, chloroform as less polar solvent can extract some interesting pigments (flavonoids and carotenoids), vitamin E and unsaturated fatty acids with higher antioxidant activities[33]. So, this global antioxidant activity would likely be bound to the quality rather than the quantity of antioxidant molecules present in the extract.

Table-2: Total antioxidant capacity (TAC), radical-scavenging activity, reducing power and β-carotene bleaching test (BCBT) in *Halocnemum strobilaceum*. Means (three replicates) followed by at least one same letter are not significantly different at P< 0.05.

Fractions	TAC (mg of GAE/g DW)	DPPH test (IC_{50} µg/ml)	Reducing power (EC_{50} µg/ml)	BCBT (IC_{50} µg/ml)
Polar	4.17 [b]	107.5[b]	4400 [b]	3000 [b]
Apolar	5.54[a]	61 [a]	530 [a]	940 [a]

DPPH radical-scavenging activity: The apolar and polar fractions of *H. strobilaceum* scavenging effect on the DPPH radical in terms of IC_{50} values presented significant difference (61 and 107.5µg.ml^{-1} respectively) (Table-2). The obtained values revealed that the chloroformic extract of this halophytic specie has a greater free radical scavenging effect compared to the methanolic extract. These results confirmed previous studies, in which the chloroformic extract of the medicinal plant *Rhubarb* (*Rheum ribes*) exhibited an IC_{50} values (50.9µg.ml^{-1}) smaller than the one got in the methanolic extract (IC_{50} = 60.6 µg/ml)[32].

Thus, the difference in DPPH scavenging activity of plant fractions might be due to the difference in solvent selectivity for extracting certain phenolic groups[17]. Several studies showed that solvent nature, especially polarity, influence significantly the extracting power of the latter on plants phenolic compounds[32].

Iron reducing power: As shown in Table-2, the reducing power of the chloroformic extract, expressed as EC_{50}, was clearly more important than that of the methanolicone. These results were in agreement with previous studies led by Ozturk et al. which revealed that the chloroformic extract of *Rhubarb* (*Rheum ribes*) possessed an iron reducing power more interesting than that one of the methanolicextract[32]. However, *H.strobilaceum* exhibited a weaker reducing power comparing to the other halophytic species such as *Mesembryanthemum edule* (EC_{50}=126µg.ml^{-1}) and *Limoniastrum monopetalum* (EC_{50}=330 µg.ml^{-1})[28,35]. Consequently, this antioxidant activity is depending on the solvent nature and the quality of the natural antioxidants present in both extracts.

***β*-carotene bleaching (BCB) antioxidant activity:** Table-2 results showed that *H.strobilaceum* fractions adding to this system stops the *β*-carotene bleaching at different degrees. Moreover, the extract concentration providing 50% inhibition (IC_{50}) presented more interesting ability (IC_{50} = 940µg.ml^{-1}) in the chloroformic fraction compared to the methanolicone (IC_{50} = 3000µg.ml^{-1}). The linoleic acid oxidation in his fraction, generates hydroperoxide-derived free radicals attacking the *β*-carotene chromophore, which induces the reaction emulsion bleaching. It has been reported in literature that an extract able to limit or stop *β*-carotene oxidation could be designated as a primary antioxidant and free radical scavenger[36].

As for the *β*-carotene-linoleic acid bleaching results, the chloroformic fractions exhibited free radicals scavenging potency in a complex heterogeneous medium. Such propriety would be promoting for the incorporation of this extract in emulsion type systems as antioxidative preservative.

Anti-microbial activity: Agar diffusion methodology was implemented for the antibacterial potencies of apolar and polar shoot fractions measure against some selected pathogenic bacteria specifically: *P. aeruginosa, E. coli, E. faecium S. aureus,* and *S.typhimurium* are resumed in Table-3. Results showed that the studied extracts could inhibit bacterial proliferation according to the fraction nature and strains resistance. In fact, the methanolic fraction inhibited the growth of only one bacterium, whereas chloroform fraction exhibited better activity against the test pathogens. These results are in agreement with those of Meot-Duros et al. which studied the effect of *Crithmum maritimum*, *Eryngium maritimum* and *Cakile maritima* chloroformic and methanolic extracts against 12 pathogens[37].

Their research revealed that the apolar fraction possess generally stronger activity than the polar one since it is capable to inhibit the growth of 9 bacteria, whereas the polar fraction inhibited only two strains. Therefore, the apolar extract potency is due to the presence of some interesting and bioactive molecules presenting an inhibition activity for some resistant strains particularly the negative gram (eg. *Echerchia coli*…). Numerous researchers ascribed the antimicrobial activity of plant extracts to their phenolic content[37]. Those findings could be attributed to the selectivity of each solvent which affects the nature of extracted compounds. The bactericidal activity of apolar fraction can be accredited to the presence of low polarity compounds such as essential oil, carotenoids, lipophilic flavonoids, and unsaturated fatty acids. In fact, it was highlighted that lipophilic flavonoids can preserve plants against microorganism's attacks, as several have been shown to have antibacterial and/or antifungal activities[39].

The antibacterial action of chloroformic fraction would be due to the presence of long-chain fatty acids in particularly unsaturated and polyunsaturated fatty acids. In the same context, other investigations highlighted that long-chain UFA like oleicacid, linoleic acid, and linolenic acid showed an interesting antibacterial action, while long-chain saturated fatty acids, containing palmitic acid and stearic acid are lessactive[40,41]. Accordingly, to improve the antibacterial activity, it is required to concentrate and purify the phenolic compounds.

Purified components may be used as natural antimicrobials in food systems, as well as to prevent the growth of food borne bacteria resulting in extension of the shelf life of processed foods.

Table-3: Antibacterial activities of *Halocnemum strobilacum* shoot fractions (polar and apolar) against five human pathogenic bacteria at 100 µg/ml concentrations. Inhibition zone calculated in diameter around the disc (mm ± SD)

Bacterial strain	*H. strobilacum*		Gentamicin
	Polar	Apolar	
Escherichia coli	-	9 ±0.03	27
Pseudomonas aeruginosa	-	10 ±0.00	16
Staphylococcus aureus	-	10±0.00	22
Enterococcus faecalis	8 ±0.01	10 ±0.00	16
Salmonella typhimurium	-	11 ±0.01	26

SD: standard deviation. IZ: inhibition zone. The diameter of disc was 6mm. No antimicrobial activity (–), inhibition zone <1 mm. Weak inhibition zone, inhibition zone 1mm. Slight antimicrobial activity, inhibition zone 2 to 3mm. Moderate antimicrobial activity, inhibition zone 4 to 5mm. High antimicrobial activity, inhibition zone 6 to 9mm. Strong antimicrobial activity, inhibition zone >9mm.

Antioxidants identification: Phenolic compounds identification: The authentic standards retention time are resumed in the Table-4.

The HPLC chromatogram of *H. strobilaceum* shoot extract is illustrated in Figure-1.

The obtained result revealed twelve phenolic compounds that five among them were successfully identified (four phenolic acids: *p*-coumaric, *o*-coumaric, rosmarinic and 3,4-dimethoxybenzoic acid) and one flavonoid (rutin hydrate). Retention times, spectral characteristics of the corresponding peaks as well as sample spiking have been considered for the identification of phenolic compounds against standards.

Table 4 Retention time (RT) of thirty standards of phenolic acids and flavonoids

Standards	RT
Gallic acid	3.993
Gallocatechin	5.465
Protocatechuic acid	6.407
3,4-dihydroxyphenol acetic acid	6.835
Epigallocatechin	7.596
Catechin	7.785
Chloroginic acid	8.873
4-hydroxybenzoic acid	9.293
2,5-dihydroxybenzoic acid	9.701
Vanillic acid	10.441
2,5-dihydroxybenzoic acid	9.701
3,5 dimethoxy-4-hydroxybenzoic acid	10.919
Epigallocatecchin-3-o-gallate	11.512
P-coumaric acid	14.771
Rutinhydrat	15.679
Rutintrihydrat	15.700
Trans-4-hydroxy-3-methoxycinnamic acid	16.000
Sinapic acid	16.026
3,4-Dimethoxybenzoic acid	16.234
O-coumaric acid	19.325
Trans-2-hydroxycinnamic acid	19.384
Rosmarinic acid	20.231
Salicylic acid	20.422
Naphtoresorcinol	24.039
Quercitindihydrat	25.988
Trans-cinnamic acid	25.999
4-methoxycinnamic acid	26.812
Apigenine	27,273
4',5,7-trihydroxyflavone	29.386
Kaempferol	30.963

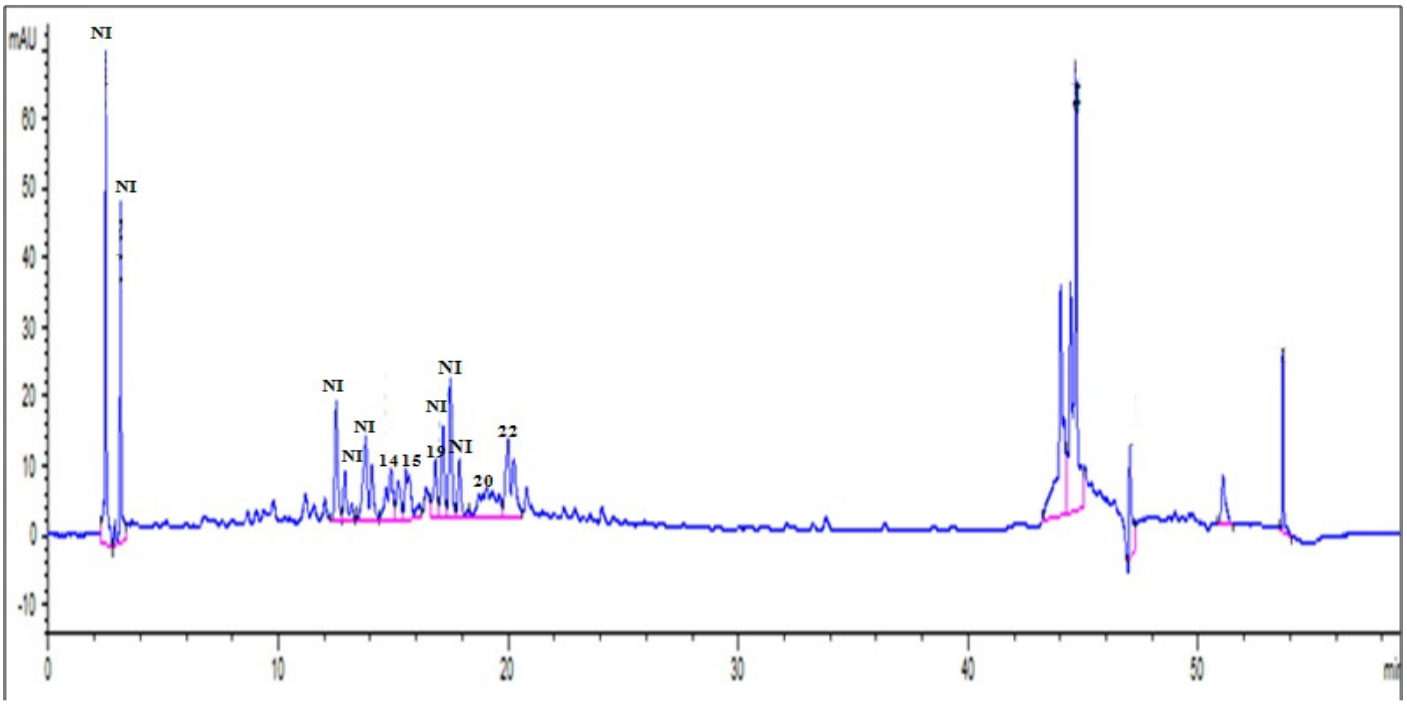

Figure-1: HPLC profiles of phenolic acids and flavonoids standards of *Halocnemum strobilaceum* shoot extract monitored at 280 nm. The peak numbers correspond to: 14 *P*-coumaric acid; 15, rutinhydrat; 19, 3,4-Dimethoxybenzoic acid; 20, *O*-coumaric acid; 22, rosmarinic acid, NI: not identified.

Coumaric acid is a hydroxycinnamic acid, an organic compound that is a hydroxyl derivative of cinnamic acid. There are three isomers, *o*-coumaric acid, *m*-coumaric acid, and *p*-coumaric acids that differ by the position of the hydroxyl substitution of the phenyl group. *p*-coumaric acid is the most abundant isomer of the three in nature. *p*-coumaric acid can be found in a wide variety of edible plants such as peanuts, tomatoes, carrots, and garlic[41]. In addition, *p*-coumaric acid had antibacterial and antioxidant properties and is believed to reduce the risk of stomach cancer by reducing the formation of carcinogenicnitrosamines[42]. In the same context, rosmarinic acid is a polyphenol found in many plants, especially in high concentrations in oregano, rosemary, lemon balm, sage and marjoram. It is one of the aroma compounds in these plants. It has antioxidant, anti-inflammatory and antimicrobial activities[42]. The antioxidant activity of rosmarinic acid is stronger than that of vitamin E. Rosmarinic acid helps to prevent cell damage caused by free radicals, thereby reducing the risk for cancer and atherosclerosis. Rosmarinic acid is also used for food preservation. In Japan the perilla extracts, rich in rosmarinic acid, is used the garnish and improve the shelf life of fresh seafood[42]. Moreover, the 3,4-dimethoxybenzoic acid is occurring, together with veratrine, in the root of white hellebore and in sabadilla seed, extracted as a white crystalline substance which is related to protocatechuic and veratricacids[43]. It is preferably used as one of the principal anti-microbial preservatives in foods and beverages (its concentration is usually limited and not exceeding 0.1%), since it is about 200 times more soluble than benzoic acid. In addition, this acid is used in medications, anti-fermentation additives and tableting lubricant for pharmaceuticals[44]. Rutin hydrate is a polyphenolic flavonoid that acts as an antioxidant and not as scavenger. It can attenuate peroxide production in glial cells by acting as a free radical scavenger and protect renal cells from oxidative injury. Inclusion of rutin in the diet of rats significantly reduced the appearance of single-strand breaks in nuclear DNA caused by hepatocarcinogen saflatoxin B1 and N-nitrosodimethylamine[45]. This protection from DNA damage was found to be due to a reduction in the induction of repair enzymes polymerase, DNA polymerase β and DNA ligase. Since DNA damage and inefficient repair are thought to initiate the process of carcinogenesis, effects of rutin on these functions suggests a protective role of this flavonoid against carcinogenesis induced by chemical carcinogens[45]. For those reasons, plant antioxidants such as phenolic compounds are usually used as food additives to stabilize and preserve the quality[7]. Antioxidant mechanisms generally detected for food protection from oxidation are: scavenging of free radicals via the desactivation of singlet oxygen and metal ions, orvia an electron or an hydrogen atom donation[33]. They have also a great interest for their potential role as functional foods or nutraceuticals[46].

Composition of fatty acids: The results for fatty acid composition of crude *H. strobilaceum* shoot oils are resumed in Table-5. Palmitic acid (C16:0) followed by oleic acid (C18:1) and linoleic acid (C18:2) representing 43.7, 24.3 and 19.6%, respectively; were the major identified components. Others fatty acids were also detected. The main group of fatty acids

representing 56.1% was SFA, followed by MUFA 24.3% and PUFA 19.6%. The saturated/unsaturated acid ratio of *H. strobilaceum* shoot oils was 1.3 due to the considerable amount of unsaturated fatty acid such as C18:1Δ9 and C18:2Δ9. Linoleic and oleic acids possess powerful biological activities such as antioxidant and antibacterial potencies[34]. These remarkable unsaturated fatty acids are the major compounds of *Helichrysum pedunculatum* and *Schotia brachypetala* usually used for wounds healing during male circumcision rituals in South Africa[47,48]. Besides, studies carried by Hu et al. indicated that the oil wealth in unsaturated fatty acids is considered as beneficial in the dietary food[49]. In fact, these lipids have medicinal prosperities (reducing inflammation and atherosclerosis and cardiovascular disease risks), contrary to the saturated ones which are responsible of the cholesterol increase in blood and of heart problems[50].

Table-5: Fatty acid composition (%) in *Halocnemum strobilaceum* shoots.

	Peak area (%)		
	Fatty acids	Formula	*H. strobilaceum*
Saturated fatty acids	Lauric acid	C12:0	2.67
	Myristic acid	C14:0	2.35
	Palmitic acid	C16:0	43.67
	Stearic acid	C18:0	3.32
	Behenic acid	C22:0	4.12
Monounsaturated fatty acids	Oleic acid	C18:1	24.28
Polyunsaturated fatty acids	Linoleic acid	C18:2	19.59
	Σ SFA	-	56.13
	Σ MUFA	-	24.28
	Σ PUFA	-	19.59

Conclusion

The obtained results showed that the extracting solvent, significantly affected antioxidant content and biological activities of *Halocnemum strobilaceum*. Such variability between polar and apolar fractions bioactive capacities can be attributed to their active compounds content which is toughly correlated with the extraction solvent nature. Thus, this halophyte could be an important source of naturally bioactive molecules with interesting biological potencies. Those proprieties could be promising to incorporate the active compounds as preservative ingredients in food and pharmaceutical products.

Acknowledgements

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research (LR10CBBC02).

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