Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxydant and antimicrobial activities of *Ononis natrix* leaves extracts

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ABSTRACT

Background and aim: The use of synthetic antioxidants has begun to be restricted because of their induction of DNA damage and their toxicity. So, it is an interesting and useful task to find new sources for natural antioxidant and functional food. The health benefits of *Ononis natrix* leaves were widely investigated. The present study describes for the first time the antioxidant, antibacterial and antihypertensive activities of various solvent extracts of *Ononis natrix* leaves. **Method:** The aerial parts (leaves) of *O. natrix* were collected from the South of Tunisia and different solvent extracts were prepared. The antioxidant activities of extracts at different concentrations were evaluated using various in vitro antioxidant assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, β-carotene bleaching and DNA nicking assays. The antibacterial and angiotensin I-converting enzyme (ACE) inhibitory were also investigated. **Results:** All the extracts showed strong antioxidant activity. The radical scavenging activities and reducing powers of all the extracts were close to those of the synthetic antioxidant BHA. The chloroform extract exhibited high inhibition of β-carotene bleaching and also showed a well DNA protection against degradation by hydroxyl radicals. Moreover, the antimicrobial activities of the extracts were tested against nine species of microorganisms, and the results obtained showed significant antibacterial activity against the Gram-positive and Gram-negative bacteria. *O. natrix* **e**xtract showed also important antihypertensive activities. Conclusion: The obtained results demonstrated the health benefit features of *Ononis natrix* leaves and their potential uses as feedstock of bioactive molecules.

Key words: ACE inhibitory activity, Antioxidant, Antimicrobial, Chemical composition, Extracts, *Ononis natrix*.

INTRODUCTION

Oxidation processes are intrinsic to the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms.[1] However, the excessive production of free radicals and the unbalanced mechanisms of antioxidant protection result in the onset of numerous chronic disorders: cancer, cardiovascular disease, diabetes, and other ageing-related diseases.[2] In the last decades, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in stabilization of foods in agro-alimentary industry are suspected to have negative effects on consumer's health.[3] Accordingly, attention is focused on the use of naturally occurring safe antioxidants and biologically active compounds from medicinal plants. Several plants were found to exhibit antioxidant properties, which are mainly attributed to a variety of active natural antioxidants including flavonoids, polyphenols, alkaloids, anthocyanins, terpenoids, carotenoids and vitamins.[4] Numerous studies have been published on the antiviral, antimicrobial and cytotoxic activities of plant extracts. Some of these studies claim that the phenolic compounds present in plant extracts might also play a major role in their biological effects.[5-6] Recently, the ability of phenolic substances from plant

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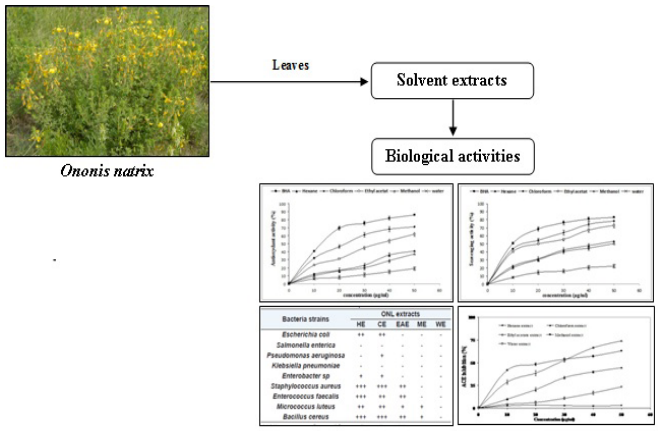


Graphical Abstract

material, including flavonoids and phenolic acids, to act as antioxidants has been extensively investigated.[7,8] So, many researchers have shown a great interest in plants because of their safety and related total antioxidant potential.[9] Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents and hydrogen donors. So, plants containing high level of polyphenols have a great importance as natural antioxidants.

Besides, because of the acquired bacterial resistance to antibiotics, there is a great interest in looking for new natural antimicrobial drugs. Biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for such new drugs.[6]

The species Ononis natrix comprises three subspecies that grow in the Iberian Peninsula: *Ononis natrix*, *Ononis hispanica* and *Ononi Ramosissima***.** *Ononis natrix* (Leguminosae, tribe Trifolieae) is a small plant known for its medicinal properties which are similar to those of *Ononis Spinosa*. The infusion of its roots has diuretic and ant rheumatic properties and has been used for the treatment of certain disturbances of the urinary tract.

This study aim was to determine the total phenolic and flavonoid contents and to evaluate the antioxidant, antimicrobial and ACE-inhibitory properties of different solvent extracts of *Ononis natrix* leaves. The antioxidant activity was investigated by various methods including DPPH scavenging activity, ferric-reducing activity, b-carotene bleaching assay and DNA nicking assay.

MATRIALS AND METHODS

Reagents

Angiotensin I-converting enzyme from rabbit lung, the ACE synthetic substrate hippuryl-L-histidyl-L-leucine (HHL), Chemicals required for the assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), β-carotene and L-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium hydroxide, FeCl3, ferrozine and other solvents, were of analytical grade. All solutions were freshly prepared in distilled water.

Materials

The aerial parts (stems and leaves) of *O. natrix* were collected from the South of Tunisia on January 2012 (Sfax). The plant material was dried at room temperature in the shadows, for 2 weeks until constant weight. The dried preparation was ground further to obtain a fine powder, and then stored at ambient temperature in a dry and dark place until use.

Preparation of *O. natrix* leaves (ONL) extracts

The dried powder of ONL (50 g) was extracted sequentially by adding solvents of increasing polarity: hexane, chloroform, ethyl acetate, methanol and water. The powder was first extracted by stirring with 500 mL of hexane at 30°C for 24 h. The extract was filtered through Whatman filter paper in a Buchner funnel. The filtrate was evaporated to dryness under reduced pressure in a rotatory vacuum evaporator at 40°C. The remaining residues were successively extracted with chloroform, ethyl acetate, methanol and water under the same conditions.The water extract was freeze dried. The dried sample of each extract was weighed and the yield of soluble constituents was determined. The dried extracts were kept in the dark at 4°C until further analyses.

Total phenolic content

The total phenolics content (TPC) of each extract was determined by the Folin-Ciocalteu method.[10] A 0.5 mL

aliquot of diluted extract solution was mixed with 0.5 mL of Folin-Ciocalteu's reagent. After the mixture was shaken for 5 min, 0.5 mL of 200 g/L sodium carbonate solution was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 5 mL by adding distilled water. The control reaction contained all reagents except the extract. The reaction mixture was then incubated in the dark at 25°C for 90 min, and the absorbance of the resulting colour was measured at 760 nm against a distilled water/sodium carbonate blank. Gallic acid monohydrate was used as standard for the calibration curve. TPC was expressed as mg gallic acid equivalent (GAE) per gram extract. Values presented are the average of three measurements.

Total flavonoid content

The total flavonoid content of ONL extracts was determined by the method of Zhishen *et al*.[11] Briefly, 250 µL of each ample were mixed with 1 mL of distilled water and subsequently with 150 µL of 150 g/L sodium nitrite solution. After 6 min, 75 µL of a 100 g/L aluminium chloride solution was added, and the mixture was allowed to stand for a further 5 min before 1 mL of 40 g/L NaOH solution was added. The mixture was immediately made up to 2.5 mL with distilled water and well mixed. The absorbance of the mixture was then measured at 510 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE)/g dried extract. Values presented are the average of three measurements.

Antioxidant activity

DPPH assay

The DPPH radical-scavenging activity of ONL extracts was determined by the method of Kirby and Schmidt[12] with some modifications. A volume of 500 µL of each extract at different concentrations (12.5 to 200 µg/mL) was added to 375 µL of 99% ethanol and 125 µL of DPPH solution (0.2 mM in ethanol) as free radical source. The mixtures were incubated for 60 min in the dark at room temperature. Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. BHA was used as positive control. DPPH radical-scavenging activity was calculated as:

$$DPPH\ radical-scavenging\ activity\ (\%) = \frac{A\ control + Ablank - A\ sample}{A\ control} \times 100$$

Where *A control*, *A blank* and *A sample* are, respectively, the absorbance of the control reaction (containing all reagents except the sample), the ONL extract without DPPH solution and the ONL extract with DPPH solution. The test was carried out in triplicate.

Ferric-reducing activity

The reducing power of ONL extracts was determined by the method of Yildirim and Mavi.[13] Sample solutions (0.5 mL) containing different concentrations of dried extract (12.5 to 200 µg/mL) were mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of 10 g/L potassium ferricyanide solution. The mixtures were incubated for 30 min at 50°C. After incubation, 1.25 mL of 100 g/L TCA was added and the reaction mixtures were centrifuged for 10 min at 3000 g. A 1.25 ml aliquot of the supernatant from each sample mixture was mixed with 1.25 mL of distilled water and 0.25 mL of 1.0 g/L ferric chloride solution in a test tube. After a 10 min reaction time, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power. The control was conducted in the same manner, except that distilled water was used instead of sample. Values presented are the mean of triplicate analyses.

ß-carotene bleaching assay

The ability of ONL extracts to prevent β-carotene bleaching was assessed as described by Koleva *et al*.[14] A stock solution of β-carotene/linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene, 25 µL of linoleic acid and 200 µL of Tween 40 in 1 mL of chloroform. The chloroform was completely evaporated under vacuum in a rotatory evaporator at 40 °C, then 100 mL of bi-distilled water were added, and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. Aliquots (2.5 mL) of the β-carotene/linoleic acid emulsion were transferred to test tubes containing different ONL extract concentrations. After two hours incubation at 50 °C, the absorbance of each sample was measured at 470 nm. BHA was used as positive standard. The control tube contained no sample.

Antioxidant activity in b-carotene bleaching model in percentage was calculated with the following equation:

$$Antioxidant\ activity\ \% = [1 - (A_0 - A_t) / (A'_0 - A'_t)] \times 100$$

Where A_0 and A'_0 are the absorbances of the sample and the blank, respectively, measured at time zero, and A_t and A'_t are the absorbances of the sample and the blank, respectively, measured after 2 h. Tests were carried out in triplicate. The same procedure was repeated with BHA as positive control.

DNA nicking assay

DNA nicking assay was performed using pYES2.1 TOPO plasmid (invitrogen) by the method of Lee *et al*[15] with slight modifications. A mixture of 10 µL of ONL extracts at the concentration of 100 µg/mL and plasmid DNA (0.5 µg/

well) were incubated for 10 min at room temperature followed by the addition of 10 µL of Fenton's reagent (30 mM H_2O_2, 50 mM L-ascorbic acid and 80 µM $FeCl_3$). The mixture was then incubated for 5 min at 37°C. The DNA was visualized on ethidiumbromide stained agarose gels using the GelDocXR Gel Documentation System (BioRad). Average band density was determined by using Quantity One 1-D Analysis Software (BioRad).

Antimicrobial activity

Microbial strains

Antibacterial activities of ONL extracts were tested against eight strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus cereus* (ATCC 11778) and *Enterococcus faecalis* (ATCC 29212).

Agar diffusion method

Antimicrobial activities of the different extracts were tested according to the method described by Berghe and Vlietinck.[16] ONL extracts (50 mg) were dissolved in 100% dimethylsulfoxide (DMSO) (1 mL). Culture suspension (200 mL) of the tested microorganisms 10^6 colony-forming units (cfu)/mL of bacteria cells (estimated by absorbance at 600 nm) were spread on Luria Bertani agar and. Then, bores (7 mm depth, 6 mm diameter) were made using a sterile borer and were loaded with 60 µL of ONL extracts at 50 mg/mL. A well with only 60 mL of DMSO (without extract) was used as a negative control. Cycloheximide was used as positive reference for bacteria. The Petri dishes were kept, first for 4 h at 4°C to allow extract diffusion in the agar, and then incubated for 24 h at 37°C. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones in millimetres (including well diameter of 6 mm). The test was carried out in triplicate, and the values presented are the averages of three replicates.

Determination of the angiotensin I-converting enzyme (ACE) inhibition activity

The ACE inhibition activity was measured as reported by Nakamura *et al*.[17] A sample solution (80 µl), of different concentrations (25, 50, 75, 100 and 150 µg/mL) of ONL extracts, was added to 200 µL containing 5 mM hippuryl-L-histidyl-L-leucine (HHL), and then preincubated for 3 min at 37°C. ONL extracts and HHL were prepared in 100 mM borate buffer, pH 8.3, containing 300 mM NaCl. The reactions were then initiated by adding 20 µL of 0.1 U/mL ACE from rabbit lung prepared in the same buffer and incubated for 30 min at 37 °C. The enzymatic reaction was terminated by the addition of 250 µL of 1.0 M HCl. The released hippuric acid (HA) was quantified by RP-HPLC on a Vydac C18 column connected to a system composed of a Waters TM 600 automated gradient controller pump module, a WaterWisp 717 automatic sampling device and a Waters 996 photodiode array detector. The sample was thereafter eluted using an acetonitrile gradient from 0 to 28% and from 28 to 47% in 0.1% trifluoroacetic acid (TFA) (v/v) for 50 and 20 min, respectively. The eluate was followed at 228 nm and the spectral and chromatographic data were stored on a NEC image 446 computer. Millennium software was used to acquire, analyze and plot chromatographic data. The average value estimated from three determinations at each concentration was used to calculate the ACE inhibition rate as follows:

$$\text{ACE inhibition}\,(\%) = \left[\frac{B - A}{B - C}\right] \times 100$$

Where A is the absorbance of HA generated in the presence of ACE inhibitor component, B the absorbance of HA generated without ACE inhibitors and C is the absorbance of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay).

Statistical analysis

Values were expressed as means ± standard deviation of three parallel measurements. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with SPSS 11 (Statistical Package for the Social Sciences) program. Differences at $P<0.05$ were considered statistically significant. Correlation and regression analysis was carried out using EXCEL program.

RESULTS AND DISCUSSION

Yield, total phenolic and flavonoid contents

The ONL components were fractionated by extraction with solvents of increasing polarity: hexane, chloroform, ethyl acetate, methanol and water. The yield of extractable compounds relative to the weight of dried plant material ranged from 16 g/kg (ethyl acetate extract) to 152 g/kg (methanol extract) (Table 1). It is well known that phenolic substances and flavonoids contribute directly to the antioxidant activity of plant materials.[18] Phenolic compounds exhibit considerable free radical-scavenging activities (through their reactivity as hydrogen- or electrondonating agents) and metal ion-chelating properties.[18] Flavonoids are a class of secondary plant

Table 1: Yield and Total Phenolic and Flavonoid Contents of Extracts from *ononis natrix* leaves

Extract	Yield (g/kg dried extract)	TPC[a] (mg GAE/g extract)	TFC[b] (mg QE/g extract)
Hexane	26.12 ± 3.04	50.2 ± 7.2	20.34 ± 2.98
Chloroform	27.55 ± 2.1	286.35 ± 19.03	126.75 ± 14.3
Ethyl Acetate	16.22 ± 0.91	268.06 ± 22.6	66.77 ± 8.2
Methanol	152.78 ± 7.29	45.05 ± 6.31	1.5 ± 0.22
Water	131.52 ± 11.45	11.12 ± 2.07	0.57 ± 0.07

Values are expressed as mean ± standard deviation of three independent determinations; [a]Total phenolic content as gallic acid equivalent (GAE); [b]Total flavonoid content as quercitin equivalent (QE).

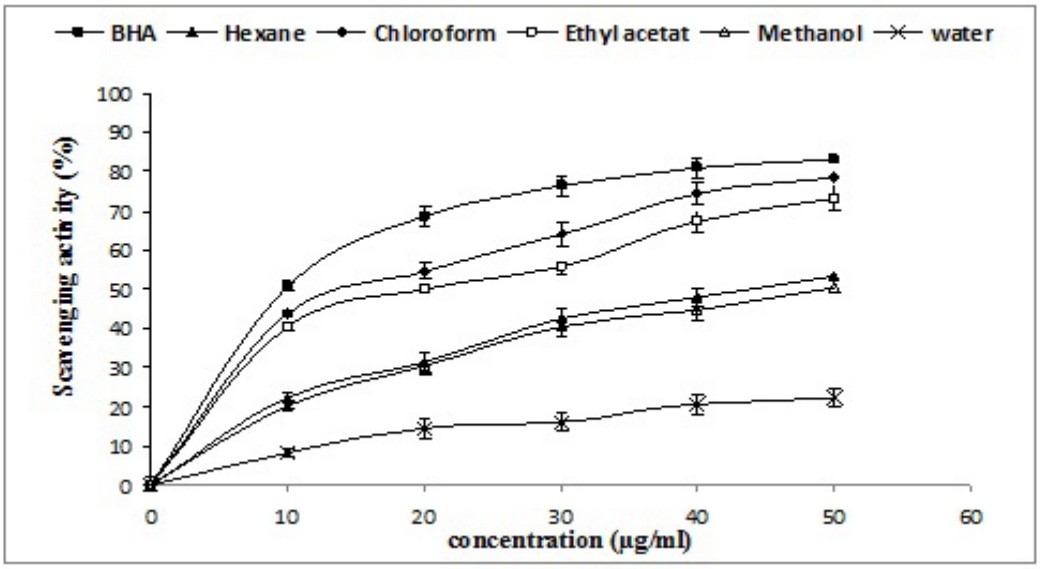


Figure 1: DPPH free radical-scavenging activities of the ONL extracts at different concentrations. BHA was used as reference antioxidant

Values are means ± SD (n=3)

phenolics with powerful antioxidant activities.[19] Therefore the amounts of total phenols and flavonoids in ONL extracts were determined (Table 1).

As shown in Table 1, the chloroformic extract (CE) had the highest phenolic content (286.35 mg of GAE/g) followed by the ethyl acetatic extract (268.06 mg of GAE/g). Variations in the yields and phenolic contents of the different extracts may be attributed to the polarities of compounds present in the ONL extracts.

The results in Table 1 show that the chloroformic extract (CE) had the highest flavonoid content (126.75 mg QE/g extract). In the aqueous extract, the flavonoids were present at low concentration (0.565 QE/g extract). This result is in agreement with the report of Chirinos *et al*.[20] who mentioned that the use of water as the only solvent yields an extract with a high content of impurities (e.g. organic acids, sugars, soluble proteins), which could interfere in the phenolic identification and quantification.

Antioxidant activity

In this study, various antioxidant assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, reducing power, β-carotene bleaching and DNA nicking assays were employed to evaluate the antioxidant activity of the ONL extracts.

DPPH free radical-scavenging activity

DPPH is a stable free radical which can be reduced by a proton-donating substrate such as an antioxidant, causing the decolorization of the DPPH and reducing the absorbance at 514 nm.[21]

DPPH radical scavenging activities of all ONL extracts and BHA (used as positive control) as shown in Figure 1 are concentration-dependent. The chloroformic extract was the strongest radical scavenger with an IC_{50} of 13.45 µg/mL. The IC_{50} values of ethyl acetatic extract, hexanic extract and methanolic extracts were 20.03; 43.92 and 49.97 µg/mL, respectively.

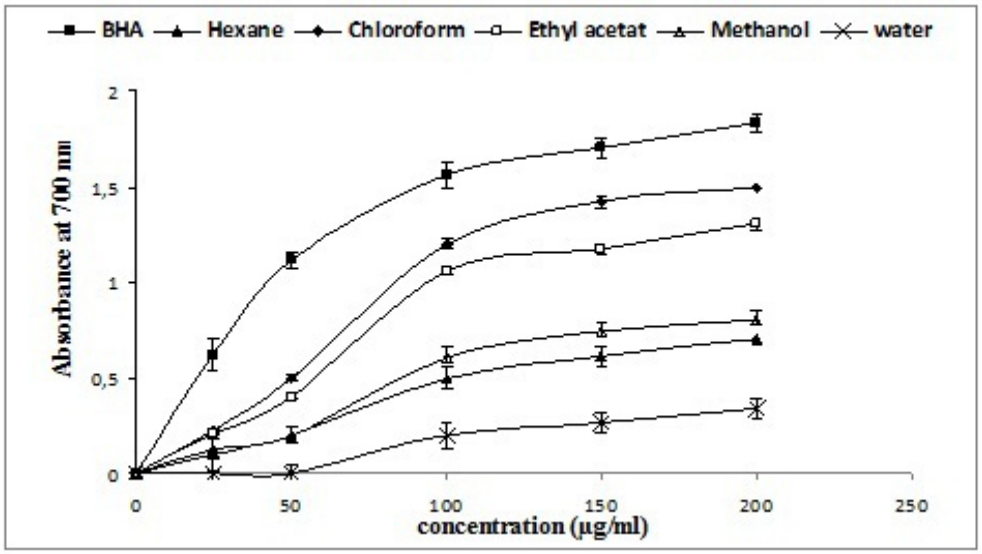


Figure 2: Reducing power of the ONL extracts at different concentrations. BHA was used as reference antioxidant

Values are means ± SD (n=3)

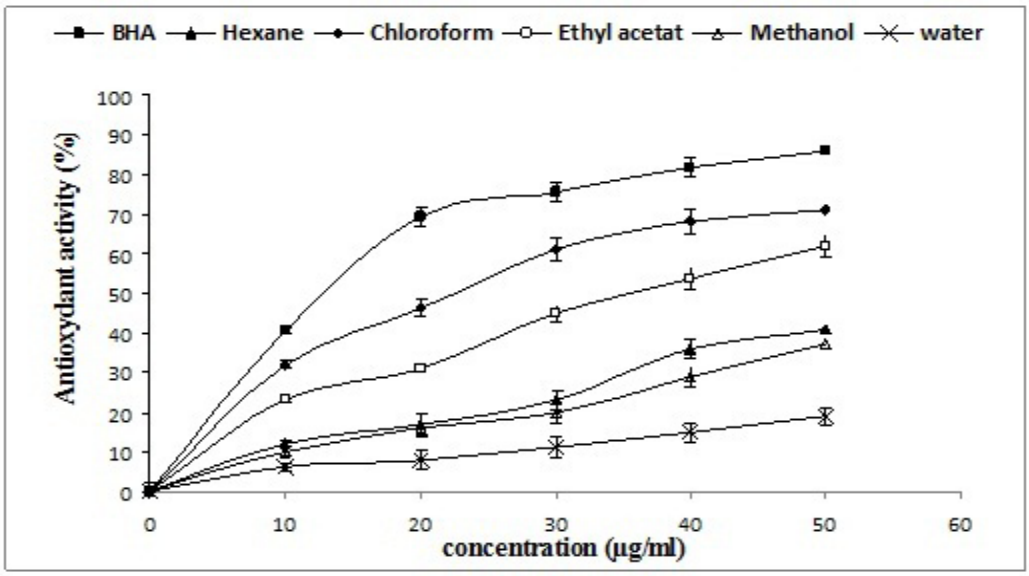


Figure 3: Antioxidant activities of the ONL extracts at different concentrations measured by the β-carotene bleaching method. BHA was used as reference antioxidant

Values are means ± SD (n=3)

It is interesting to note that under the same conditions, the chloroform extract showed nearly similar free radical-scavenging activity as BHA over the entire concentration range tested. The obtained results suggested that some components within the chloroformic extract are significantly strong radical scavengers. It has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample.[22]

Reducing power

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron or hydrogen.[13] Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain bioactive compounds. In this assay, the ability of ONL to reduce Fe^{3+} to Fe^{2+} was determined.

Figure 2 shows the reducing power activities (as indicated by the absorbance at 700 nm) of the different extracts compared with BHA as standard. The higher the absorbance of the reaction mixture, the higher is the reducing power. All extracts showed some degree of electron donation capacity, and the reducing power of all extracts increased with increasing amount of sample. The

Table 2: Antibacterial activity of ONL solvent extracts

Bacteria strains	ONL extracts				
	HE	CE	EAE	ME	WE
Escherichia coli	++	++	-	-	-
Salmonella enterica	-	-	-	-	-
Pseudomonas aeruginosa	-	+	-	-	-
Klebsiella pneumoniae	-	-	-	-	-
Enterobacter sp	+	+	-	-	-
Staphylococcus aureus	+++	+++	++	-	-
Enterococcus faecalis	+++	++	++	-	-
Micrococcus luteus	++	++	+	+	-
Bacillus cereus	+++	+++	++	+	-

Inhibition zones: +++: > 2 cm; ++: 1-2 cm, + : <1 cm, and -: no activity.

chloroform extract, which contained the highest amount of total phenolics and flavonoids, was the most potent reducing agent, followedby the water extract.

The reducing powers of the chloroform, ethyl acetate, methanol, hexan and water extracts at a concentration of 200 µg/mL were 1.5, 1.3, 0.8, 0.7 and 0.34 respectively. The ferric-reducing activity correlated well with the content of total phenols and flavonoids. A correlation between reducing power and total phenols present in plant extracts has been reported in the literature.[23-24]

β-carotene –linoleate assay system

The β-carotene–linoleic bleaching inhibition assay simulates membrane lipid oxidation and can be considered a good model for membrane based lipid peroxidation. In this oil–water emulsion-based system, linoleic acid acts as a free radical generator that produces peroxyl radicals under thermally induced oxidation. The produced free radicals attack the β-carotene chromophore resulting in bleaching effect, which can be inhibited by a free-radical scavenger.[25]

The antioxidative activities of the three ONL extracts, as well as the positive control BHA, as measured by the bleaching of β-carotene, are presented in Figure 3. In this assay, the antioxidant activity of ONL extracts increased with increasing extract concentration. The ONL chloroform extract showed the highest capacity to prevent bleaching of b-carotene, while the water extract exhibited the lowest activity. The antioxidant activities of the chloroform, ethyl acetate, methanol, hexan and aqueous extracts at a concentration of 50 µg/mL were 71.82%, 62.5%, 37%, 42.2% and 19.03%. The control, without sample, rapidly oxidized and the absorbance at 470 nm tends to zero. Therefore, the high detected antioxidant activity of ONL extracts suggests their possible efficiency in preventing the oxidative degradation of membrane lipids and suggests the lipophilic property of chloroform extract.

DNA nicking assay

Chandrasekara and Shahidi[26] reported that hydroxyl radical is extremely reactive, although it is shortlived. This radical has been known as a highly damaging free radical in cells. Oxidative damage to the DNA may occur at both the phosphate backbone and the nucleotide bases. This results in a wide variety of modifications, including strand scission, sister chromatid exchange, DNA-DNA and DNA-protein cross-links as well as base modification.[27]

The inhibition of supercolied plasmid DNA strand scission oxidation induced by hydroxyl radicals in the presence of different ONL extracts is depicted in Figure 4. The assay was used to evaluate the antioxidant activity of ONL extracts, based on their protection of supercoiled DNA strand from scission by hydroxyl radicals into the nicked circular form. In the presence of peroxyl and hydroxyl radicals, the scission of supercoiled DNA strand took place and was converted to nicked circular form.

Lane (-) showed an untreated plasmid with its two forms: the upper one is open-circular (nicked) DNA and the faster migrating band corresponded to supercoiled (closed circular) plasmid. Incubation of plasmid DNA with Fenton's reagent in the absence of extracts (lane (+)) resulted in the complete degradation of the two DNA bands (native supercoiled circular DNA and open circular

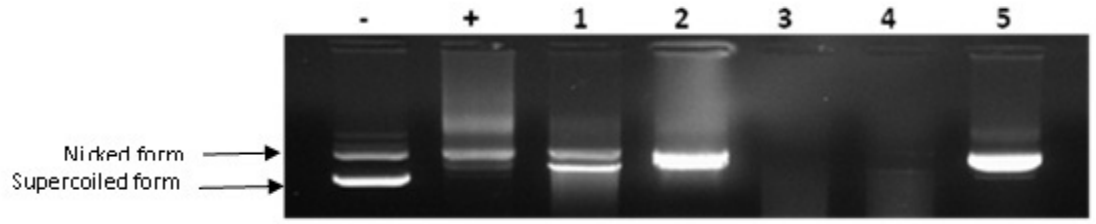


Figure 4: Gel electrophoresis pattern of the plasmid pCRII™TOPO incubated with Fenton's reagent in the presence and absence of ONL extracts. Lane 1: untreated control: native pCRII™TOPO DNA (0.5 µg); lane 2: DNA sample incubated with Fenton's reagent; lanes 3, 4, 5, 6, 7: Fenton's reagent + DNA + 100 µg HE, CE, EAE, ME and WE, respectively

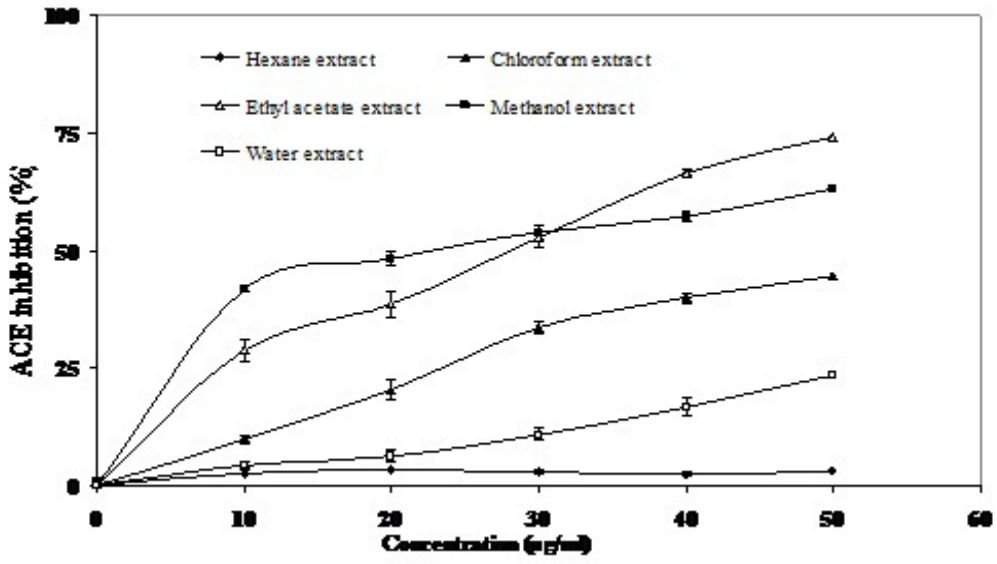


Figure 5: Angiotensin I-converting enzyme inhibitory effect of the ONL extracts at different concentrations. Values presented are the means of triplicate analysis

form). This DNA damage was reduced in the presence of the majority of ONL's extracts. At a concentration of 100 μg/mL, hexan extract showed the best protection to both nicked and supercoiled form. Both chloroform and aqueous extracts showed a moderate protection to supercoiled form and a complete protection to nicked form. The ethyl acetate and methanol extracts showed lower protection. Surguladze *et al*.[28] also reported the scission of supercoiled DNA strand to nicked circular form by free radicals. The rate of nicking correlated with the iron content and was strongly inhibited by radical scavengers and chelators. Oxidative stress in cells caused by ROS such as hydroxyl and peroxyl radicals generally leads to DNA damage, which is implicated in mutagenesis and carcinogenesis, among other pathological processes.[26,29]

Antimicrobial activity

The phenolic compounds are well-known for their antimicrobial activity.[30] The antibacterial activity of ONL extracts was tested on nine strains: five Gram-negative (*E. coli*, *S. enterica*, *P. aeruginosa*, *K. pneumoniae* and *E. sp*) and four Gram-positive (*S. aureus*, *E. faecalis*, *M. luteus* and *B. cereus*). The antibacterial activity was measured as the diameter of the clear zone of growth inhibition.

According to the results given in Table 2, the extracts of the investigated species showed an important *in vitro* potential of antimicrobial activities against the ten bacteria tested. The data obtained indicated that the ONL extracts displayed a variable degree of antimicrobial activity on the different tested strains. Our data indicated that Gram-positive *S. aureus* was the most sensitive tested strain to the ONL extracts. The results showed that only the aqueous extract has no antibacterial activity against the tested strain. Moreover, the results obtained are of great importance, particularly in the case of *B. cereus* and *S. aureus*, which are well-known for being resistant to a number of phytochemical compounds and for the production of several types of enterotoxins that cause gastroenteritis.[31]

ACE inhibitory activity

The inhibition of angiotensin I-converting enzyme (ACE) by dietary anti-hypertensive agents is potentially an important strategy to manage hypertension. With this regard, it was demonstrated that the ACE inhibition is considered as a useful therapeutic approach in the treatment of high blood pressure. Since synthetic ACE inhibitors may cause adverse side effects, plants extracts could be used as natural and economical ACE inhibitors for hypertension prevention and treatment. The ONL extracts were then tested for ACE inhibition activity. As shown in Figure 5, ONL extracts exhibited dose dependent ACE inhibitory activities.

The ONL methanol extract showed the highest ACE inhibitory activitiy, while the hexan extract exhibited the lowest activity. The IC_{50} value of the ethyl acetate and methanol extracts were calculated to be 28 μg/mL and 22.86 μg/mL, respectivly. Several chemical classes of ACE inhibitors compounds derived from plant extracts have been described such as tannins,[32] flavonoids[33] and peptides.[34] Furthermore, Tundis *et al*.[35] reported a 72.56%

and 52.56% ACE inhibition by the methanol and ethyl acetate extracts of *Senecio samnitum* at 330 µg/mL.

CONCLUSION

Many plant species are currently used as sources of natural additives because of their beneficial properties. The results presented in this study are the first information on the antioxidant and biological activities of extracts from O. *natrix* leaves. The ONL extracts, rich in phenolic and flavonoid compounds, were found to be an effective antioxidant in different *in vitro* assays. A dose-dependent effect between ONL extracts concentration and antioxidant activity was found. The results of this study indicate that ONL extracts exhibited a good antioxidant activity compared to that of positive controls like BHA and they can be used in food systems as natural antioxidants. ONL extracts possess also a bioactive potential as shown by the DNA nicked assay. ONL extracts showed moderate antimicrobial activities and important ACE inhibitory activity. The extracts of ONL may be an alternative additive in food, pharmaceuticals and cosmetic preparations instead of many toxic synthetic molecules.

CONTRIBUTION DETAILS

The first author contributed to the sample's collection, experimental studies and drafting the paper, the second and third authors contributed to the manuscript preparation and its revision, the fourth author and fifth authors contributed to the concept, design and definition of intellectual content and review the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

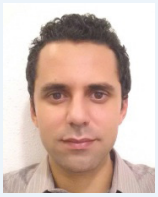
ABBREVIATION

ACE: Angiotensin I-Converting Enzyme
ONL: Ononis Natrix Leaves
TPC: Total Phenolics Content
GAE: Gallic acid Equivalent
QE: Quercetin Equivalent
ONL: OnonisNatrix Leaves
ACE: Angiotensin I-Converting Enzyme
TPC: Total Phenolics Content
TFC: Total flavonoid content
GAE: Gallic acid Equivalent
QE: Quercetin Equivalent
CE: Chloroform
HE: Hexan extract
EAE: Ethyl acetat extract
ME: Metanol extract
WE: Water extract
DPPH: 1,1-diphenyl-2-picrylhydrazyl
BHA: Butylated hydroxyanisole
BHT: Butylated hydroxytoluene;
HHL: Hippuryl-L-histidyl-L-leucine
TCA: Trichloroacetic acid
BSTFA: Bis-(trimethylsilyl) acetamide
DMSO: Dimethylsulfoxide
HA: Hippuric acid
TFA: Trifluoroacetic acid

Highlights of the paper

- This is the first report of the preparation of various solvent extracts of *Ononis natrix* growing in Tunisia.
- The *O. natrix* leaves extracts showed higher radical scavengers and lipid peroxidation inhibitory activities.
- These extracts possess an inhibitory activity towards angiotensin I converting enzyme and exerted strong antimicrobial activity.
- The *O. natrix* extracts could be a useful as natural food preservatives.

About Authors



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