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Original Research Article

Antioxidant and antibacterial activity of extract and phases from stems of *Spartium junceum* L. growing in Algeria

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Abstract

This work aimed to evaluate the antioxidant and antibacterial activities of the hydroalcoholic (80% methanol) extract and *n*-hexane (*n*-Hex), chloroform (Chl), ethyl acetate (EtAc), and *n*-butanol (*n*-But) phases from *Spartium junceum* L. stems collected in Algeria. Preliminary phytochemical investigations on phenolic compounds have been carried out.

The total phenolic content, spectrophotometrically determined, ranged from 71.8095 ± 3.7136 mg GAE/g (extract) to 0.0582 ± 0.0106 mg GAE/g (*n*-Hex). By HPLC-PDA analysis flavonoids (flavone derivatives), p-hydroxybenzoic acid, p-hydroxycinnamic acid, and cinnamic acid derivatives were identified both in the extract and phases.

S. junceum extract showed a noticeable free radical scavenging effect in the DPPH test (IC_{50} = 0.6833 ± 0.0240 mg/mL), mild reducing power, and strong chelating activity (IC_{50} = 0.2292 ± 0.0138 mg/mL). Among the phases, *n*-But displayed the best effect both in the DPPH test and reducing power assay, whereas *n*-Hex resulted the most active in the ferrous ions chelating activity assay. A positive relationship between DPPH radical scavenging activity and total phenolic content was found. Both the extract and phases exhibited antimicrobial activity against Gram-positive bacteria only. *Staphylococcus aureus* ATCC 6538 was the most susceptible strain (MIC range: 15.60-250.00 µg/mL), and the Chl phase showed the greatest efficacy. *S. junceum* extract resulted non-toxic against *Artemia salina*.

The obtained results demonstrate the potential of *S. junceum* stems as safe sources of natural antioxidant and antimicrobial compounds.

Keywords: *Spartium junceum* L., Phenolic compounds, Antioxidant activity, Antibacterial activity.

Introduction

Spartium junceum L. (syn. *Genista juncea* L. Scop.), known as Spanish broom or Weaver's broom, is a plant belonging to the Fabaceae family. It is the sole species included in the genus *Spartium*, but is closely related to the other brooms in the genera *Cytisus* and *Genista*. It is a perennial, leguminous shrub native to the Mediterranean region in Southern Europe, Southwest Asia and Northwest Africa and cultivated as an ornamental plant [1]. The plant typically grows up to 3 m (or more in cultivation), with main stems up to 5 cm thick, rarely 10 cm. Leaves are sparse, 1 to 3 cm long and up to 5 mm broad, oblong-linear to lanceolate, glabrous above,

appressed-sericeous beneath. The yellow flowers, sweet-scented, are arranged in terminal racemes [2].

S. junceum has been used as a traditional medicine in some countries such as Iran, Turkey, and Syria [3]. The flowers are components of the herbal tea known as "Zahraa", widely consumed in Syria after meals as a digestive and to promote good health [4]. A decoction of the whole plant is utilized in Northern Peru for the treatment of arthritis and bone pain [5]. In Italy, a plaster of *S. junceum* bark tied tightly with the stems is utilized in veterinary medicine for limbs fractures; moreover, the latex of young stems without flowers is used as keratolytic to remove warts [6,7].

Several studies focused on the biological properties of *S. junceum* flowers; it has been shown that they possess mild sedative, diuretic, antiulcer, anti-inflammatory, analgesic and antitumour activities [3,8,9]. The potent antioxidant activity of flavonoid-rich fractions obtained from *S. junceum* flowers has been also reported [10]. To the best of our knowledge there are no reports about the phytochemical composition and the biological activities of the stems of this plant.

This work aimed to evaluate the antioxidant and antibacterial activities of the hydroalcoholic extract of *S. junceum* stems collected in Algeria; furthermore, preliminary phytochemical investigations on phenolic compounds have been carried out. In order to establish the correlation between the observed activity and the phenolic content, the crude extract was successively partitioned with solvents of increasing polarity, and the obtained phases were subjected to the same investigations.

Material and Methods

Chemicals and Reagents

LC-MS grade water (H_2O), acetonitrile (ACN), cinnamic acid and naringin were obtained from Sigma-Aldrich/Supelco (Milan, Italy). LC-MS grade acetic acid was attained from Riedel-de Haën (Seelze, Germany). Folin-Ciocalteau reagent and sodium carbonate were purchased from Merck chemicals; methanol from Baker Analysed Reagent; $FeCl_2$ was obtained from Carlo Erba (Milan, Italy). Müeller Hinton Broth (MHB) was supplied from Oxoid (Basingstoke, UK). Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Plant material

The stems of *S. junceum* were collected in June 2011 at Constantine (Algeria) and identified by Prof Samir Benayache, Laboratoire de phytochimie et analyses physico-chimiques et biologiques, Université Mentouri, Constantine, Algérie.

The dried stems (1.5 Kg) were powdered and subjected at maceration in 80% methanol at room temperature for 5 days. After filtration, the extractive solution was evaporated to dryness by rotary evaporator (40 °C). The extract was suspended in distilled water and then partitioned between *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The yield of the extract, referred to 100 g of dried stems, was 17.44%. The yields of the phases, referred to 100 g extract (dw), were 0.45%, 2.05%, 1.02%, and 12.64% for *n*-hexane (*n*-Hex), chloroform (Chl), ethyl acetate (EtAc), and *n*-butanol (*n*-But), respectively.

Phytochemical investigations

Determination of total phenolic content

The total phenolic content of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases was determined by Folin-Ciocalteau method, referring to calibration curve of gallic acid, phenol compound used as a standard [11]. Briefly, 100 μL of each sample solution were mixed with 0.2 mL Folin-Ciocalteu reagent, 2 mL of H_2O, and 1 mL of 15% Na_2CO_3, and the absorbance was measured at 765 nm, after 2 h incubation at room temperature, with a model UV-1601 spectrophotometer (Shimadzu, Milan, Italy). The total polyphenols were estimated as gallic acid equivalent (GAE) and expressed in mg GAE/g extract (dw) ± standard deviation (SD). The data were obtained from the average of three independent determinations.

Identification of phenolic compounds by HPLC-PDA analysis

HPLC-PDA analyses were performed on a LC system (Shimadzu, Milan, Italy) equipped with 2 LC-10AD$_{VP}$ pumps, a CTO-20AC column oven, a SCL-10A$_{VP}$ system controller, and SPD-M10A$_{VP}$ photo diode array (PDA) detection. Data acquisition was performed by Shimadzu LabSolution Software ver. 1.12. For chromatographic separations, an Ascentis Express C18 column (15 cm x 4.6 mm I.D.) packed with 2.7 μm partially porous particles, was employed (Supelco, Bellefonte, PA, USA). The injection volume was 2 μL, and the mobile phase consisted of water/acetic acid (0.075 %) at pH=3 (solvent A) and ACN/acetic acid (0.075 %) (solvent B), respectively in the following linear gradient mode: 0 min, 0% B; 60 min, 40% B; 80 min, 100% B; 81 min, 0% B. The mobile phase flow rate was 1.0 mL/min. PDA wavelength range was 190-400 nm and the chromatograms were extracted at 254 nm (sampling frequency: 6.25 Hz, time constant: 0.32 s). *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases (10 mg) were dissolved in 1 mL of chloroform, methanol or methanol:methyl tertiary-butyl ether (MTBE) (4:1 *v/v*) and filtered through a 0.45 μm membrane filters (Whatman, Clifton, USA).

Antioxidant activity

Free Radical Scavenging Activity

Free radical scavenging activity of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases has been evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) test [12]. The extract was tested at different concentrations (0.0625-1.5 mg/mL); the phases were

tested at concentrations corresponding to 0.0625-1.5 mg/mL extract, calculated on the basis of the fractionation yield. An aliquot (0.5 mL) of each sample solution was added to 3 mL of daily prepared methanol DPPH solution (0.1 mM). The optical density change at 517 nm was measured, 20 min after the initial mixing, with a model UV-1601 spectrophotometer (Shimadzu). Butylated Hydroxytoluene (BHT) was used as reference. The scavenging activity was measured as the decrease in absorbance of the samples *versus* DPPH standard solution. Results were expressed as radical scavenging activity percentage (%) of the DPPH, defined by the formula $[(A_o - A_c)/A_o]x100$, where A_o is the absorbance of the control and A_c is the absorbance in the presence of the sample or standard. The results were obtained from the average of three independent experiments and are reported as mean percentage (%) ± SD. The results were also expressed as mean 50% Inhibitory Concentration (IC_{50}) ± SD, determined graphically by interpolation of the dose-response curve.

Reducing Power Assay

The reducing power of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases was determined according to the method of Oyaizu [13]. Different amounts of the extract (0.0625-1.5 mg/mL) or of each phase (concentrations corresponding to 0.0625-1.5 mg/mL extract) in 1 mL solvent were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrycyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min. The resulting solution was cooled rapidly, mixed with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The resulting supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% fresh $FeCl_3$, and 10 min after the absorbance was measured spectrophotometrically at 700 nm; increased absorbance of the reaction mixture indicated increased reducing power. As blank, an equal volume (1 mL) of water was mixed with a solution prepared as described above. BHT was used as reference standard. The results were obtained from the average of three independent experiments and are expressed as mean absorbance values ± SD.

Ferrous ions (Fe^{2+}) chelating activity

The Fe^{2+} chelating activity of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases was estimated by the method of Decker and Welch [14]. Different concentrations of extract (0.0625-1.5 mg/mL) and phases (dose corresponding to 0.0625-1.5 mg/mL extract), in 1 mL solvent, were mixed with 0.5 mL of distilled water and 0.05 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 0.1 mL of 5 mM ferrozine. Then the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The control contains $FeCl_2$ and ferrozine, complex formation molecules. Ethylenediaminetetraacetic acid (EDTA) was used as reference standard. The percentage of inhibition of the ferrozine-(Fe^{2+}) complex formation was calculated by the formula $[(A_o - A_c)/A_o] \times 100$, where A_o is the absorbance of the control and A_c is the absorbance in the presence of the sample or standard. The results were obtained from the average of three independent experiments and are expressed as mean percentage (%) ± SD and as mean IC_{50} ± SD.

Antimicrobial Activity

Microbial strains and culture conditions

The antimicrobial activity of *S. junceum* methanol extract, *n*-Hex, Chl, EtAc, and *n*-But phases was investigated against a representative range of standard and clinical microbial strains. The microorganisms used for antimicrobial sensitivity testing included six standard strains of Gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Enterococcus durans* ED010 (from the Faculty of Pharmacy library) *Enterococcus hirae* ATCC 10541, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 49134; seven standard strains of Gram-negative bacteria: *Enterobacter cloacae* EC02 (from the Faculty of Pharmacy library), *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 10536, *Proteus mirabilis* PM02 (from the Faculty of Pharmacy library), *Serratia marcescens* ATCC 19980, *Salmonella tiphy* ATCC 13311 and *Klebsiella pneumoniae* KP01 (from the Faculty of Pharmacy library); 5 clinical isolates of *S. aureus* obtained from specimens of skin infections and surgical infections. Bacteria were grown in MHB at 37 °C for 18-24 hours.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration values (MIC_s) were determined using the in broth micro dilution method according to the standard protocols previously reported [15]. Tests were performed in 2x MHB for antimicrobial activity. Cultures of each strain were prepared overnight in appropriate growth medium; microorganism suspensions were therefore adjusted with sterile medium to give approximately $1x10^6$ CFU/mL. Prior to analysis, all extracts were weighed (2 mg) and then dissolved in DMSO (1%) and MHB to obtain a final concentration of 2 mg/mL. Two-fold serial dilutions were prepared in a 96-well plate. Positive and negative controls were also included. The MIC was defined as the lowest concentration (mg/mL) of extract which completely inhibit the visible growth of microorganisms in broth.

Artemia salina Leach lethality bioassay

The *Artemia salina* Leach (brine shrimp) lethality bioassay was employed to predict the toxic of *S. junceum* extract. Medium lethal concentration (LC_{50}) determination was carried according to the method of Meyer et al. [16]. Each sample, opportunely dissolved and then diluted in artificial seawater, was tested at the final concentrations of 10, 100, 500 and 1000 µg/mL prepared in single vials. Ten brine shrimp larvae, taken 48 h after initiation of hatching in artificial seawater, were transferred to each sample vial, and artificial seawater was added to obtain a final volume of 5 mL. After

24 h of incubation at 25-28 °C, the vials were observed using a magnifying glass, and surviving larvae were counted. The assay was carried out in triplicate, and LC_{50} was determined using the *probit* analysis method. Extracts are considered non-toxic when all brine shrimp larvae survive at a concentration of 1000 µg/mL.

Results & Discussion

Phytochemical investigations

Determination of total phenolic content

The Folin-Ciocalteau assay is one of the oldest method developed to determine the content of total phenols [17]. Numerous examples of the application of this assay to characterize natural products may be found in the literature. In most cases, total phenolics quantified by this method are correlated with the antioxidant capacities confirming the value of the Folin-Ciocalteau test [18]. In this work, the total polyphenol content of *S. junceum* stems hydroalcoholic extract, *n*-Hex, Chl, EtAc, and *n*-But phases was determined. A linear calibration curve of gallic acid, in the range 125-500 µg/ml with r^2 value of 0.998, was constructed. The total phenolic content decreases in the following order: *S. junceum* extract > *n*-But > Chl > EtAc > *n*-Hex (Table 1).

Table 1. Total phenolic content, Free radical scavenging activity (DPPH test), and Fe^{2+} chelating activity of *S. junceum*.

	Total Phenolics (mg GAE/g)	**DPPH test** IC_{50} (mg/mL)	**Fe^{2+} chelating activity** IC_{50} (mg/mL)
S. junceum extract	71.8095 ± 3.7136	0.6833 ± 0.0240	0.2292 ± 0.0138
n-Hex	0.0582 ± 0.0106	nd	1.9938 ± 0.0854
Chl	2.1192 ± 0.0457	13.0052 ± 1.0649	nd
EtAc	1.7115 ± 0.0181	nd	nd
n-But	14.6252 ± 0.6595	2.1223 ± 0.1559	nd
Standard		0.0966 ± 0.0063[a]	0.0064 ± 0.0001[b]

The results were obtained from the average of three independent experiments and are expressed as the mean ± SD.
n-Hex: *n*-hexane, Chl: chloroform, EtAc: ethyl acetate, and *n*-But: *n*-butanol. nd: not determined. Standard: [a]BHT, [b]EDTA.

Identification of phenolic compounds by HPLC-PDA analysis

The HPLC-PDA analysis of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases revealed the presence of various polyphenolic compounds, belonging to different chemical classes *viz.* p-hydroxybenzoic acid, cinnamic acid, flavones and flavanone derivatives (Figure 1a, b, c). Cinnamic acid and naringin were positively identified in the Chl and EtAc phase, respectively, thanks to the match with elution order and reference material data.

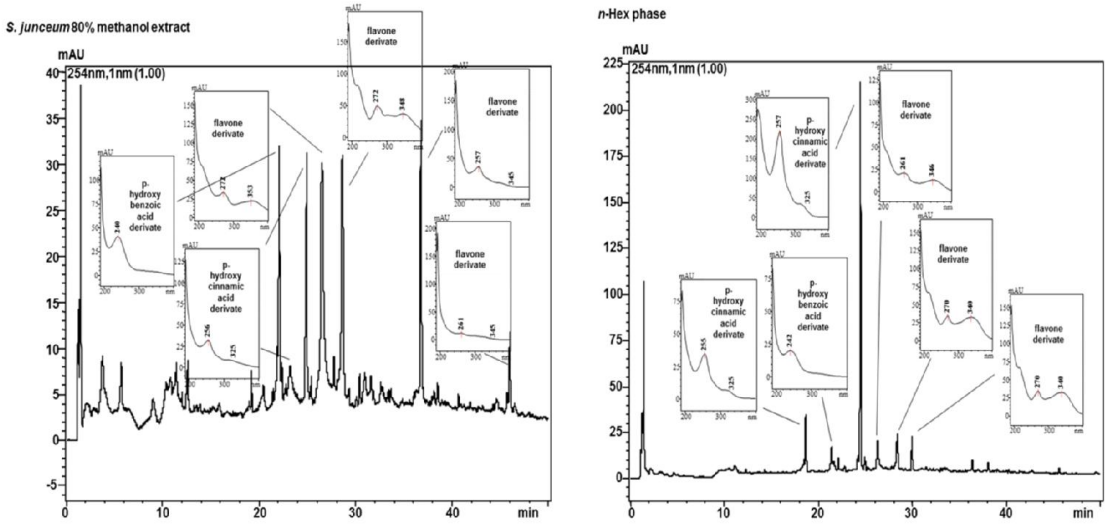


Figure 1.a. HPLC-PDA chromatograms (254 nm) of *S. junceum* 80% methanol extract and *n*-hexane (*n*-Hex) phase.

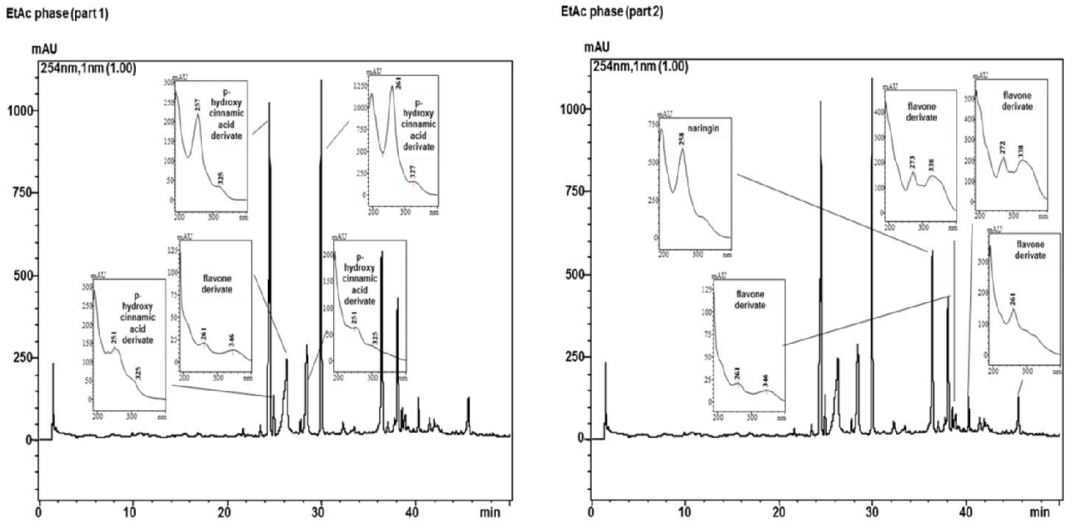


Figure 1.b. HPLC-PDA chromatograms (254 nm) of *S. junceum* ethyl acetate (EtAc) phase.

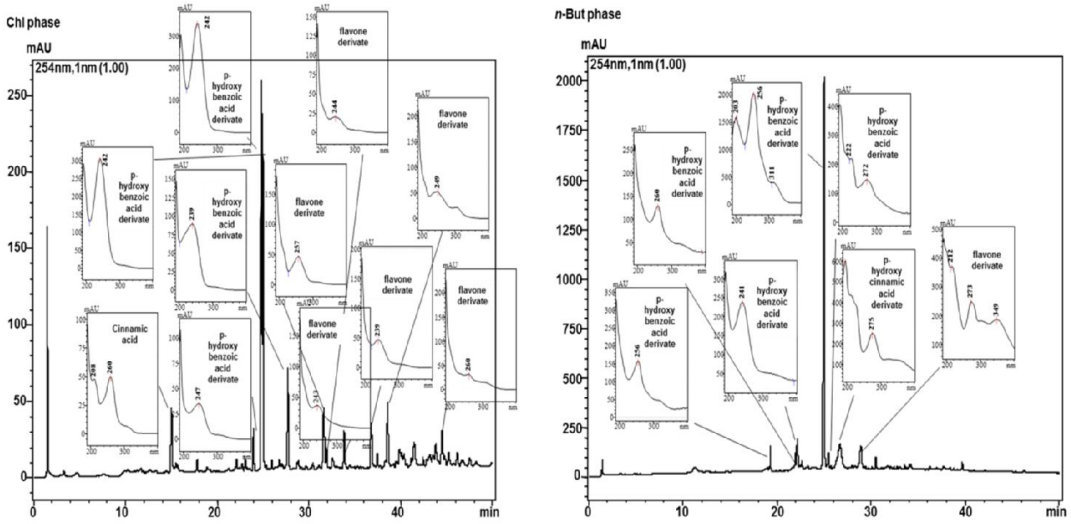


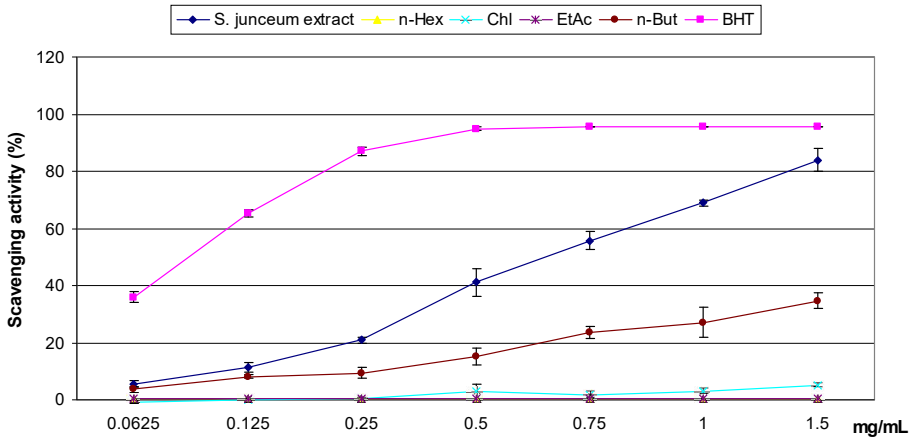
Figure 1.c. HPLC-PDA chromatograms (254 nm) of *S. junceum* chloroform (Chl) and *n*-butanol (*n*-But) phases.

Antioxidant activity

The interest in natural antioxidants has increased considerably in recent years because many antioxidants exhibit beneficial biological effects, including antibacterial, antiviral, antiallergic, antithrombotic and because they are linked to lower incidence of cardiovascular disease and certain types of cancer disease [19]. The commercial development of plants as sources of antioxidants that can be used to enhance the properties of foods, for both nutritional purposes and preservation, is currently of major interest [20]. Antioxidant activity occurs by different mechanisms, such as prevention of chain initiation, reducing capacity, radical scavenging and decomposition of peroxides. Therefore, the use of a variety of methods for the determination of antioxidant capacity of plant derived phytocomplexes or isolated compounds is suggested [21]. In order to evaluate the *in vitro* antioxidant effectiveness of *S. junceum* extract and phases, three tests based on different approaches and mechanisms were utilized. DPPH is a stable free radical which presents a deep purple colour and a strong absorption band in the range of 515-520 nm. In the presence of antioxidant compounds, DPPH can accept an electron or a hydrogen atom from the antioxidant scavenger molecule to be converted to a more stable molecule. As the reduced form of DPPH is pale yellow, it is possible to determine the antioxidant activity by studying the change of colour spectrophotometrically. The greater the free radical scavenging capacity of an antioxidant compound, the more reduction of DPPH and the less purple colour there is in the sample, and this is reflected in a lower IC_{50} value [22].*S. junceum* extract showed a noticeable effect on scavenging free radicals, dose-dependent, resulting close to that of the standard BHT at the highest tested concentration (1.5 mg/mL). Concerning the phases, *n*-But displayed a moderate effect, Chl showed a very scarce activity, whereas EtAc and *n*-Hex did not show any free radical scavenging ability (Figure 2). Based on IC_{50} values, the scavenging effect of extract, active phases and BHT on DPPH radical decreases in the order: BHT > *S. junceum* extract > *n*-But > Chl (Table 1). Linear regression analysis revealed a positive relationship between DPPH radical scavenging activity and total phenolic content ($r^2 = 0.5144$). Different studies have indicated that the electron donation capacity, reflecting the reducing power, of bioactive compounds is associated with antioxidant activity [23]. The reducing power assay measures the ability of single compounds as well as plant extracts to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion through the donation of an electron; the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of the antioxidant sample. By monitoring spectrophotometrically at 700 nm, an increase in absorbance of the reaction mixture would indicate an increase in reducing capacity. Such an assay may indicate just how easily a given antioxidant donates electrons to reactive free radicals species, thus promoting the termination of free radical chain reactions [24]. The results of the reducing power assay showed that *S. junceum* extract and *n*-But phase possess mild activity, as compared with the standard BHT, which increases in a dose dependent manner; the other phases did not display any activity (Figure 3).The Fe^{2+} chelating properties of *S. junceum* extract, *n*-Hex, Chl, EtAc, and *n*-But phases were estimated by the method of Decker and Welch [14], with slight modifications. Ferrozine can quantitatively form complex with Fe2+. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. S. junceum extract displayed very good Fe^{2+} chelating activity, if compared to the standard EDTA; nonetheless, it does not appear to be dose-dependent. Among the phases, only the *n*-Hex showed moderate effect, whereas the other phases did not display any activity, as highlighted by the IC_{50} values, too (Figure 4, Table 1). No correlation between total phenolic content and chelating activity was

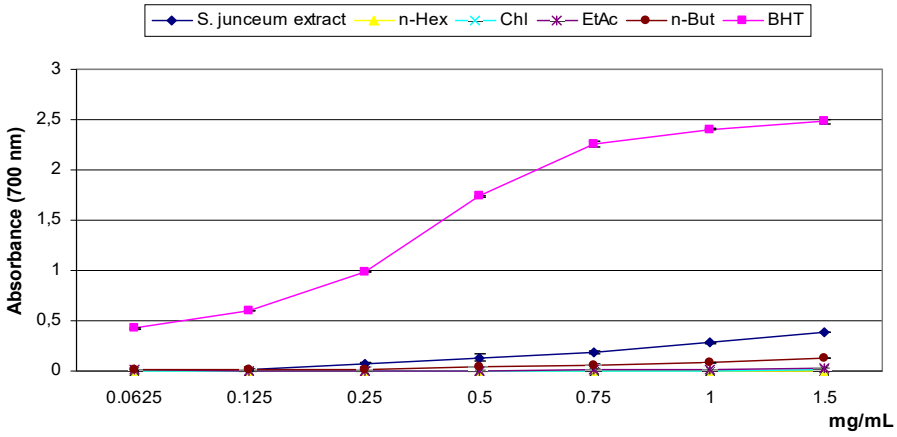
found. Polyphenols can be considered as the most abundant plant secondary metabolites with highly diversified structures, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. It has been proposed that polyphenols can act as antioxidants by a number of potential mechanisms; the free radical scavenging, in which these compounds can break the free radical chain reaction, as well as suppression of the free radical formation by regulation of enzyme activity or chelating metal ions involved in free radical production are reported to be the most important mechanisms of their antioxidant activity [25]. Flavonoids have powerful antioxidant activities *in vitro*, being able to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide $O_2^{\bullet-}$, hydroxyl radical $OH^{\bullet}$, peroxyl radicals $RO_2^{\bullet}$, hypochlorous acid (HOCl), and peroxynitrous acid (ONOOH). Flavonoids can also chelate metal ions, often decreasing the pro-oxidant activity of metal ions [26]. On the basis of the obtained results it could be hypothesized that the radical scavenging properties of *S. junceum* stems hydroalcoholic extract are related, almost in part, to the phenolic compounds. By contrast, the strong chelating properties do not appear to depend on this class of compounds; thus, the chelating ability may be ascribed to other phytochemicals which are extracted together with polyphenols. Bioassay-guided fractionation procedure will be performed to characterize the active constituents responsible for the chelating activity of the extract.

Figure 2. Free radical scavenging activity of *S. junceum* extract and phases.



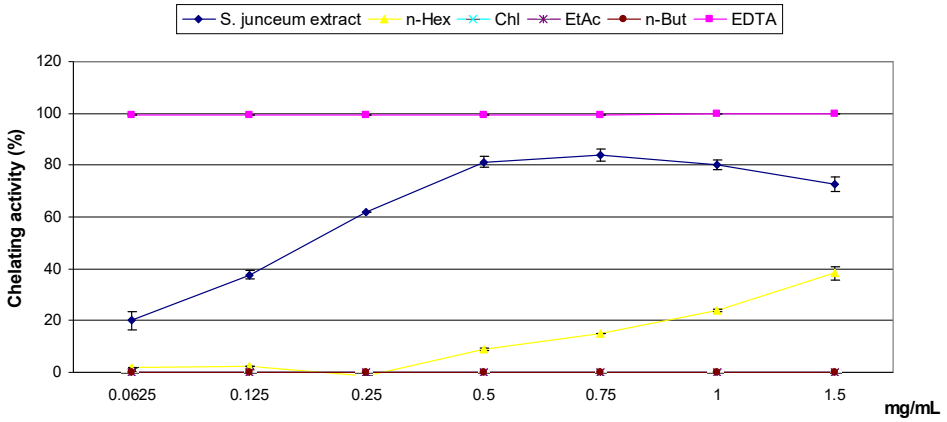
The results were obtained from the average of three independent experiments and are expressed as the mean percentage (%) ± SD. *n*-Hex: *n*-hexane, Chl: chloroform, EtAc: ethyl acetate, and *n*-But: *n*-butanol.

Figure 3. Reducing power of *S. junceum* extract and phases.



The results were obtained from the average of three independent experiments and are expressed as the mean absorbance ± SD. *n*-Hex: *n*-hexane, Chl: chloroform, EtAc: ethyl acetate, and *n*-But: *n*-butanol.

Figure 4. Chelating activity of *S. junceum* extract and phases.



The results were obtained from the average of three independent experiments and are expressed as the mean percentage (%) ± SD. *n*-Hex: *n*-hexane, Chl: chloroform, EtAc: ethyl acetate, and *n*-But: *n*-butanol.

Antimicrobial activity

The results obtained from the antimicrobial tests indicated that both *S. junceum* extract and phases display antimicrobial efficacy against Gram-positive bacteria, whereas they were not active against the Gram-negative. The MIC values of extract and phases for sensitive Gram-positive bacteria are reported in Table 2; *S. junceum* extract and Chl phase showed greater efficacy than the other phases. *S. aureus* ATCC 6538 was the most sensitive strain to the hydroalcoholic extract (MIC: 125 µg/mL), followed by *S. epidermidis* (MIC: 1000 µg/mL). The greatest antimicrobial activity was highlighted for Chl phase; *S. aureus* ATCC 6538 was the most susceptible strain (MIC: 15.60 µg/mL), followed by *L. monocytogenes* (MIC: 31.25 µg/mL), and clinical strains of *S. aureus* (MIC range: 500-125 µg/mL).

Polyphenols are known to be synthesized by plants in response to microbial infection; thus it should not be surprising that they have been found *in vitro* to be effective antimicrobials against a wide array of microorganisms. Flavonoid-rich plant extracts from different species have been reported to possess antimicrobial activity [27]. Despite of this, the antibacterial activity of *S. junceum* stems extract seems not to be related to its phenolic content. In fact the Chl phase, which exhibited the best activity, contains a very low amount of phenolic compounds. Thus, the observed effect may depend on other classes of phytochemicals.

Table 2. The MIC values of *S. junceum* extract and phases for sensitive Gram-positive bacteria.

	MIC values (µg/mL)				
Gram positive bacteria	***S. junceum* extract**	**Chl**	**EtAc**	***n*-Hex**	***n*-But**
S. aureus ATCC 6538	125.00	15.60	62.50	250.00	250.00
S. epidermidis ATCC 49134	1000.00	na	na	na	na
L. monocytogenes ATCC 7644	na	31.25	na	na	na
Clinical isolates of *S. aureus* (5)	na	500.00-125.00*	na	na	na

*MIC range

n-Hex: *n*-hexane, Chl: chloroform, EtAc: ethyl acetate, and *n*-But: *n*-butanol; na: no activity.

Artemia salina Leach lethality bioassay

For the preliminary assessment of the toxicity of *S. junceum* extract towards *Artemia salina* (brine shrimps) larvae, LC_{50} value was determined. The extract did not display any toxicity against brine shrimps; in fact a LC_{50} value higher than 1000 µg/mL was found. Haugh et al. reported that, from a pharmaceutical point of view, it is an advantage when antibacterial drugs have no effect on eukaryotic cells [28].

Conclusion

In the current work we report the study of the antioxidant and antibacterial properties of the hydroalcoholic extract and phases obtained from *S. junceum* stems collected in Algeria.

S. junceum extract displayed good antioxidant activity both in DPPH test and in chelating activity assay. The radical scavenging properties of *S. junceum* stems extract are related to the phenolic content, whereas the chelating ability seems to depend on other phytochemicals.

S. junceum extract showed antimicrobial efficacy against Gram-positive bacteria, and *S. aureus* ATCC 6538 was the most sensitive strain; anyway, the observed effect does not appear to be linked to

the phenolic content of the extract, but more probably to other secondary metabolites. Finally, the extract resulted non-toxic against *A. salina*.

In conclusion, on the basis of the results of this investigation, the stems of *S. junceum* collected in Algeria could be considered as promising candidate from which relatively safe antioxidant and antimicrobial constituents might be obtained.

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References

[1]. Millspaugh CF. American Medicinal Plants. General publishing Co. Toronto;1974.

[2]. Heywood VH. *Spartium* L.. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, editors. Flora Europea. Vol. II. Cambridge at the University Press; 1968. p. 101.

[3]. Yesilada E, Takaishi Y, Fujita T, Sezik E. Anti-ulcerogenic effects of *Spartium junceum* flowers on in vivo test models in rats. J Ethnopharmacol. 2000;70(3):219-226.

[4]. Carmona MD, Llorach R, Obon C, Rivera D. Zahra, a Unani multicomponent herbal tea widely consumed in Syria: components of drug mixtures and alleged medicinal properties. J Ethnopharmacol. 2005;102:344-350.

[5]. Bussmann RW, Glenn A. Fighting pain: Traditional Peruvian remedies for the treatment of asthma, rheumatism, arthritis and sore bones. Indian J Tradit Know. 2011;10(3):397-412.

[6]. Guarrera PM, Salerno G, Caneva G. Folk phytotherapeutical plants from Maratea area (Basilicata, Italy). J Ethnopharmacol. 2005;99(3):367-378.

[7]. Scherrer AM, Motti R, Weckerle CS. Traditional plant use in the areas of Monte Vesole and Ascea, Cilento National Park (Campania, Southern Italy). J Ethnopharmacol. 2005;97:129-143.

[8]. Mosaddegh MH, Ghasemi N, Mosaddegh A, Hejazian SH. Antinoniceptive effects of *Spartium junceum* L. extract on mouse formaline test. World Appl Sci J. 2008;3(2):223-226.

[9]. Cerchiara T, Blaiotta G, Straface VS, Belsito E, Liguori A, Luppi B, Bigucci F, Chidichimo G. Biological activity of *Spartium junceum* L. (Fabaceae) aromatic water. Nat Resour. 2013;4:229-234.

[10]. Yesilada E, Tsuchiya K, Takaishi Y, Kawazoe K. Isolation and characterization of free radical scavenging flavonoid glycosides from the flowers of *Spartium junceum* by activity-guided fractionation. J Ethnopharmacol. 2000;73:471-478.

[11]. Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V. Changes in antioxidant effects and their relationship to phytonutrients in fruit of the sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agric Food Chem. 2000;48(5):1485-1490.

[12]. Ohnishi M, Morishita H, Iwahashi H, Shitzuo T, Yoshiaki S, Kimura M, Kido R. Inhibitory effects of chlorogenic acids on linoleic acid peroxydation and haemolysis. Phytochemistry. 1994;36(3):579-583.

[13]. Oyaizu M. Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. Jpn J Nutr. 1986;44(6):307-315.

[14]. Decker EA, Welch B. Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem. 1990;38:674-677.

[15]. Bisignano C, Filocamo A, Ginestra G, Giofrè SV, Navarra M, Romeo R, Mandalari G. 3,4-DHPEA-EA form *Olea europea* L. is effective against standard and clinical isolates of *Staphylococcus* sp.. Ann Clin Microbiol Antimicrob. 2014;13(24):1-4.

[16]. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-34.

[17]. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol. 1999;299:152-178.

[18]. Roginsky V, Lissi EA. Review of methods to determine chainbreaking antioxidant activity in food. Food Chem. 2005;92:235-254.

[19]. Nilsson J, Pillai D, Önning G, Persson C, Nilsson Å, Åkesson B. Comparison of the ABTS and FRAP methods to assess the total antioxidant capacity in extracts of fruit and vegetables. Mol Nutr Food Res. 2005;49:239-246.

[20]. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci - Reviews. 1997;2(4):152-159.

[21]. Salazar R, Pozos ME, Cordero P, Pérez J, Salinas MC, Waksman N. Determination of the antioxidant activity of plants from Northeast Mexico. Pharm Biol. 2008;46:166-170.

[22]. Taviano MF, Marino A, Trovato A, De Pasquale R, Miceli N. Antioxidant and antimicrobial activities of berries extracts of five *Juniperus* species under *Juniperus* Section from Turkey. In: Hai-Xue Kuang editor. Phytochemicals: Occurrence in Nature, Health Effects and Antioxidant Properties. Nova Science Publishers, Inc. New York; 2013. p. 55-68.

[23]. Taviano MF, Marino A, Trovato A, Bellinghieri V, La Barbera TM, Güvenç A, Hürkul MM, De Pasquale R, Miceli N. Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. Pharm Biol. 2011;49(1):1014-1022.

[24]. Lue B-M, Skall Nielsen N, Jacobsen C, Hellgren L, Guo Z, Xu X. Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays. Food Chem. 2010;123(2):221-230.

[25]. Vladimir-Knežević S, Blažeković B, Štefan MB, Babac M. Plant polyphenols as antioxidants influencing the human health. In: Rao V, editor. Phytochemicals as nutraceuticals - Global approaches to their role in nutrition and health. InTech Publisher; 2012. p. 155-180.

[26]. Halliwel B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? Arch Biochem Biophys. 2008;476(2):107-112.

[27]. Barreca D, Bellocco E, Laganà G, Ginestra G, Bisignano C. Biochemical and antimicrobial activity of phloretin and its glycosilated derivatives present in apple and kumquat. Food Chem. 2014;160:292-297.

[28]. Haug T, Stensvåg K, Olsen ØM, Sandsdalen E, Styrvold OB. Antibacterial activities in various tissues of the horse mussel, *Modiolus modiolus*. J Invertebr Pathol. 2004;85(2):112-119.