Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1126–1134
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts

El Akrem Hayouni [a,*], Manaf Abedrabba [b], Marielle Bouix [c], Moktar Hamdi [a]

[a] *Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de la Technologie (INSAT), B.P. 676, 1080 Tunis, Tunisia*
[b] *Unité de Recherche, Physico-chimie et Moléculaire, IPEST, La Marsa, Tunisia*
[c] *Laboratoire de Microbiologie Industrielle, ENSIA, 1 avenue des Olympiades, F-91744 Massy cedex, France*

Received 8 October 2006; received in revised form 10 December 2006; accepted 14 February 2007

Abstract

The effects of different extracting solvents, used in two extraction methods, on the total polyphenol contents of *Quercus coccifera* L. and *Juniperus phoenicea* L. fruits were studied. The antioxidant and antibacterial activities of these extracts were evaluated. Water and organic solvents, used individually, such as acetone and chloroform, or in mixtures: acetone/water/acetic acid (90/9.5/0.5) and ethyl acetate/methanol/water (60/30/10), significantly affected total polyphenol content. The antioxidant activity of these extracts was investigated by the widely used $ABTS^{\cdot+}$ method and the β-carotene bleaching test. The results showed that solvents with different polarities had significant effects on antioxidant activity. A few of these extracts are as strong as some common synthetic antioxidants. Extracts were also tested for their antibacterial effects on spoilage and pathogenic bacteria. Results showed that acetone/water/acetic acid extracts exhibited the highest antibacterial activities against all tested germs. It is also noteworthy that the Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, were inhibited more easily than were the Gram-negative ones. Since a strong correlation between polyphenol contents and biological activities was noted (correlation ranging between $R^2 = 0.76$ and $R^2 = 0.84$), we suggest the use of these extracts to prevent the deterioration of stored foods by bacteria, if any resulting organoleptic effects are acceptable.

Keywords: Polyphenols; $ABTS^{\cdot+}$ method; BCB test; Antioxidant; Antibacterial

1. Introduction

Interest in the phytochemical content of fruits vegetables and grains has increased due to consumer awareness of its various health and nutraceutical benefits. There is intense interest in plant polyphenols, as witnessed by numerous papers devoted to various aspects of these compounds (Tura & Roberts, 2002; Yanishlieva & Marinova, 2001). The use of plants and herbs as antioxidants in processed food is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (Madsen & Bertelsen, 1995). Plant antioxidants tend to be water-soluble, because they frequently appear combined as glycosides and they are located in the cell vacuole (Harborne, 1998). These compounds, among the most common groups of photochemicals, are of considerable physiological and morphological importance to plants. Phenolics are antioxidants with redox properties, allowing them to

Abbreviations: $ABTS^{\cdot+}$, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); ANOVA, Analysis of variance; BCB, β-carotene bleaching method; CFU, colony forming unit; GAE, gallic acid equivalent; NA, nutrient agar; PBS, phosphate buffer saline; Vit-C, Vitamin C (ascorbic acid).

* Corresponding author. Tel.: +21 697471160; fax: +21 672660300.
E-mail address: Akrem.Hayouni@isetzg.rnu.tn (E.A. Hayouni).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.02.010

act as reducing agents, hydrogen donators, and singlet and triplet oxygen quenchers (Pietta, 2000). They also have metal chelation properties (Kähkönen et al., 1999). Interesting new findings, regarding their biological activities, provide the basis for the present rapidly increasing interest in the use of natural antioxidants as functional food ingredients and/or food supplements. For example, Yanishlieva and Marinova (2001) have reviewed the use of phenolic compounds from plant sources as natural antioxidants in a number of edible oils, such as corn, cottonseed, fish, olive, peanut, rapeseed, soybean and sunflower oil. Accordingly, research to identify antioxidative compounds is an important issue, although it remains unclear which of the compounds in medical plants are the active ones. Even though a variety of herbs and plants are known to be sources of phenolic compounds, studies aimed at isolating polyphenols, and evaluating their antioxidative and antimicrobial effects, have rarely been carried out.

Spoilage of food, due to the presence of bacterial and fungal infection, has been a major concern for decades, and causes a considerable economic loss, worldwide. The demand for non-toxic, natural preservatives has been rising with increased awareness and reports of the ill-effects of synthetic chemicals present in foods. Furthermore, emergence of food-borne pathogens has lately become a major public health concern (WHO, 2002). Many compounds in plants have been reported to be biologically active, antimicrobial, allopathic antioxidants and to have bioregulatory properties. Extracts from herbs and spices are the most common plant materials evaluated for antioxidant and antimicrobial use (Dorman & Deans, 2000). However, compared to herbs, spices, vegetables and beverages, there is limited research on the inhibitory effects of various extracts from plants (Harborne, Baxter, & Moss, 1999).

Different solvent systems have been used for the extraction of polyphenols from plant material (Pinelo, Rubilar, Sineiro, & Nunez, 2004). Extraction yield is dependent on the solvent and the method of extraction (Goli, Barzegar, & Sahari, 2004). The extraction method must allow complete extraction of the compounds of interest, and it must avoid their chemical modification (Zuo, Chen, & Deng, 2002). Water, and aqueous mixtures of ethanol, methanol and acetone, are commonly used in plant extraction (Sun & Ho, 2005). Wang and Helliwell (2001) reported that aqueous ethanol was superior to methanol and acetone for extracting flavonoids from tea. However, in another work, water was found to be a better solvent, for extracting tea catechins, than were 80% methanol or 70% ethanol (Khokhar & Magnusdottí, 2002). Moreover, in the extraction of polyphenol, a single extraction compared to multiple extraction procedure is not sufficient (Goli et al., 2004).

Despite the medicinal potential of plants in Tunisia being considerable, knowledge of this area and studies on these plants remain scarce. The choice of our investigated plants is based on two criteria: first, that these plants have ethnopharmacological data indicating their utilization in folk medicine; second, that in this domain, there is no study in Tunisia which deals with the biological activities of various extracts of such evergreen sclerophyll species. Thus, the purpose of our study was to investigate the effects of different extracting solvents, employed in two different extraction procedures, on total polyphenols and antioxidants, as well as antibacterial capacity/activity. This evaluation, related to the total phenolic contents and biological activities, may help us to find new potential sources of natural additives (antioxidants, antibacterial agents).

2. Materials and methods

2.1. Plant materials

Fruits of *Quercus coccifera* L. (Fagaceae) and *Juniperus phoenicea* L. (Cupressaceae) were used in this study. Fruits were collected in November, 2005, from different places around the town of Thala in mid-west Tunisia at 1200 m altitude. Specimens were identified by Dr. Nadia Ben Salem at the Department of Botany, National Institute of Agronomic Research (INRAT, Tunis) and voucher specimens were deposited at the Herbarium of the Department of Botany in the cited institute.

2.2. Chemicals

All chemicals were purchased from Sigma (USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

2.3. Extraction of polyphenols

Fruits were ground to a fine powder with a grinder. Two methods were adopted to extract total polyphenols from these powders. In the first method (method #1), powders (100 g) were extracted in a Soxhlet extractor with hexane for 6 h at 65 °C to remove the fatty materials. Then, the defatted powder was divided into five fractions. Each fraction was separately re-extracted in a Soxhlet apparatus for 5 h with 250 ml of one of these solvents: absolute chloroform, absolute acetone; mixture 1; acetone:water:acetic acid (90:9.5:0.5), mixture 2; ethyl acetate:methanol:water (60:30:10), respectively. For water extraction, the fifth fraction was infused with 100 ml of freshly boiled distilled water for 10 min. The infusion was filtered through Whatman No. 1 paper and rapidly cooled under tap water. In the second extraction method (method #2), maceration of crude powder was carried out in a one-step extraction (batch mode). Ground fruits (25 g) were extracted at room temperature overnight, separately, with 250 ml of the same solvents as above without being defatted. This time, the extract was filtered through 0.45 μm filter paper. Infusions were prepared as above, but using non-defatted powder. All obtained organic extracts were concentrated by rotary evaporation under vacuum at 45 °C, using a HACH UV–Vis spectrophotometer (Model DR/4000, Colorado,

USA), to get crude extracts, whereas infusions were lyophilised.

2.4. Determination of total phenolic compounds by Folin–Ciocalteu method

The amount of total phenolics was determined with the Folin–Ciocalteu reagent using the method of Lister and Wilson (2001). This method was employed to evaluate the phenolic content of the samples. A calibration curve of gallic acid (ranging from 0.005 to 0.05 mg/ml) was prepared (in 80% of methanol), and the results, determined by the regression equation of the calibration curve ($y = 62.94x - 0.67$, $R^2 = 0.99$), were expressed as mg gallic acid equivalents per gram dry weight of raw material (mg GAE/ g dry weight). In this method, 100 μl of sample (diluted to obtain absorbance in the range of the prepared calibration curve) were dissolved in 500 μl (1/10 dilution) of the Folin–Ciocalteu reagent and 1 ml of distilled water.

The solutions were mixed and incubated at room temperature. After 1 min, 1.5 ml of 20% sodium carbonate (Na_2CO_3) solution were added. The final mixture was shaken thoroughly and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a HACH UV–Vis spectrophotometer.

2.5. Quantification of total antioxidant activity

2.5.1. ABTS assay

ABTS radical-scavenging activity of extracts was determined according to Re et al. (1999). In this test, we measured the relative capacity of antioxidants to scavenge the $ABTS^{\cdot+}$ radical compared to the antioxidant potency of L(+) ascorbic acid (vitamin C) used as a standard.

The $ABTS^{\cdot+}$ radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate ($K_2S_2O_8$) solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in the dark. Before usage, this solution was diluted to get an absorbance of 0.700 ± 0.020 at 734 nm with PBS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl). The spectrophotometer was first blanked with PBS. The final reaction mixture of standard group was made up to 1 ml with 950 μl of $ABTS^{\cdot+}$ solution and 50 μl of Vit-C. Similarly, in the test group, 1 ml of reaction mixture comprised 950 μl of $ABTS^{\cdot+}$ solution and 50 μl of the extract solution. The reaction mixture was vortexed for 10 s and its absorbance at 734 nm was recorded each minute after initial mixing. Appropriate solvent blanks were run in each assay, and all measurements were done within at least 6 min. The results, determined from regression equation of the calibration curve ($y = 0.0446x - 0.0076$, $R^2 = 0.987$), were expressed as mg ascorbic acid equivalents per gram dry weight of raw material (mg Vit-C/g dry weight).

2.5.2. β-Carotene bleaching test

The antioxidant activity of fruit extracts was evaluated according to a slightly modified version of the β-carotene bleaching method (Pratt, 1980). β-Carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween 40 (polyoxyethylene sorbitan monopalmitate) were mixed with 0.5 ml of chloroform. Chloroform was removed at 45 °C, under vacuum, using a rotary evaporator (Heidolph, Germany). The resulting mixture was immediately diluted with 10 ml of triple-distilled water and was mixed well for 1–2 min. The emulsion was further made up to 50 ml with oxygenated distilled water. Aliquots (4 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of test samples. BHA was used for comparative purposes. A control, containing 0.2 ml of corresponding solvent and 4 ml of the above emulsion, was prepared. The tubes were placed, at 50 °C, in a water bath. Absorbances of all the samples at 470 nm were taken at zero time ($t = 0$), measurement of absorbance was continued, until the colour of the β-carotene disappeared in the control reaction ($t = 180$ min), at 15 min intervals. A mixture prepared as above, without β-carotene, served as blank. All determinations were performed in triplicate. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula: $AA = \%\ \text{inhibition} = 100[1 - (A_0 - A_t)/A_0^\circ - A_t^\circ]$ where A_0 and A_0° are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A_t° are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

2.6. Determination of antibacterial effect

2.6.1. Bacterial strains

The extracts were individually tested against a panel of 8 spoilage and pathogenic bacteria including *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas morgani*, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella anatum*, *Klebsiella pneumoniae*, *Listeria monocytogenes*.

2.6.2. Disc-diffusion assay

The agar disc diffusion method was employed for the determination of antibacterial activities of the extracts, as recommended by NCCLS (1997). Briefly, a suspension of the tested bacteria (100 μl of suspension containing 10^8 CFU/ml) was spread on nutrient agar (NA). Filter paper discs (12.7 mm in diameter) were impregnated with 100 μl of 3 mg/ml extracts (300 μg/disc) and placed on the inoculated plates. Negative controls were prepared using the corresponding solvent. Streptomycin B (30 μg/disc) was used as a positive reference standard to determine the sensitivity of one strain/isolate in each bacterial specie tested. The inoculated plates were incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested bacteria. All tests

were performed in triplicate and experiments were repeated twice.

2.7. Statistical analysis

All data were expressed as means ± standard errors of triplicate measurements. One-way analysis of variance (ANOVA) was carried out to test any differences between the solvents used. Statistical comparisons between variables (e.g., yields, phenolic contents and antioxidant activity with method #1 and method #2) were performed with Student's *t*-test. Correlation between the antioxidant activity and total phenolic content was carried out using the correlation and regression in the EXEL programme. Differences were considered significant at $p \leqslant 0.05$.

3. Results and discussion

3.1. Extraction yields

Yields of different extracts obtained by both methods were examined and presented in Table 1. It is clear that the highest yields were recorded when extractions were performed in the one-step batch mode (method #2). Statistically, all the results were at least significant ($p \leqslant 0.05$), which means that there was a significant difference between yields obtained by each of the used methods from both plant materials. This remains true, no matter what the solvent used. The difference between extraction yields obtained by the two methods depended on the raw material analyzed. Variation in the yields of various extracts is attributed to polarities of different compounds present in the fruits and such differences have been reported in literature concerning fruit seeds (Jayaprakasha, Singh, & Sakariah, 2001). The highest yield in sequential extractions (method #1) was achieved by the polar solvents. For *J. phoenicea*, the order of the extracts, obtained by method #1, was: acetone > mixture 1 > mixture 2 > infusion > chloroform. When method #2 was used, the order completely changed and became as follows: mixture 1 > mixture 2 > chloroform > acetone > infusion. These results were the same when *Q. coccifera* fruits were used, except that, when method #2 was adopted, mixture 2 gave a higher yield than did mixture 1.

3.2. Amount of total phenolics

The amount of total phenolics varied in the different extracts and ranged from 45.0 to 201 mg GAE/g of dry material (Table 2). Researchers at "The Local Food-Nutraceuticals Consortium" (School of Pharmacy, University of London) found 103 mg g^{-1} ethanolic extract from *Quercus ilex* L. subsp. *Rotundifolia* (Lam.) fruits (The Local Food-Neutraceuticals Consortium, 2005). In another work, Djeridane et al. (2006) recorded 12.7 mg GAE/g dry weight of 70% aqueous ethanol extract from *Juniperus oxycedrus*. This extract was prepared using the one-step extraction method. The amount of total phenolic compounds, in all tested extracts, was higher than those of some Asian vegetables (Kaur & Kapoor, 2002).

The highest total phenolic levels in *J. phoenicea* were detected when mixture 1, water and acetone, was used for extraction in method #1 as well as in method #2. We can establish the order of all the extracts with highest value

Table 1
Extraction yield (EY) of solvent extracts, obtained by two different methods, from *J. phoenicea* L. and *Q. coccifera* L. fruits

		Water	Acetone	Chloroform	Mixture 1	Mixture 2
J. phoenicea	Method #1	6.32[a]	9.65[a]	4.14[a]	7.21[a]	6.88[a]
	Method #2	10.8[b]	15.2[b]	21[b]	36.2[b]	31.8[b]
Q. coccifera	Method #1	4.21[c]	5.75[c]	3.7[c]	5.3[c]	4.32[c]
	Method #2	9.32[d]	15.4[d]	19.2[d]	19.8[d]	26.4[d]

EY: values are expressed as % of dry fruit powder.
For each solvent, values in the same column bearing different letters are significantly different at $p \leqslant 0.05$ (at least).

Table 2
The effect of different solvents on polyphenol content in *J. phoenicea* L. and *Q. coccifera* L. fruit extracts obtained by both methods

	Juniperus phoenicea		*Quercus coccifera*	
	Method #1	Method #2	Method #1	Method #2
Water	167 ± 0.68	189 ± 0.51	74.9 ± 0.24	99.0 ± 0.77
Chloroform	89.6 ± 0.11	66.1 ± 1.20	65.5 ± 0.36	45.0 ± 1.32
Acetone	143 ± 1.02[A]	106 ± 0.65	173 ± 1.21[A]	97.5 ± 0.85
Mixture 1	188 ± 0.33	202 ± 0.43	123 ± 0.17	139 ± 1.83
Mixture 2	96.8 ± 1.45[a,B]	94.1 ± 0.46[a,C]	103 ± 1.66[b,B]	101 ± 0.52[b,C]

Values are expressed as mg GAE/g dry weight (means ± standard deviation of three measurements).
For each solvent, values in the same line bearing different letters are not significantly different ($p > 0.05$).
Values bearing capital letters are the cases where polyphenol contents, obtained with a given method, are higher in *Q. coccifera* L. extracts.

of polyphenol content by method #1 as follows: mixture 1 > infusion > acetone > mixture 2 > chloroform. The same order was noted when extracts were obtained by method #2. In the case of *Q. coccifera*, the order of polyphenol content of extracts was completely different. The highest amount of polyphenol in extracts prepared by method #1 was found in the acetone extract, closely followed by mixture 1, mixture 2, infusion and chloroform, respectively. On the contrary, the highest polyphenol content in extracts prepared according to method #2 was found in mixture 1 and the order was as follows: mixture 1 > mixture 2 > infusion > acetone > chloroform. In all cases, chloroform was the least effective solvent. Similar results were noted when the lowest amount of phenolics was recorded in non-polar (chloroformic) subfraction of methanol extract from aerial parts of *Salvia tementosa* (Bektas, Dimitra, Atalay, Munevver, & Moschos, 2005). These results showed that, for both methods, polyphenol content was strongly dependent on the solvents. Polar fractions had more phenolics in them than had non-polar fractions. As mentioned above, our results clearly showed that a higher content of polyphenols was obtained with an increase in the polarity of the solvent used. Aqueous acetone (mixture 1) and water gave the highest levels of polyphenols. In the literature, it is reported that aqueous acetone (70%), with or without acid, is more efficient than is absolute acetone for recovery of a maximum amount of condensed tannins from different peas (Chavan, Shahidi, & Naczk, 2001). In another work, it had been found that, among the solvents tested, methanol and a mixture of ethanol and water (17:3) were the most effective and gave the greatest level of total phenolics from grape seeds (Jayaprakasha et al., 2001). Polyphenol contents in extracts prepared by both methods, from *J. phoenicea*, were substantially higher than those

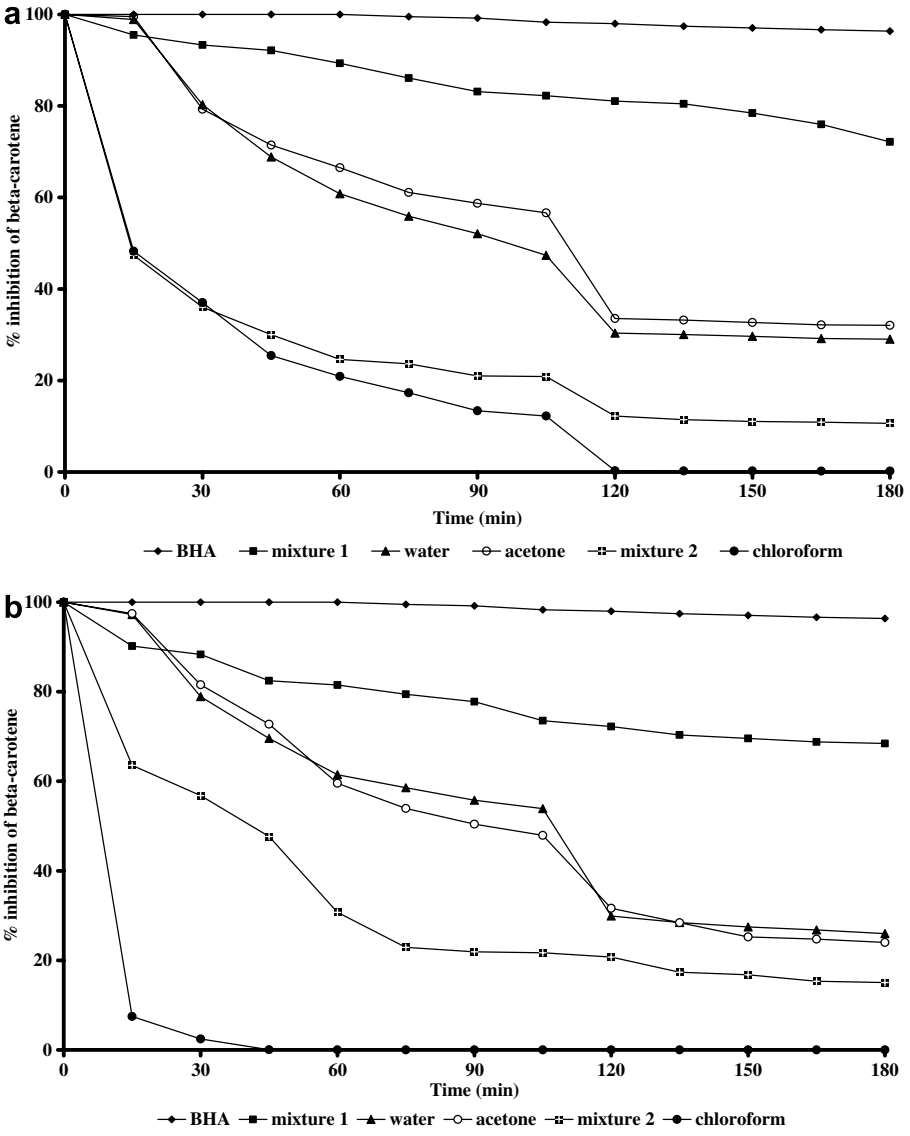

Fig. 1. The antioxidant activities of *J. phoenicea* L. extracts as determined by the β-carotene bleaching test. (a) Extracts prepared according to method #1. (b) Extracts prepared according to method #2. (Results are expressed as means ± standard deviation of three measurements.)

from *Q. coccifera*, and a significant difference was found between them. Nevertheless, some exceptions were noted (values bearing the same capital letters A, B or C in Table 2). The results also indicated that, for a given raw material, the total polyphenols, prepared either with method #1 or with method #2, were significantly different. Some exceptions were observed, particularly when mixture 2 was used as extracting solvent (values bearing the same letters a or b in Table 2).

3.3. Antioxidant activity

In light of the differences among the wide number of the systems available, the results of a single method can give only a reductive suggestion of the antioxidant properties of the extracts (Gianni et al., 2005). For that reason, we combined two complementary techniques, based on bleaching of β-carotene (Fig. 1a and b) and scavenging of the $ABTS^{\cdot+}$ radical (Table 3).

According to the results presented in Fig. 1, we can note that extracts from *J. phoenicea* obtained by mixture 1 showed more than 70% inhibition, which is as strong as the synthetic BHA. Meanwhile, extracts obtained by chloroform were less effective in minimizing the oxidation of lipids. Similar results were found by Djeridane et al. (2006). Thus, it can be seen that extracts prepared by different solvents and different techniques exhibited varying degrees of antioxidant activity. The same observations were noted with extracts from a *Q. coccifera* sample (data not shown). It can be concluded that the extracts obtained using high polarity solvents were considerably more effective radical-scavengers than were those using low polarity solvents, indicating that antioxidants or active compounds of different polarity could be present in the extracts with high antioxidant capacity. Change in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences activity estimation (Zhou & You, 2004). Nevertheless, compared with extracts from *J. phoenicea*, those from *Q. coccifera* exhibited less antioxidant activity, whatever the extracting solvent or the extraction method (Table 3). The antioxidant capacity of the extracts can be ordered as follows according to the results of $ABTS^{\cdot+}$ radical bleaching: mixture 1 > acetone > water > mixture 2 > chloroform when method #1 was used. However, when method #2 was used, the order was as follows: mixture 1 > water > acetone > mixture 2 > chloroform.

Furthermore, content of polyphenols in all the extracts (except a few) correlates with their antioxidant activity, confirming that polyphenols are likely to contribute to the radical-scavenging activity of these plant extracts. Similar results were reported for different plants by various studies (Miliauskas, Vensjutonis, & Van Beek, 2004; Yu, Ahmedna, & Goktepe, 2005). The correlation between the total phenolics and antioxidant activity of all extracts ranges between $R^2 = 0.76$ and $R^2 = 0.84$. This result suggests that between 76% and 84% of the antioxidant capacity of extracts is due to the contribution of phenolics. Similar correlation was reported by Djeridane et al. (2006) who found $R^2 = 0.79$ as the correlation factor between antioxidant activity and total phenolics in some ethanolic extracts from Algerian medicinal plants, including *Juniperus oxycedrus*. In a few other cases (namely with mixture 2), no correlation was noted. The unclear relationship between the antioxidant activity and the total phenolics may be explained in numerous ways. In fact, the total phenolics content does not incorporate all the antioxidants. Their redox properties allow them to act as reducing agents, hydrogen donators, and singlet and triplet oxygen quenchers (Pietta, 2000). Nevertheless, they may exhibit strong metal chelation properties (Kähkönen et al., 1999). In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity, not only dependent on the concentration, but also on the structure and interaction between antioxidants (Rice-Evans, Sampson, Bramley, & Holloway, 1997). The polar fractions may have more polyhydroxyl phenolics, such as tannins, which may act synergistically with other compounds (Haslam, 1996).

Table 3
The effect of different solvents on antioxidant capacity in *J. phoenicea* L. and *Q. coccifera* L. fruit extracts obtained by both methods

	Juniperus phoenicea		*Quercus coccifera*	
	Method #1	Method #2	Method #1	Method #2
Water	14.4 ± 0.44^{a}	15.2 ± 1.01^{a}	10.8 ± 0.67^{b}	8.34 ± 1.21^{c}
Chloroform	6.01 ± 1.34^{a}	3.03 ± 0.21^{b}	4.21 ± 0.29^{c}	2.22 ± 0.22^{d}
Acetone	16.4 ± 0.15^{a}	14.1 ± 0.4^{a}	13.2 ± 0.81^{b}	11.2 ± 0.81^{c}
Mixture 1	28.2 ± 0.32^{a}	26.7 ± 0.94^{a}	22.2 ± 0.57^{b}	18.2 ± 1.01^{c}
Mixture 2	09.9 ± 0.55^{a}	7.24 ± 0.94^{a}	7.13 ± 0.79^{b}	6.41 ± 0.15^{b}

Results of the $ABTS^{\cdot+}$ assay are expressed as mg equivalents of vitamin C/g dry weight (means ± standard deviation of three measurements).
For each solvent, values in the same line bearing different letters are significantly different at $p \leqslant 0.05$ (at least).

The antibacterial effects of the extracts, obtained by both methods, using water, acetone and chloroform on tested bacteria are presented in Table 4, whereas, the effects of mixtures of extracts, on the same bacteria, are presented in Fig. 2. All extracts were used at 300 μg/disc.

The different extracts inhibited growth to variable extents, depending on the bacterium in question. Aqueous extracts, from both plants, showed the best effectiveness good broad-spectrum action, followed by chloroform and acetone extracts. Similar trends for inhibition of bacterial growth have been observed in earlier studies with other plant extracts (Negi & Jayaprakasha, 2003). It seems that *J. phoenicea* extracts obtained by method #1 and method #2 exhibited a wider spectrum against all tested bacteria than did the corresponding extracts from *Q. coccifera*. This could be attributed to the higher levels of phenolics in *J. phoenicea* extracts. Furthermore, on comparing the methods of extractions, it is clear that the aqueous extracts obtained by method #1 were more effective against all the

Table 4
The antibacterial activities of extracts obtained by two different methods and different extracting solvents

	Water				Acetone				Chloroform				Streptomycin[a]
	Q. coccifera		*J. phoenicea*		*Q. coccifera*		*J. phoenicea*		*Q. coccifera*		*J. phoenicea*		
	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	
Pseudomonas aeruginosa ATCC 9027	22.5	23	25	25.5	13.5	NA	14	13	15	13	17.5	14	29
Pseudomonas morgani[b]	23	24.5	24	25.5	14	13	15.5	13	14.5	13.5	16	14.5	27
Staphylococcus aureus ATCC 25923	25	25	26	27	15.5	NA	18	16.5	16	15.5	18.5	17	28.5
Enterococcus faecalis ATCC 29212	19.5	19.5	20	23.5	NA	NA	16	NA	18.5	15	18	17.5	25
Escherichia coli ATCC 25922	18	19.5	20	22	NA	NA	13	NA	13.5	14	15.5	15	24
Salmonella anatum[c]	17	21	19.5	21.5	13	NA	13.5	13.5	14.5	NA	16	13.5	25
Klebsiella pneumoniae[b]	19.5	21	21	22	13.5	13	14	13	15	NA	17.5	NA	24
Listeria monocytogenes[c]	20.5	22.5	21	24.5	NA	NA	14	NA	14.5	14.5	14.5	NA	26.5

Results are expressed as diameter (in mm) of inhibition zone minus diameter of inhibition zones of negative control as determined by the disc diffusion method.
NA: not active.
[a] Streptomycin B at 30 μg/disc was used as positive control.
[b] Clinical strain.
[c] Food spoilage strain.

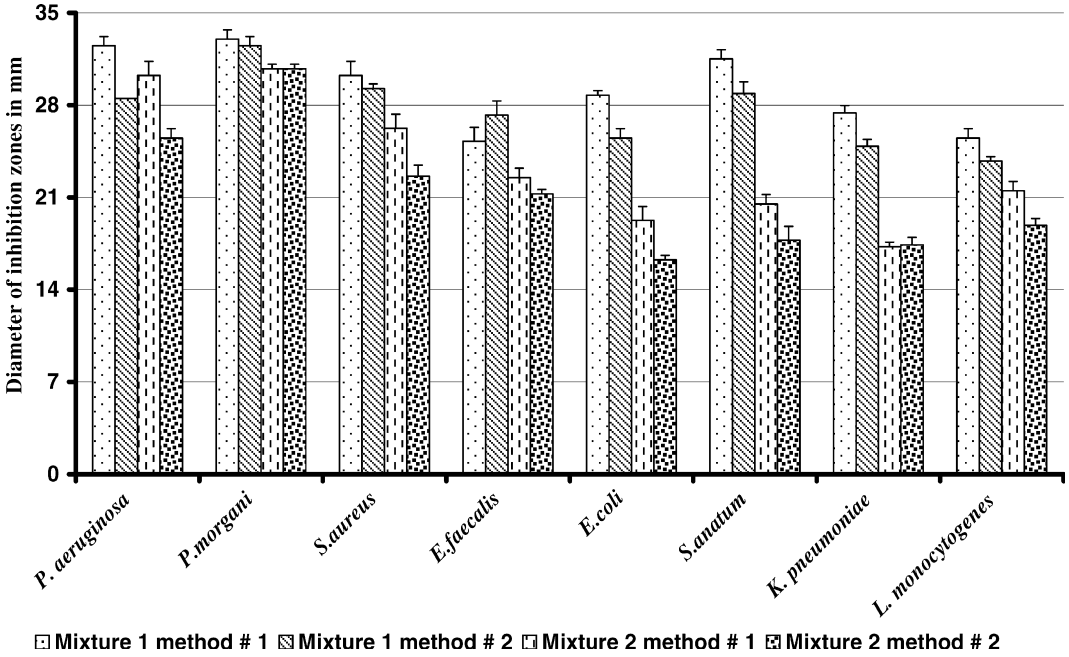

Fig. 2. The effect of mixture solvents on antibacterial activity of *J. phoenicea* L. and *Q. coccifera* L. fruit extracts obtained by both methods. (Results are expressed as diameter (mm) of inhibition zone including the paper disc diameter. Values are given as means ± SEM.)

bacteria than were those obtained by method #2. This observation remained true for aqueous extracts from *J. phoenicea*, as well as from *Q. coccifera*. In all other cases, the extracts showed similar behaviour, since the diameters of inhibition zones remained very close. Moreover, *E. coli* ATCC 25922 and *L. monocytogenes* were not inhibited by acetone extracts of *J. phoenicea* fruits obtained by both methods, whereas, *S. anatum* and *S. aureus* ATCC 25923 were not inhibited by acetone extract prepared from *Q. coccifera* using method #2.

As shown in Table 4, the Gram-positive bacteria *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 seemed to be more easily inhibited than were the Gram-negative bacteria, namely *L. monocytogenes* and *E. coli*. This may be attributed to lipopolysaccharides in the outer membrane of the Gram-negative bacteria, which make them inherently resistant to external agents, such as hydrophilic dyes, antibiotics and detergents (Negi & Jayaprakasha, 2003). Similar results have been observed in earlier studies using other plant extracts (Candan et al., 2003; Negi & Jayaprakasha, 2003). Nevertheless, it should be taken into account that the inhibition area depends on the ability of the antibacterial compound to diffuse uniformly through the agar. This phenomenon was noted in many reports (Rauha et al., 2000).

As we noted in Fig. 2, the extracts obtained by mixture 1 (using either method #1 or method #2) were more effective, in inhibiting all tested bacteria, than those obtained by

mixture 2. Meanwhile, inhibitions recorded with all these extracts were higher than were those recorded using all the other solvents (water, acetone and chloroform). Similar findings were reported by Baydar, Özkan, and Sağdiç (2003). They found that 20% defatted grape seed extracts, obtained by the same mixtures as used in this work, inhibited some food spoilage bacteria, such as *E. feacalis* (34 mm with mixture 1 and 28 mm with mixture 2), *S. aureus* (29 mm with mixture 1 and 26 mm with mixture 2).

4. Conclusion

The technique of extraction, as well as the extracting solvent, significantly affected extraction yield, total polyphenol and biological activities (antioxidant and antibacterial) of several extracts from *J. phoenicea* L. and *Q. coccifera* L. fruits. Rankings in polyphenol contents of extracts, varied, depending on the nature of solvent, the method of extraction used and the raw material. The most efficient solvents for polyphenol extraction were the polar solvents, such as: acetone/eau/acetic acid (95/4.5/0.5), ethyl acetate/methanol/water (60/30/10) and water. Regardless of the method used, the one-step extraction method allowed higher yields. Meanwhile, the sequential method, using a defatted material, provided extracts with stronger antioxidant and antibacterial capacity/activity. As observed, extracts with higher antioxidant capacity and antibacterial activity also had higher polyphenol contents. It can be concluded that the extracts obtained using higher polarity solvents were more effective radical-scavengers and bacterial inhibitors than were those obtained using less polar solvents. Furthermore, it is notable that the extracts from *J. phoenicea* were more efficient as antibacterial agents and exhibited stronger antioxidant capacity than did the corresponding ones from *Q. coccifera*. These results indicate that selective extraction from natural sources, by appropriate solvents and suitable methods, is important for obtaining fractions with high antioxidant and antibacterial activities. Results presented here suggest that the extracts obtained from *J. phoenicea* and *Q. coccifera* possess antioxidant and antibacterial properties and therefore, they can be used as preservative ingredients in the food and/or pharmaceutical industry, if any resulting organoleptic effects are acceptable. These plants contain high amounts of phenolics and have very high levels of gallic acid equivalents. In order to confirm the antioxidative effect of these plants, a further survey, which uses other kinds of antioxidant assay, should be undertaken. Moreover, arising from this work, we can conclude that studies using foodstuff models are needed to further confirm the advantageous quality of these extracts.

Acknowledgements

We are very grateful to Dr. Nadia Ben Salem, head of the Department of Botany, National Institute of Agronomic Research (INRAT, Tunis, Tunisie) for identification of plants. The authors equally thank Dr. Raïs Salwa and Mss. Nazek M. (Institut Pasteur de Tunis, Tunisia) for providing us with bacteria. This study received financial support from Ministère de la Recherche Scientifique, de la Technologie et du développement des compétences, Tunisia.

References

Baydar, N. G., Özkan, G., & Sağdiç, O. (2003). Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control, 14*, 141–143.

Bektas, T., Dimitra, D., Atalay, S., Munevver, S., & Moschos, P. (2005). Antimicrobial and antioxidant activities of essential oils and various extracts of *Salvia tementosa* Miller (Lamiaceae). *Food Chemistry, 90*, 333–340.

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., et al. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. Millefolium Afan. (Asterraceae). *Journal of Ethnopharmacology, 87*, 215–220.

Chavan, U. D., Shahidi, F., & Naczk, M. (2001). Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. *Food Chemistry, 75*, 509–512.

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 Chemistry, 97*, 654–660.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*, 308–316.

Gianni, S., Silvia, M., Mariavittoria, M., Martina, S., Stefano, M., Matteo, R., et al. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry, 91*, 621–632.

Goli, A. H., Barzegar, M., & Sahari, M. A. (2004). Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chemistry, 92*, 521–525.

Harborne, J. B. (1998). Phenolic compounds. In J. B. Harborne (Ed.), *Phytochemical methods: A guide to modern techniques of plant analysis* (pp. 40–160). London: Chapman & Hall.

Harborne, J. B., Baxter, H., & Moss, G. P. (Eds.). (1999). *Phytochemical dictionary: Handbook of bioactive compounds from plants* (2nd ed.). London: Taylor & Francis.

Haslam, E. (1996). Natural polyphenols (vegetables tannins) as drug: Possible mode of action. *Journal of Natural Products, 59*, 205–215.

Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chemistry, 73*, 285–290.

Kähkönen, M. P., Hopia, A. I., Heikki, J. V., Rauha, J.-P., Pihlaja, K., & Kujala, T. S. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Kaur, C., & Kapoor, H. C. (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *Ineternational Journal of Food Science and Technology, 37*, 153–161.

Khokhar, S., & Magnusdottí, S. G. M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry, 50*, 565–570.

Lister, E., & Wilson, P. (2001). *Measurement of total phenolics and ABTS assay for antioxidant activity* (personal communication). Lincoln, New Zealand: Crop Research Institute.

Madsen, H. L., & Bertelsen, G. (1995). Spices as antioxidants. *Trends in Food Science and Technology, 6*, 271–277.

Miliauskas, G., Vensjutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*, 231–237.

National Committee for Clinical Laboratory Standards (NCCLS). (1997). *Performance standards for antimicrobial disk susceptibility test* (6th ed.). Approved Standard. M2-A6, Wayne, PA.

Negi, P. S., & Jayaprakasha, G. K. (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of Food Science, 68*, 1473–1477.

Pietta, P.-G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*, 1035–1042.

Pinelo, M., Rubilar, M., Sineiro, J., & Nunez, M. J. (2004). Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chemistry, 85*, 267–273.

Pratt, D. E. (1980). Natural antioxidants of soybean and other oil-seeds. In M. G. Simic & M. Karel (Eds.), *Autoxidation in food and biological systems* (pp. 283–292). New York: Plenum Press.

Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology, 56*, 3–12.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorizing assay. *Free Radicals in Biology and Medicine, 26*(9/10), 1231–1237.

Rice-Evans, C. A., Sampson, J., Bramley, P. M., & Holloway, D. E. (1997). Why do we expect carotenoids to be antioxidants *in vivo*? *Free Radical Research, 26*, 381–398.

Sun, T., & Ho, C. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry, 90*, 743–749.

The Local Food-Neutraceuticals Consortium (2005). Understanding local Mediterranean diets: Multidisciplinary pharmacological and ethnobotanical approach. *Pharmacological Research, 52*, 353–366.

Tura, D., & Roberts, K. (2002). Sample handling strategies for the determination of biophenols in food and plants. Review. *Journal of Chromatography A, 975*, 71–93.

Wang, H., & Helliwell, K. (2001). Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International, 34*, 223–227.

WHO (2002). Food safety and foodborne illness. World Health Organization Fact sheet 237, Geneva. Revised January 2002.

Yanishlieva, N. V., & Marinova, E. M. (2001). Stabilisation of edible oils with natural antioxidants. *European Journal of Lipid Science and Technology, 103*, 752–767.

Yu, J., Ahmedna, M., & Goktepe, I. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chemistry, 90*, 199–206.

Zhou, K., & You, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebennsmittel-Wissenschaft und-Technologie, 37*, 717–721.

Zuo, Y., Chen, H., & Deng, Y. (2002). Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-erh teas using HPLC with photodiode array detector. *Talanta, 57*, 307–316.