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Full Length Research Paper

Antioxidant and antimicrobial properties of *Frankenia thymifolia* Desf. fractions and their related biomolecules identification by gas chromatography/ mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC)

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***Frankenia thymifolia* Desf. is an endemic xero-halophyte species common in the salted and arid region of Tunisia. In this study, two kinds of *Frankenia* shoot fractions were assessed on their polyphenol contents and biological activities. Then, the main phenolic and fatty acid compositions were identified. Results showed that polar fraction contains a highest polyphenol, flavonoïd and tannin contents (14.2 mg GAE g^{-1} DW, 4.8 and 4.6 mg CE g^{-1} DW, respectively). The higher phenolic content in this fraction reflect the best total antioxidant capacity (8.8 mg GAE g^{-1} DW), antiradical activity, β-carotene bleaching and Fe-reducing tests with the lowest IC_{50} and EC_{50} values as compared to apolar fraction. However, chloroformic fraction was more efficient against pathogen strains. In fact, this fraction was active against all strains. Whereas, polar extract exhibited slight and moderate antibacterial activity (*Staphylococcus aureus* and *Enterococcus faecium*, respectively). The high performance liquid chromatography (HPLC) analysis showed that salicylic and transcinnamic acids were the major phenolics. The major fatty acids were palmitic, elaïdic and linoleic acids. Such variability in antioxidant and antimicrobial capacities between the two fractions can be explained by different bioactive compounds contain in each fraction and might be of great importance in terms of valorizing this halophyte as a source of bioactive molecules for cosmetic and pharmaceutical industries.**

Key words: Biological activities, fatty acid, *Frankenia thymifolia*, phenolic, fractionation, high performance liquid chromatography (HPLC).

INTRODUCTION

A renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical, cosmetic and nutritional industries (Oktay et al., 2003; Wangensteen et al., 2004) with the recognition that plant-derived products have great potential as sources of pharmaceuticals (Cragg et al., 1996; Borchardt et al., 2008). This is mainly due to their high biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis (Suhaj, 2006). In addition, antioxidants are used to preserve food quality mainly

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because they arrest oxidative deterioration of lipids (Akinmoladun et al., 2007). These factors have inspired the widespread screening of new species for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Jayaprakasha et al., 2001; Gulcin et al., 2002). Among them, halophytes and/or xerophytes could be the good candidates to serve as a genetic source for this purpose. In fact, the potential offered by a number of these naturally tolerant plants in functional foods, as nutraceuticals or ingredients industries is huge because of their exceptional richness in polyunsaturated fatty acids (such as ω-3 and ω-6 fatty acids), β-carotene and other pigments, sterols, essential oils as well as bioactive molecules, for example, phenolic compounds and more specifically phlorotannins, terpenes and alkaloids, liable to be endowed with antioxidant, antimicrobial, anti-inflammatory and anti-tumoral activities (Balasundram et al., 2006; Ksouri et al., 2009).

Doubtlessly, these relevant substances found in xero-halophytes, possess pertinent physiological functions and valuable biological activities and are considered key-compounds in the fight against various diseases (for example, cancer, chronic inflammation, atherosclerosis and cardiovascular disorder) and ageing processes (Kohen and Nyska, 2002). In plant, unfavorable environmental conditions (salinity, drought, heat/cold, luminosity and other hostile conditions) lead to increased production and accumulation of reactive oxygen species (ROS) (Menezes-Benavente et al., 2004).

These ROS are highly reactive because they can interact nonspecifically with various molecules and metabolites, eliciting peroxidative reactions and causing considerable damage to vital molecules such as proteins, lipids and nucleic acids. Xero-halophytic species are naturally adapted to high salinity and drought conditions in the soil (Vicente et al., 2004; Meot-Duros and Magné 2009), and are known for their ability to quench these toxic ROS, since they are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components (Ksouri et al., 2008). Among the various kinds of natural antioxidants, vitamins, terpenoids, polyphenols and polyunsaturated fatty acids are of great importance, owing to their multiple applications in food industry, cosmetic, pharmaceutical and medicinal materials (Maisuthisakul et al., 2007). In xero-halophytic plants, polyphenol synthesis and accumulation is generally stimulated in response to biotic/ abiotic stresses (Naczk and Shahidi, 2004), such as salinity (Navarro et al., 2006), leading one to think that secondary metabolites may play a role in the adaptation of halophytic species to this constraint (Ksouri et al., 2007). In fact, the physiological benefits of these compounds have been attributed to their potential role in inhibiting lipid peroxidation, modulating cell signal transduction pathways and inducing apoptosis (Hou et al., 2004; Tabart et al., 2007) and have an important effect on microbial safety (Tadhani et al., 2007).

Evaluation of antioxidant and antiradical activities of fruits, vegetables, and other plant products cannot be carried out accurately by any single universal method or extraction solvent system due to the complex nature of phytochemicals present (Shahidi and Naczk, 2004; Prior et al., 2005). Numerous tests have been proposed to evaluate and estimate different aspects of antioxidant potency of plant components (Decker et al., 2005; Prior et al., 2005). They can be categorized into two groups: assays for radical-scavenging ability and assays for the ability to inhibit lipid oxidation under accelerated aging conditions. In fact, the features of an oxidation process are a substrate, an oxidant and an initiator, as well as intermediates and final products (Ksouri et al., 2009). Measurement of any of these parameters can be used to assess antioxidant activity (Antolovich et al., 2002). In addition, several studies showed that polyphenol content differed with solvents polarities. Different solvent systems have been used for extraction of polyphenols from plant materials (Chavan et al., 2001). In this context, Trabelsi et al. (2010) found that extraction solvent systems (pure or mixture) of varying polarities differ significantly in their extraction capacity and selectivity for leaf phenolic contents and antioxidant activities evaluation in the halophyte *Limoniastrum monopetalum*. Consequently, the solubility of phenolic compounds is actually governed by the type of solvent used, the degree of polymerization of phenolics, as well as by the interaction of phenolics with other food constituents and formation of insoluble complex (Djeridane et al., 2006).

In Tunisia, a considerable diversity of xero and/or halophytes species with multiple interests including therapeutic practices occurs, and a number of them have not been subject to chemical investigations. For example *Frankenia thymifolia* Desf. is an endemic xero-halophyte of North Africa (Ozenda, 1991), common in the ‘‘Hauts Plateaux’’ salted grounds and the southern part of Tunisia (arid region), in particular at the edge of the Chotts. The genus it has not been the subject of many chemical investigations. The only phytochemical studies consist to the identification of diverse flavonoid and phenolic sodium sulfates in *F. thymifolia* Desf. (Harkat et al., 2007), *F. pulverulenta* L. (Harborne, 1975) and *F. laevis* L. (Hussein, 2004). In other hand, Zampini et al. (2009) showed an important antibacterial activity against *Morganell morganii*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* in *Frankenia triandra* extract.

The aims of this work were to assess the potential effects of mixture extracting solvents on *F. thymifolia* shoot phenolic contents (total polyphenol, flavonoids and tanins) and their antioxidant and antimicrobial activities and at the last, to identify the main phenolic compounds and fatty acids composition present in *F. thymefolia* shoot by high performance liquid chromatography (HPLC) and gas chromatography/ mass spectrometry (GC/MS)

respectively.

MATERIALS AND METHODS

Plant material and preparation for extract

F. thymifolia samples were collected from Sabkhat EL Adhibet (Mednine locality) (600 km south Tunis; arid bioclimatic stage; mean annual rainfall < 200 mm) in May 2009. The harvested organs 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 of solvents mixture (chloroform/methanol/water) (12/5/3). Extraction was repeated two times. The two fractions (polar: aqueous and apolar: chloroformique) were separated after water addition and decantation. Apolar fraction was evaporated under reduced pressure and then placed in a vacuum oven at not more than 40°C for about 24 h to remove any residual solvent. Dry powder was weighed and removed in pure methanol. Two fractions were then stored at 4°C until analysis (Meot-Duros and Magné, 2009).

Total phenolic contents

Total phenolic compounds were assayed by the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). An aliquot (0.125 ml) of suitable diluted extracts was added to 0.5 ml of distilled water and 0.125 ml of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 ml of 7% Na_2CO_3 solution. The solution was then diluted with deionised water to a final volume of 3 ml and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 760 nm. Total phenolic content of shoots (three replicates for each fraction) was expressed as mg gallic acid equivalents (GAE) g^{-1} DW through the calibration curve with gallic acid. The calibration curve range was 50 to 400 µg/ml (R^2 = 0.99).

Total flavonoid contents

Total flavonoids were measured according to Dewanto et al. (2002). An aliquot (0.25 ml) of diluted samples was added to 0.075 ml of $NaNO_2$ solution (5%), mixed and left for 6 min, before adding 0.15 ml of a freshly prepared $AlCl_3$ solution (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoids were expressed as mg (+)-catechin/g DW (mg CE/g DW), through the calibration curve of (+)-catechin. The calibration curve range was 0 to 400 µg/ml. Samples were analyzed in triplicate.

Condensed tannin contents

Procyanidins were measured using the modified vanillin assay described by Sun et al., (1998). To 0.05 ml of suitably diluted sample and 3 ml of methanol vanillin solution (4%), 1.5 ml of HCl was added. The mixture was maintained at ambient temperature for 15 min. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW (mg CE/g DW). The calibration curve range was 0 to 400 µg/ml. All samples DO were measured in three replicates at 500 nm against methanol as a blank.

Antioxidant activities

Evaluation of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Prieto et al., 1999). An aliquot (0.1 ml) of plant extract of each fraction was combined to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. After, the mixture had cooled to room temperature; the absorbance of each solution was measured at 695 nm (Anthelie Advanced 2, SECOMAN) against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0 to 500 µg/ml. Each fraction was analyzed in triplicate.

DPPH radical-scavenging activity

DPPH quenching ability of organ extracts was measured according to Hanato et al. (1988). One milliliter of the extract at known concentrations was added to 0.25 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm and corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The antiradical activity was expressed as IC_{50} (µg ml^{-1}), the extract dose required to induce a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenging effect} = [(A_0 - A1)/A_0] \times 100 \quad (1)$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. Each fraction was analyzed in triplicate.

Iron reducing power

The method of Oyaizu (1986) was used to assess the reducing power of *F. thymifolia* shoot. Methanol extracts (1 ml) at different concentrations (20 to 500 µg ml^{-1} for the polar fraction and 20 to 2000 for the apolar fraction) were mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide $K_3Fe(CN)_6$, then incubated in a water bath at 50°C for 20 min. After that, the mixture was centrifuged at 650 × *g* for 10 min and 2.5 ml of 10% trichloroacetic acid were added. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue-green appearing colour was measured at 700 nm. The EC_{50} value (µg ml^{-1}) for the reducing power is the extract concentration at which the absorbance was 0.5, and ascorbic acid was used as a positive control.

β-Carotene bleaching test (BCBT)

A modification of the method described by Koleva et al., (2002) was employed. B-Carotene (2 mg) was dissolved in 20 ml chloroform and to 4 ml of this solution, linoleic acid (40 mg) and tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 ml of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Sample extract and reference compounds (BHT and BHA) were prepared in ethanol. An aliquot

(150 µl) of the β-carotene: linoleic acid emulsion was distributed in each of the wells of 96-well microtitre plates and ethanolic solutions of the test samples (10 µl) were added. Three replicates were prepared for each of the samples. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured using a model EAR 400 microtitre reader (Labsystems Multiskan MS) at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 120 min of incubation. The antioxidant activity (AA) of the extracts was evaluated in term of β-carotene blanching using the following formula:

$$AA\ (\%) = [(A_0 - A1)/A_0] \times 100 \quad (2)$$

where A_0 and A_1 have the same meaning as in Equation (1). The results are expressed as IC_{50} values (µg/ml).

Screening for antimicrobial activity

The antibacterial activity of shoot extracts of each fraction (polar and apolar) was assessed by the agar disk diffusion assay (Bagamboula et al., 2003) against five human pathogenic bacteria: Gram-positive cocci including *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecium* (ATCC 19436) and Gram-negative bacteria including *Escherichia coli* (DH5α), *Salmonella typhi* (ATTCC 25922) and *P. aeruginosa* (ATCC 27853). The bacterial strains were first grown respectively on Muller Hinton medium at 37°C for 24 h prior to seeding onto the respective nutrient agar. One or several colonies of the indicator bacteria were transferred into API suspension medium (BioMérieux) and adjusted to the 0.5 McFarland turbidity standard with a Densimat (BioMérieux). A sterile filter disc with 6 mm of diameter (Whatman paper No. 3) was placed on the infusion agar seeded with bacteria, and 10 µl of several extract concentrations were dropped onto each paper disc, representing 2, 4, and 100 mg per disc. The treated Petri dishes were kept at 4°C for 1 h, and incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Standard discs of gentamycin (10 UI) served as positive antibiotic controls according to CASFM 2005 guidelines. Discs with 10 µl of pure methanol were used as negative controls. For the antifungal activity of the same *Frankenia* extracts, the agar-disc diffusion method was used as previously described (Cox et al., 2000). One Candida strain (*C. albicans* (ATCC10281)) was first grown on Sabouraud chloramphenicol agar plate at 30°C for 18 to 24 h. The colony was transferred into Api suspension medium and adjusted to two McFarland turbidity standard. The inocula of the yeast was streaked onto Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterile filter disc, diameter 6 mm (Whatman paper No. 3) was placed in the plate. Ten microlitres of different extract concentrations (representing 4, 2, and 1 mg /ml) were dropped on each paper disc. The treated Petri dishes were placed at 4°C for 1 to 2 h and then incubated at 37°C for 18 to 24 h. As for the antibacterial activity, the antifungal one was evaluated by measuring the diameter of the growth inhibition zone around the discs. The susceptibility of the standard was determined using a disc paper containing 10 UI of gentamycin. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Identification of phenolic compounds using HPLC

Hydrolysis of dried areal parts was realized according to slightly modified method of Proestos et al. (2006). Briefly 40 ml of methanol containing BHT (1 g/L) was mixed with 0.5 g of dried *F. thymifolia* shoot and 10 ml of 6 mol/l HCl. The mixture was stirred carefully, sonicated for 15 min, and finally incubated in a water bath at 90°C for 2 h. The obtained mixture was filtered through a 0.45 µm membrane filter and injected to HPLC.

The separation of phenolics was performed with an Agilent apparatus equipped with an autosampler model 1100, a Prostar Pump model 1100, diode array detector model 1100. A C18 column (Prontosil, 250 × 4.0 mm, 5 µm, Bischoff) was used for analysis. The mobile phase was composed of two solvents: A, 0.025% TFA in H_2O and B, MeCN. The sample was dissolved in MeOH/H_2O (1/1) and filtered through a 0.45 µm Millipore filter. The elution program at 1 ml/min was as follows: 10 to 50% B (0 to 40 min), 50 to 100% B (40 to 41 min), 100% B (41 to 50 min), 100-10% B (50 to 51 min), 10% B (55 to 59 min), each sample was directly injected and chromatograms were monitored at 280 and 306 nm.

Fatty acid extraction and composition analysis

Oil extraction

Arial parts of *F. thymifolia* were ground in a grinder. Oil was extracted using hexane in a Soxhlet extractor for 8 h (35 g sample in 200 ml hexane). Shoot oil was stored at -20°C for further analysis.

Fatty acid methyl ester analysis (FAMEs)

Extract (0.2 ml) was saponified with 3 ml of 0.5 N NaOH in methanol, and the mixture was incubated for 15 min in a boiling water bath at 60°C. The fatty acids were later converted to methyl esters with 3 ml of a Boron trifluoride-methanol complex (14%) reagent, and the whole incubated at 60°C for 15 min. The fatty acid methyl ester was washed with 2 ml of water and extracted with 10 ml of petroleum ether. The identification of FAMEs was performed using an HP-5980 Series II instrument, equipped with HP-5MS capillary column (30 m x 0.25 mm; 0.25 µm film thickness), split/splitless injector (220°C). The oven temperature was held at 150°C, then programmed at 15°C/ min up to 220°C, and held isothermally at 220°C for 5 min. Helium was the carrier gas at an initial flow rate of 1 ml/min. Split ratio was 20:1. Injection volume was 2 µl. Quantification of fatty acid methyl esters, expressed as percentage, was obtained directly from GC-MS peak area integration. The components were identified by comparing their relative retention times and mass spectra with the data from the library of essential oil constituents, Wiley, Mass-Finder and Adams GC/MS library.

Statistical analysis

In all cases, three replicates were used for all assays. Results were processed by using the one-way analysis of variance (ANOVA) using the STATI-CF statistical program. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Total polyphenol, flavonoid, and condensed tannin contents

Results showed that phenolic content varied greatly among the two fractions (Table 1), and the polar fraction (methanol:water) contains the highest amount of polyphenol, flavonoïd as well as condensed tannin (14.18 mg of GAE g^{-1} DW, 4.79 and 4.61 mg of CE g^{-1} DW,

Table 1. Total polyphenol, flavonoid, and condensed tannin contents in *Frankenia thymifolia* shoot extract using two fractions. Means of three replicates followed by at least one same letter are not significantly different at $P < 0.05$.

Fraction	Polyphenol content (mg of GAE/g DW)	Flavonoid content (mg of CE/g DW)	Tannin content (mg of CE/g DW)
Methanol/water	14.18[a]	4.79[a]	4.51[a]
Chloroform	0.32[b]	0.52[b]	2.51[b]

Table 2. Total antioxidant capacity (mg GAE g^{-1} DW) and antioxidant properties against $DPPH^{\cdot}$ radical and β-carotene bleaching test (IC_{50} in µg ml^{-1}) and iron reducing power (EC_{50} = µg/ml) of shoot fractions and authentic standards (BHT, BHA and ascorbic acid) expressed in IC_{50} value (µg ml^{-1}).

Fraction	Total antioxidant capacity	$DPPH^{\cdot}$ scavenging activity	Iron reducing power	β-Carotene bleaching test
Met/water	8.78[a]	99[b]	120[b]	11[b]
Chloroform	2.72[b]	120[a]	>1000[a]	>1000[a]
BHT	*	11.5	*	75
BHA	*	*	*	48
Ascorbic acid	*	*	37.33	*

respectively). These values were respectively 44, 9 and 1.8-fold higher than the chloroformic one, demonstrating clearly the influence of the solvent on the extractability of phenolic compounds, in particular on the extractability of other constituents and formation of insoluble complex. In addition, similar trends were observed with previous studies which showed that solvent nature exert a great power in phenolic extraction capacities in many species (Akowuah et al., 2005; Turkmen et al., 2006; Trabelsi et al., 2010). Independently of solvent, polyphenol content in methanolic extract of *Frankenia* was higher than in several species cited in the literature. Velioglu et al. (1998) reported a level ranging from 0.2 to 10.6 g 100 g^{-1} DW in fruits, grains and vegetables, and Zhou and Yu (2006) found 13 and 10.6 mg GAE g^{-1} DW in spinach and broccoli respectively. In this context the level of phenolic compound of methanolic fraction seems to be higher than those reported by Djeridane et al. (2007) in some medicinal halophytic plant *Thapsia garganica*, *Rhamnus alaternus*, *Teucrium polium* and *Thymelea hirsuta* (2.5, 6, 12.6 and 12.7 GAE g^{-1} DW, respectively). Accordingly, previous studies suggested that abiotic stresses (salinity, luminosity, water deficit, etc.) widely present in the arid zone may enhance phenolic synthesis as a response to the oxidative stress generated by the formation of reactive oxygen species in these hostile environments (Navarro et al., 2006; Ksouri et al., 2008).

Antioxidant activities of shoot extracts

Considering the multifaceted aspects of antioxidants and their reactivity, several antioxidant assays were applied. Among them, total antioxidant activity, DPPH assays, reductive potential, β-carotene linoleate system are the most commonly used for the determination of antioxidant activities of plant extracts.

Total antioxidant capacity

Global antioxidant activity of *F. thymifolia* was expressed as the number of gallic acid equivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex. Results showed in Table 2 reveal that the antioxidant activities of the two fractions were significantly different. This ability was 3.2 fold higher in polar extract (8.78 mg GAE g^{-1}DW) as compared to chloroform fraction (2.72 mg GAE g^{-1}DW). The better antioxidant activity of methanolic fraction in *F. thymifolia* was correlated with highest level of phenolic compound. The significant differences in antioxidant potential between the two solvents used in this experiment was essentially due to the difference in polarity, and thus different extractability of the antioxidative compounds (Maisuthisakul et al., 2007).

DPPH radical-scavenging activity

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radical was determined by the decrease in

absorbance induced by plant antioxidants.

Tested for antiradical properties against DPPH, the chloroformic extract was less potent than methanolic extract. In fact, polar shoot extracts showed the best IC_{50} value (99 µg ml^{-1}) as compared to apolar one. However, the positive standard, BHT, was statistically even more powerful as a hydrogen donor (IC_{50} = 11.5 µg ml^{-1}). Thus, the difference in DPPH scavenging activity of plant extracts might be due to the difference in solvent selectivity for extracting certain phenolic groups (Djeridane et al., 2006). Several studies showed that solvent natures, notably polarity, have significantly different extraction capacities and qualities for phenolic compounds in plants (Parida et al., 2004; Turkmen et al., 2006).

Iron reducing power

Table 2 showed that the Fe^{3+} reducing power of shoot extract differs greatly depending on polarity of solvent. The EC_{50} value was 120 µg ml^{-1} for methanol extract, and was absent for chloroformic extract until 1000 µg ml^{-1}. Although, the positive control (Vitamin C) showed a stronger activity (EC_{50} = 37.33 µg ml^{-1}) compared to plant extract. The higher phenolic content in methanolic fraction (ca. 44-times higher than in chloroformic extract) reflected the highest total antioxidant (8.78 mg of GAE g^{-1} DW) and the antiradical activities and Fe-reducing power. In fact, Maisuthisakul et al. (2007) consider that polyphenol compounds contribute directly to the antioxidant capacities. In this context, previous reports showed also a significant correlation between antioxidant activity and total phenolic content of Algerian and Chinese medicinal plants (Djeridane et al., 2006; Wong et al., 2006).

Antioxidant assay using β-carotene linoleate system

Result displayed that the addition of *Frankenia* extracts to this system prevents the bleaching of β-carotene at different degrees. Fraction extracts hindered the extent of β-carotene bleaching on a dose dependent manner. In term of β-carotene bleaching effect, the extract concentration providing 50% inhibition (IC_{50}) showed a higher ability in methanolic fraction (IC_{50} = 11 µg/ml) than in the chloroformic fraction (IC_{50} = 1000 µg/ml). Moreover, methanolic extract exhibited a marked antioxidant activity close to that of BHA (48 µg ml^{-1}) and BHT (75 µg ml^{-1}). In this methanolic fraction, oxidation of linoleic acid produces hydroperoxide-derived free radicals which attack the chromophore of β-carotene, resulting in a bleaching of the reaction emulsion. Liyana-Pathirana and Shahidi (2006) reported that an extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant. According to the β-carotene-linoleic acid bleaching data, the methanolic fractions are capable of scavenging free radicals in a complex heterogenous medium. This suggests that the extracts may have potential use as antioxidative preservatives in emulsion-type systems.

Antimicrobial activities

Table 3 shows the antibacterial activities of shoot polar and apolar fractions measured by the agar diffusion method against selected pathogenic bacteria. The disk diffusion method was used to determine the antimicrobial activity towards selected food borne pathogens namely: *Escherichia coli, P. aeruginosa, S. aureus, E. faecium Salmonella thyphi,* and *Candida albicans*. Results displayed that shoot extracts inhibited microorganism growth, depending on the strains sensibility and extract concentration. In fact, the aqueous methanolic fraction have a slight antibacterial activity against *S. aureus* and moderate against *E. faecium*, while no inhibitory effect was observed against the other bacteria. Moreover, the inhibition zone against these two last strains increased when the concentration increased from 1 to 4 mg ml^{-1}. However, chloroformic extract was active against all bacteria as compared to methanolic one. The inhibition zone against all bacteria treated with chloroformic extracts increased from 7 to 10.5 mm as function of the concentration (1 to 4 mg ml^{-1}). In addition, the concentration of 1 mg ml^{-1} of apolar fraction was efficient to inhibit all microorganism growth and corresponding to the MCI (minimal concentration of inhibition). Concerning antifungal test, methanolic extract failed to show any activity against *C. albicans* in the three concentrations (1, 2 and 4 mg ml^{-1}) while chloroformic fraction was weak active at 1 mg ml^{-1} and exhibited a moderate activity at 4 mg ml^{-1} (Table 3). Accordingly, Meot-Duros et al. (2008) reported that the antimicrobial activities of apolar (chloroformic) fractions were more active than polar (methanolic) ones of the halophytes *Eryngium maritimum* L., *Crithmum maritimum* L. and *Cakile maritima* Scop.

As compared to the polar fraction which showed the highest phenolic compounds and antioxidant activities, apolar fraction was more efficient to inhibit bacterial and fungal. These observations can be explained by different active compounds being extracted with each solvent. These antibacterial actions of *F. thymifolia* apolar fraction can be attributed to the presence of lipophilic flavonoids, carotenoids, essential oil and fatty acids. For instance, it was reported that lipophilic flavonoids may protect plants against infection by microorganisms, as several have been shown to have antibacterial (Harborne and Baxter, 1993) or antifungal activities (Tomás-Barberán et al., 1988). The antibacterial activity of chloroformic fraction can be also attributed to the presence of long-chain fatty acids like elaidic acid (C18:1t9), palmitic acid (C18:0) and particularly linoleic acid (C18:2). In this context, previous studies reported that long-chain UFA containing oleic acid, linoleic acid, and linolenic acid exhibited an antibacterial action, while long-chain saturated fatty

Table 3. Antibacterial activities and antifungal test of *F. thymifolia* shoot fractionss (using the methanol/water, and chloroform) against five human pathogenic bacteria and one Candida species at different concentrations (mg/ml). Inhibition zone (IZ) calculated in diameter around the disc (mm ± SD).

Bacterial strain	mg/ml	Met/water	Chloroform	Gentamycin (10 UI)
Staphylococcus aureus	4	8.6±0.00	8±0.00	30
	2	8±0.00	7.8±0.00	
	1	7.8±0.00	7±0.00	
Enterococcus faecium	4	9.5±0.03	8.5±0.00	30
	2	9.3±0.01	8.5±0.00	
	1	9.1±0.00	7.5±0.00	
Salmonella typhi	4	6±0.00	10.5±0.02	20
	2	6±0.00	10±0.01	
	1	6±0.00	8±0.00	
Pseudomonas aeruginosa	4	6±0.00	8.1±0.00	20
	2	6±0.00	8±0.00	
	1	6±0.00	8±0.00	
Echerichia coli	4	6±0.00	10±0.02	20
	2	6±0.00	9.5±0.01	
	1	6±0.00	9±0.01	
Candida albicans	4	6±0.00	9.5±0.00	27
	2	6±0.00	8.5±0.00	
	1	6±0.00	7.5±0.00	

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

acids, including palmitic acid and stearic acid, are less active (Knapp and Melly, 1986; Sun et al., 2003; Seide and Taylor, 2004;). Additionally, the efficient inhibition of growth of *S. aureus* only in chloroformic fraction can be attributed to the presence of linoleic acid. In fact, different researches indicated that linoleic acid show a strong antibacterial activity against *S. aureus* (Knapp and Melly, 1986). However, the precise mechanism for this antimicrobial activity remains unclear. Zheng et al. (2005) found that linoleic acid inhibited bacterial enoyl-acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, which has served as a promising target for antibacterial drugs.

Identification of phenolic compounds by HPLC assay

The free forms of phenolic compounds are rarely present in plants. More often, they occur as esters, glycosides or are bound to the cell wall. For this reason, before HPLC analysis, hydrolysis of glycosides or esters was necessary, so that phenolic compounds can be identified, since a considerable fraction is in bounded form. Moreover, BHT, a powerful antioxidant, was added to prevent degradation of phenolics during hydrolysis (Nardini and Ghiselli, 2004). Table 4 shows the retention time of authentic standards, and a typical HPLC chromatogram of *F. thymifolia* shoot extract is presented in Figure 1. The result obtained revealed a shoot phenolic fingerprint composed of six phenolic acids (chlorogenic acid, 2,5-dihydroxybenzoic acid, vanillic acid, trans-2-hydroxycinnamic acid, salicylic acid and transcinnamic acid) and two flavonoids (catechin and epigallocatechin-o-3-gallate). These compounds have been identified according to their retention times and spectral characteristics of their peaks against those of standards, as well as by spiking the sample with standards. Salicylic and trans-cinnamic acids were the major compounds followed by catechin and trans-2-hydroxycinnamic acid. Salicylic acid is a benzoic acid derivative naturally occurring plant hormone, acts as an important signaling molecule in plants, and the antifungal effect of this compound was clearly demonstrated (Amborabé et al., 2002). This molecule has also diverse effects on tolerance to biotic and abiotic stresses (Khan et al., 2010; Raskin, 1992; Rodríguez et al. 2008). In the same context, trans-cinnamic acid was also recognized as a marker of environmental stress in different plant species (Pina and Errea, 2008). The presence of the CH=CH–

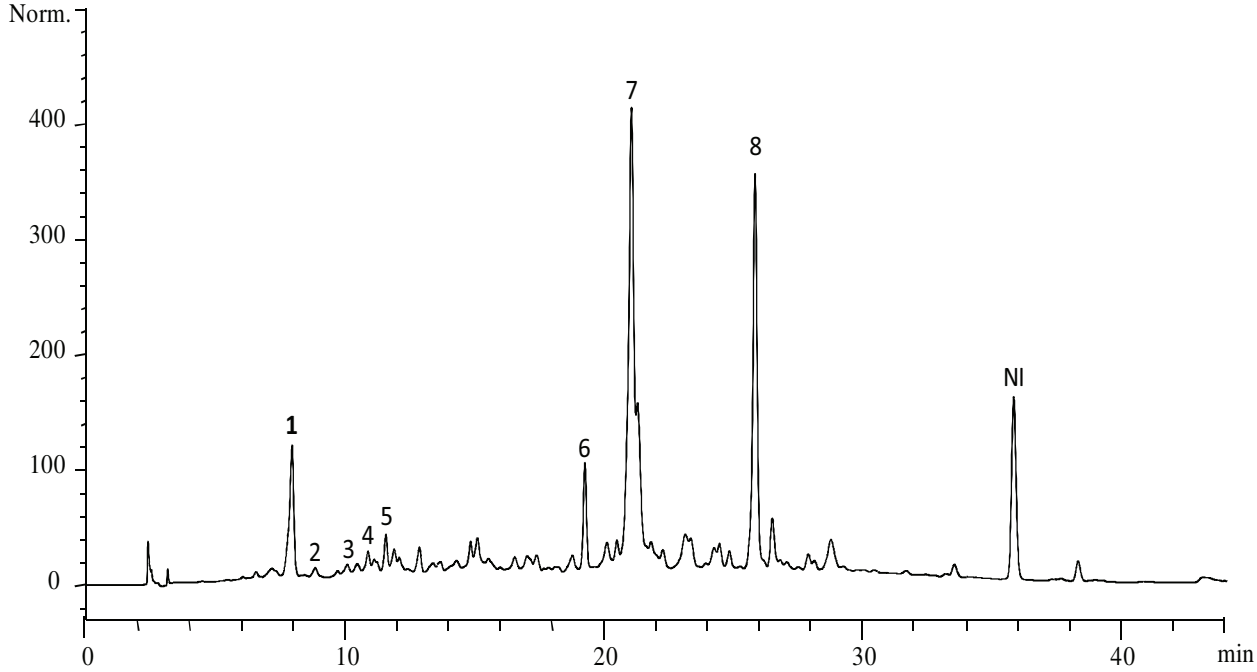

Figure 1. HPLC Chromatographic profiles of phenolic acids and flavonoids standards, of *F. thymifolia* shoot extract monitored at 280 nm. The peak numbers correspond to: 1 catechin; 2, chlorogenic acid; 3, 2,5-dihydroxybenzoic acid; 4, vanillic acid; 5, epigallocatechin-3-*o*-gallate; 6, trans-2-hydroxycinnamic acid; 7, salicylic acid; 8, trans-cinnamic acid, NI: not identified.

COOH group in the hydroxycinnamic acids found in *F. thymifolia* shoots (trans-cinnamic, trans-2-hydroxy-cinnamic, and chlorogenic acids) is considered to be key element for the antioxidative efficiency (Gombau et al., 2006). In addition, trans-cinnamic acid have a wide range of therapeutical importance, such as, antitumor activity (Bezerra et al., 2006), antioxidant activity (Chung and Shin, 2007), antibacterial activity (Naz et al., 2006) and against multi-drug resistant tuberculosis (Carvalho et al. 2008). Promising results have been shown by Rastogi et al. (1998) which reported the synergistic activity of trans-cinnamic acid in drug combinations with isoniazid. As well, catechin was found to be the most abundant flavonoid in this study. This flavonol is a well-known antioxidant, due to the presence of the o-dihydroxy and o-hydroxyketo groups (Bourgou et al., 2008; Vinson et al., 1995) that confer the capacities of quenching lipid per-oxidant, preventing DNA oxidative damage, and scavenging reactive oxygen species (Yu-Ling et al. 2008). For that, natural antioxidants such as polyphenols are often added to foods to stabilize them and prevent off-flavor development and have considerable interest for their potential role as functional foods or nutraceuticals (Espin et al. 2007; Ksouri et al. 2009). The mechanism by which antioxidants protect food from oxidation is by scavenging of free radicals via donation of an electron or a hydrogen atom, or by deactivation of metal ions and singlet oxygen.

Fatty acid composition by GC/MS

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated and polyunsaturated fatty acids (MUFA and PUFA, respectively) of crude *F. thymifolia* shoot oil are shown in Table 5. The major fatty acids were palmitic acid (C16:0) followed by elaïdic acid (C18:1n-9) and linoleic acid (ω 6; C18:2n-9), representing 28.7, 24.13 and 20.36%, respectively. Besides these three main fatty acids, eight more were identified and quantified. SFA was the main group of fatty acids, representing 47.45, followed by MUFA 26.64 and PUFA 25.87%. The ratio of saturated/unsaturated acid is 1.1, which is low because of the high level of unsaturated fatty acid such as C18:1n9 and C18:2n9. Linoleic and oleic acids are antibacterial components in the herbs (*Helichrysum pedunculatum* and *Schotia brachypetala*) used for dressing wounds during male circumcision rituals in South Africa (Dilika et al., 2000; McGaw et al., 2002).

Conclusion

Such variability in antioxidant capacities between two fractions can be explained by different active compounds being extracted with each solvent and might be of great importance in terms of valorising this halophyte as a

Table 4. Retention time (R.T) of thirteen standards of phenolic acids and flavonoids.

S/N	Standards	R.T (min)
1	Gallic acid	4.080
2	Gallocatechin	5.465
3	Protocatechic acid	6.680
4	3,4-dihydroxyphenol acetic acid	6.835
5	Epigallocatechin	7.596
6	Catechin	7.785
7	Chlorogenic acid	8.873
8	4-hydroxybenzoïc acid	9.764
9	2,5-dihydroxybenzoïc acid	10.121
10	Vanillic acid	10.897
11	Caffeic acid	11.309
12	3,5 dimethoxy- 4-hydoxybenzoïc acid	11.332
13	Epigallocatechin-3-o-gallate	11.512
14	*P*-coumanic acid	14.771
15	Rutin hydrat	15.679
16	Rutin trihydrat	15.700
17	Sinapic acid	16.493
18	Trans-4-hydroxy-3-methoxycinnamic acid	16.567
19	3,4 dimethoxybenzoïc acid	16.737
20	Trans-2-hydroxycinamic acid	19.384
21	*O*-coumaric acid	20.040
22	Rosmarinic acid	20.231
23	Salicylic acid	21.231
24	Naphtoresorcinol	24.039
25	Trans cinnamic acid	25.999
26	Quercitin dihydrat	26.760
27	Apigenine	27.273
28	4, methoxycinnamic acid	27.693
29	4',5,7 - trihydroxyflavone	30.326
30	Kaempferol	31.840

Table 5. Fatty acid profile (%) in *Frankenia thymefolia* shoots.

Fatty acid	Formula	Relative content (%)
Saturated fatty acids (SFA)		
Lauric acid (dodecanoic Acid)	C12:0	2.15
Myristic acid (tetradecanoic acid)	C14:0	4.89
Palmitic acid (hexadecanoic acid)	C16:0	28.7
Stearic acid (octadecanoic acid)	C18:0	5.79
Behenic acid (docosanoic acid)	C22:0	5.92
Monounsaturated fatty acids (MUFA)		
Oleic acid (9-octadecenoic acid)	C18:1	1.6
Elaidic acid (11-octadecenoic acid)	C18:1 trans 9	24.13
Palmitoleic acid (cis-9-hexadecenoic acid)	C16:1	0.91
Polyunsaturated fatty acid (PUFA)		
Linoleic acid (9,12- octadecadienoic acid) (ω 6)	C18:2	20.36

Table 5. Continued.

Cis, cis-linoleic acid	C18:2	3.43
α-linolenic acid (ω 3)	C18:3	2.08
ΣSFA		47.45
ΣMUFA		26.64
ΣPUFA		25.87
Ratio UFA/SFA		1.1

source of naturally bioactive molecules with high biological activities which can be used as preservative ingredients in the food and/or pharmaceutical industry.

Abbreviations: BHA, 3-tertiobutyl-4-hydroxyanisole; **BHT,** 3,5-ditertiobutyl-4-hydroxytoluène; **DPPH,** 1,1-Diphenyl-2-picrylhydrazyl; **GAE,** gallic acid equivalents; **MUFA,** monounsaturated; **PUFA,** polyunsaturated fatty acids; **ROS,** reactive oxygen species; **SFA,** saturated fatty acids; **GC/MS,** gas chromatography/ mass spectrometry; **HPLC,** high performance liquid chromatography; **ROS,** reactive oxygen species; **GAE,** gallic acid equivalents; **ANOVA,** analysis of variance; **SFA,** saturated fatty acids; **MUFA,** monounsaturated fatty acids; **PUFA,** polyunsaturated fatty acids.

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REFERENCES

Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO (2007). Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol., 6(10): 1197-1201.

Akowuah GA, Ismail Z, Norhayati I, Sadikum A (2005). The effect of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chem., 93: 311-317.

Amborabé BE, Fleurat-Lessard P, Chollet JF, Roblin G (2002). Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata*: structure–activity relationship. Plant Physiol., Biochem., 40: 1051-1060.

Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K (2002). Methods for testing antioxidant activity. The Analyst, 127(1): 183-198.

Bagamboula M, Uyttendaele J, Debevere M (2003). Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and *p*-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microb., 21: 33-42.

Balasundram N, Sundram K, Sammar S (2006). Phenolic compounds in plants and agri-industrial by-products. Antioxidant activity, occurrence, and potential uses. Food Chem., 1: 191-203.

Bezerra DP, Castro FO, Alves APN, Pessoa C, Moraes MO, Silveira ER, Lima MAS, Elmiro FJM, Costa-Lotufo LV (2006). *In vivo* growth inhibition of Sarcoma 180 by piplartine and piperine, two alkaloid amides from piper. Braz. J. Med. Biol. Res., 39: 801-807.

Borchardt JR, Wyse DL, Sheaffer CC, Kauppi KL, Fulcher RG, Ehlke NJ, Biesboer DD, Bey RF (2008). Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. J. Med. Plant Res., 2(4): 081-093.

Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B (2008). Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. C. R. Biol., 331: 48-55.

Carvalho SA, Da Silva EF, De Souza MVN, Lourenço MCS, Vicente FR (2008). Synthesis and antimycobacterial evaluation of new trans-cinnamic acid hydrazide derivatives. Bioorg. Med. Chem. Let., 18: 538-541.

Chavan UD, Shahidi F, Naczk M (2001). Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. Food Chem., 75: 509-512.

Chung HS, Shin JC (2007). Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oriza sativa* cv. Heugjinjubyeo). Food Chem., 104: 1670-1677.

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol., (88): 170–175.

Cragg GM, Simon JE, Jato JG, Snader KM (1996). Drug discovery and development at the National Cancer Institute: Potential for New Pharmaceutical Crops. In Janick J (eds) Progress in New Crops, ASHS Press, Arlington, VA., pp. 554-560.

Decker EA, Warner K, Richards MP, Shahidi F (2005). Measuring antioxidant effectiveness in food. J. Agr. Food Chem., 53: 4303-4310.

Dewanto VX, Wu K, Adom K, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agr. Food Chem., 50: 3010-3014.

Dilika F, Bremner PD, Meyer JJM (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia, 71: 450-452.

Djeridane A,·Yousfi M,·Nadjemi B, Vidal N, Lesgards JF, Stocker P (2007). Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. Eur. Food Res. Technol., 224: 801-809.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660.

Espin JC, Garcia-Conesa MT, Tomas-Barberan FA (2007). Nutraceuricals: facts and fiction. Phytochem., 68: 2986-3008.

Galvez CJ, Martin-Cordero P, Houghton AM (2005). Antioxidant activity of methanol extracts obtained from Plantago species. J. Agric. Food Chem., 53: 1927-1933.

Gombau L, Garcıa F, Lahoz A, Fabre M, Roda-Navarro P, Majano P, Alonso LJL, Pivel JP, Castell JV, Gomez-Lechon MJ, Gonzalez S. (2006). *Polypodium leucotomos* extract: Antioxidant activity and disposition, Toxicol. *In vitro,* 20: 464-471.

Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2002). On the *in vitro* antioxidant properties of melatonin. J. Pineal Res., 33: 167-171.

Hanato H, Kagawa T, Yasuhara J, Okuda T (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. Chem. Pharm. Bull., 36: 1090-1097.

Harborne JB, Baxter H (1993). Phytochemical Dictionary. Taylor and Francis, London and Washington, DC.
Harborne J.B. (1975). Flavonoids bi sulfates and their co occurrences with ellagic-acid in the Bixaceae *Frankeniaceae* and related families. Phytochem. (Oxford). 14: 1331-1338.
Harkat H, Haba H, Marcourt L, Long C, Benkhaled M (2007). An unusual lignan sulfate and aromatic compounds from *Frankenia thymifolia* Desf. Bioch. Syst. Ecol., 35: 176-179.
Hou Z, Lambert JD, Chin KV, Yang CS (2004). Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. Mut. Res., 555: 3-19.
Hussein SAM (2004). Phenolic sodium sulfates of *Frankenia leavis* L. Pharmazie. 59: 304.
Jayaprakasha GK, Sing RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73: 285-290.
Khan NA, Syeed S, Masood A, Nazar R, Iqbal N (2010). Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress, Int. J. Plant Biol., 1:e1-e5.
Knapp HR, Melly MA (1986). Bactericidal effects of polyunsaturated fatty acids. J. Inf. Dis., 154: 84-94.
Kohen R, Nyska A (2002). Oxidation of biological systems: Oxidative stress phenomena, antioxydants, redox reactions, and method for their quantification. Toxicol. Pathol., 30(6): 620-650.
Koleva II, Teris AB, Jozef PH, Linssen AG, Lyub NE (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal., 13: 8-17.
Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magné C, Abdelly C (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem. Toxicol. 47: 2083-2091.
Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol. Biochem. 45: 244-249.
Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, Abdelly C (2008). Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C. R. Biol., 331: 865-873.
Liyana-Pathirana CM, Shahidi F (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. J. Sci. Food Agr., 86(3): 477-485.
Maisuthisakul P, Pongsawatmanit R, Gordon MH (2007). Assessment of phenolic content and free-radical scavenging capacity of some Thai indigenous plants. Food Chem., 100: 1409-1418.
McGaw LJ, Jäger AK, Van Staden J (2002). Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia, 73: 431-433.
Menezes-Benavente L, Karam-Teixeira F, Alvim-Kamei CL, Margis-Pinheiro M (2004). Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). Plant Sci., 166: 323-331.
Meot-Duros L, Magné C (2009). Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. Plant Physiol. Biochem. 47: 37-41.
Meot-Duros L, Le Floch G, Magné C (2008). Radical scavenging, antioxidant and antimicrobial activities of halophytic species. J. Ethnopharmacol., 116: 258-262.
Nardini M, Ghiselli A (2004). Determination of free and bound phenolic acids in beer. Food Chem., 84: 137-143.
Navarro JM, Flores P, Garrido C, Martinez V (2006). Changes in the contents of antioxidants compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem., 96: 66-73.
Naz S, Ahmad S, Rasool SA, Sayeed SA, Siddiqi, R (2006). Antibacterial activity directed isolation of compounds from *Onosma hispidium*. Microb. Resol., 161: 43-48.
Oktay M, Gülçin I, Küfrevioglu OI (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT, 36: 263-271.
Oyaizu M (1986). Studies on products of the browning reaction prepared from glucose amine. Jap. J. Nut., 44: 307-315.
Ozenda P (1991). Flora and vegetation of the Sahara In: CNRS, Paris.
Parida AK, Das AB, Sanada Y, Mohanty P (2004). Effects of salinity on biochemical components of the mangrove, *Aeceras corniculatum*. Aquat. Bot., 80: 77-87.
Pina A, Errea P (2008). Differential induction of phenylalanine ammonia-lyase gene expression in response to in vitro callus unions of Prunus spp. J. Plant Physiol., 165: 705-714.
Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochem., 269: 337-341.
Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agr. Food Chem., 53: 4290-4302.
Proestos C, Boziaris IS, Nychas GJE, Komaitis M (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chem., 95: 664-671.
Raskin I (1992). Role of salicylic acid in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., 43: 439-436.
Rastogi N, Goh KS, Horgen L, Barrow WW (1998). Synergistic activities antibuberculous drugs with cerulenin and trans-cinnamic acid against *Mycobacterium tuberculosis*. FEMS Immunol. Med. Microbiol., 21: 149-157.
Rodríguez H, Landete JM, De las Rivas B, Munõz R (2008). Metabolism of food phenolic acids by *Lactobacillus plantarum* CECT 748^T. Food Chem., 107: 1393-1398.
Seidel V, Taylor PW (2004). *In vitro* activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. Int. J. Antimicrob. Agents, 23: 613-619.
Shahidi F, Naczk M (2004). Antioxidant properties of food phenolics. In Phenolics in Food and Neutraceuticals; CRC Press: Boca Raton, FL.
Soares JR, Dinis TCP, Cunha AP, Almeida LM (1997). Antioxidant activities of some extracts of *Thymus zygis*. Free Rad. Res., 26: 469-478.
Suhaj M (2006). Spice antioxidants isolation and their antiradical activity: a review. J. Food Comp. Anal., 19: 531-537.
Sun B, Richardo-da-Silvia JM, Spranger I (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agr. Food Chem., 46: 4267-4274.
Sun CQ, OConnor CJ, Roberton AM (2003). Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. FEMS Immunol. Med. Microbiol., 36: 9-17.
Tabart J, Kevers C, Sipel A, Pincemail J, Defraigne JO, Dommes J (2007). Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem., 105: 1268-1275.
Tadhani MB, Patel VH, Subhash R (2007). *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. J. Food Comp. Anal., 20: 323-329.
Tomás-Barberán FA, Msonthi JD, Hostettman NK (1988). Antifungal epicuticular methylated flavonoids from three Spanish *Helichrysum* species. Phytochem., 27: 753-755.
Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Bourgou S, Hajlaoui H, Abdelly, C. (2010). Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT, 43: 632-639.
Turkmen N, Sari F, Velioglu YS (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chem., 99, 835-841.
Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and green products. J. Agr. Food Chem., 46: 4113-4117.
Vicente O, Boscaiu M, Naranjo MA, Estrelles E, Belles JM, Soriano P. (2004). Responses to salt stress in the halophyte *Plantago crassifolia* (Plantaginaceae). J. Arid Env., 58: 463-481.
Vinson JA, Dabbagh YA, Serry MM, Jang J (1995). Plant flavonoids, especially tea flavonols are powerful antioxidants using an in vitro oxidation model for heart disease. J. Agr. Food Chem., 43: 2800-2802.
Wangensteen H, Samuelsen AB, Malterud KE (2004). Antioxidant activity in extracts from coriander. Food Chem., 88: 293-297.

Wong SP, Leong LP, Koh JHW (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem., 99: 775-783.
Yu-Ling L, Yang JH, Mau JL (2008). Antioxidant properties of water extracts from Monascus fermented soybeans. Food Chem., 106: 1128-1137.
Zampini IC, Cuello S, Alberto MR, Ordoñez RM, Almeida R, Solorzano E, Isla MI (2009). Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria. J. Ethnopharm., 124: 499-505.
Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH, Kim WG (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Let., 579: 5157-5162.
Zhou K, Yu L (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT, 39: 1155-1162.