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Antibacterial, anticandidal and antioxidant activities of *Salvadora persica* and *Juglans regia* L. extracts

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Salvadora persica and *Juglans regia* L. are traditionally used for the treatment of oral infections. The anticandidal; antibacterial and antioxidant activities of diluted acetone extract of *S. persica* (dry stems) and ethyl acetate extract of Tunisian *Juglans regia* L. bark were tested. The chemical composition of the volatile oil of the stems of *S. persica* was also investigated. The essential oil of *S. persica* stems was rich on benzyl isothiocyanate (52.5%) and benzyl nitrile (38.3%). Comparatively to the diluted acetone extract of *S. persica*, ethyl acetate extract of walnut's bark was more effective against Gram positive and Gram negative bacteria and the different species of *Candida* tested in the present work. Similarly, the walnut extract was rich on phenols (34.833 mg GAE.g⁻¹ DW) and tannins (16.167 mg EC.g⁻¹ DW) comparatively to the arak extract (0.443 mg GAE.g⁻¹ DW and 0.39 mg EC.g⁻¹ DW, respectively). The walnut extract displayed the highest DPPH scavenging ability with the lowest IC₅₀ value (IC₅₀, 3 µg/ml), a high capacity to quench superoxide (IC₅₀: 70 µg.ml⁻¹), the greater reducing power and bleaching of β-carotene (EC₅₀, 99 µg.ml⁻¹ and IC₅₀, 280 µg.ml⁻¹, respectively) as compared to *S. persica* extract. These findings support the interest of *S. persica* and especially walnut bark and their possible use in the developing countries due to their inexpensive cost in order to maintain oral hygiene and as a source of new antioxidant compounds.

Key words: *Salvadora persica*, *Juglans regia* L., antibacterial activity, antioxidant activities.

INTRODUCTION

The Persian toothbrush tree or Miswak (*S. persica* L.), also known as tooth brush tree belonging to the Salvadoraceae family, has been used as a brushing stick for more than 1,300 years. Nowadays chewing sticks is being used in Africa, South America, the Middle East and Asia. In Pakistan, more than the half of the rural population used chewing sticks as an oral hygiene tool. Pharmacological studies indicated that *S. persica* L. plant possess anti-microbial, anti-plaque, aphrodisiac,

alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities (Galletti and Chiavari, 1993). It has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy, gonorrhoea, boils and toothache, to treat hook worm, venereal diseases, for teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa, and as a laxative (Alali et al., 2004; Khalessi et al., 2004). It contains important phyto-constituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides (Alali et

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al., 2004; Almas, 2002; Almas et al., 2005; Rajesh et al., 2009).

Walnut species are important sources of nuts and timbers in the temperate zones across the world. In China, *Juglans regia* L. (*Juglandaceae*) is not only an agricultural commodity, but its leaves, barks, stems, pericarps, fruits, flowers and ligneous membranes are all applied for different medicinal uses. In fact, walnut leaves are considered a source of healthcare compounds, and have been intensively used in traditional medicine for treatment of venous insufficiency and for its antidiarrheic properties.

Antifungal, antibacterial and antioxidant activities of this plant have also been described (Isanga et al., 2007; Miraliakbari and Shahidi, 2008; Amaral et al., 2003). The health benefits of walnuts are usually attributed to their chemical composition. Walnuts are a good source of essential fatty acids and tocopherols (Amaral et al., 2005).

Linoleic acid is the major fatty acid, followed by oleic, linolenic, palmitic and stearic (Amaral et al., 2008); its high content of polyunsaturated fatty acids (PUFA) has been suggested to reduce the risk of heart disease by decreasing total and LDL-cholesterol and increasing HDL-cholesterol (Davis et al., 2007). In addition, walnuts have other components that may be beneficial for health including plant protein, dietary fiber, melatonin (Reiter et al., 2005), plant sterols (Amaral et al., 2005), folate, tannins and polyphenols (Li et al., 2006). Walnuts possess a high content of α -tocopherol, a vitamin E family compound, which has antioxidant activity, mainly in the prevention of lipid oxidation process (Koksak et al., 2006). Plant-derived products can also be used as antimicrobial agents, with phenolics and polyphenolic having major interest.

In Tunisia, the chewing sticks, locally known "Arak", is largely used especially by men and the leaves of walnut are also widely used by women as a mean to maintain oral hygiene and to prevent tooth decay. In a previous study, we reported that among *S. persica* and *J. regia* L. extracts, ethyl acetate *J. regia* L. extract had potent antifungal activity against all *Candida* strains. The MIC values of the *J. regia* L. against *Candida* strains were ranging from 0.006 to 0.195 mg/ml (Noumi et al., 2006).

Despite the wide use of *S. persica* (miswak) and *J. regia* L. (walnut), these plants have not received much attention and have not been intensively studied. Therefore, the present work is aimed to highlight the anticandidal, antibacterial and antioxidant activities of three different extracts of *S. persica* and Tunisian *J. regia* extracts.

MATERIALS AND METHODS

Plant material

Dried plant samples were used in this study. Dry stems of *S. persica* imported from Morocco were purchased from a local market

in the region of Sidi Bouzid (Tunisia) and the bark of *J. regia* L. from the local market of Mahdia (Tunisia) in 2009.

Plant extracts

Fresh plant samples were used. Extraction was made with three different solvent: a mixture of acetone: water (80:20; v/v), ethyl acetate and methanol. The extracts of the plant species were prepared by adding 4 g of small particle fresh plant material powder with a commercially available food blender to 40 ml solvent and allowing the mixtures to stand overnight at room temperature, after which the supernatants were filtered and dried/evaporated under a controlled temperature (40°C), and their antibacterial activities were evaluated. To make 30 mg extract-impregnated disks, 1 ml of extract solution in methanol or ethyl acetate or acetone was applied onto the sterile disks in 10 μ l increments with sufficient time in between to allow drying.

Characterization of the essential oils

Extraction of the essential oil

The air-dried and ground aerial parts of the plants were submitted for 3 h to water-distillation using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed.

Gas chromatography (GC)/MS analysis

GC/MS analyses were performed with a Varian CP- 800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μ m) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60 to 240°C at 3°C/min; carrier gas helium at 1 ml/min; injection of 0.2 μ l (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data as previously reported by Hajlaoui et al. (2009). Moreover, the molecular weights of all the identified substances were confirmed by GC/MS, using MeOH as CI ionizing.

Antimicrobial activities

Microorganisms

The tested microorganisms included the following Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Micrococcus luteus* NCIMB 8166 and Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* LT2 and *P. aeruginosa* isolated from oral cavity of a patient suffering from denture stomatitis in association with *Candida albicans* strain. A total of 48 *Candida* strains including 15 species (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. famata*, *C. kefyr*, *C. sake*, *C. holmii*, *C. lusitaniae*, *C. intermedia*, *C. atlantica*, *C. maritima*, *Pichia guilliermondii* and *Pichia jadinii*) were used in this study. Clinical isolates were taken from the oral cavity of patients by using a swabbing method. A sterile cotton swab (Nippon Menbo, Tokyo, Japan) was immediately cultured into Sabouraud Chloramphenicol agar (Bio-Rad, France) to obtain isolated colonies. All isolates were

Table 1. Antimicrobial activity of methanol, ethyl acetate and diluted acetone extracts of *S. persica* dry stems and *J. regia* bark

Compounds	%	kl	Identification
1: Benzaldehyde	2.5	963	MS, KI
2: Sabinene	0.1	972	MS, KI
3:1,8-cineole	0.3	1032	MS, KI
4: Linalool	0.1	1098	MS, KI
5: Myrcenol	0.1	1114	MS, KI
6: Benzyl isocyanate	0.3	1123	MS, KI
7: Benzyl nitrile	38.3	1162	MS, KI
8: Benzyl ester	0.1	1172	MS, KI
9: Naphtalene	0.6	1189	MS, KI
10: Methyl chavicol	0.3	1201	MS, KI
11: Aniline	0.7	1247	MS, KI
12: Trans-anethol	0.2	1287	MS, KI
13: Carvacrol	3.3	1305	MS, KI
14: Benzyl isothiocyanate	52.5	1394	MS, KI
15: Diphenyl ether	0.2	1411	MS, KI
% of identified compounds			99.5

The components and their percentages are listed in order of their elution on polar column (HP-20M). MS: Mass spectroscopy, KI: Kovats index.

incubated at 30°C for 24 to 48 h and yeast-like colonies were isolated and identified by the ID 32C (bio-Mérieux, Marcy-l'Étoile, France) assimilation kit.

Disk-diffusion assay

Antimicrobial activity was done according to the protocol described by Hajlaoui et al. (2009). For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 9 ml of Mueller-Hinton broth then incubated at 37°C for 18 to 24 h. The overnight cultures were used for the antimicrobial activity of the essential oil used in this study and the optical density was adjusted at 0.5 McFarland turbidity standards with a DENSIMAT (BioMérieux). The inocula of the respective bacteria were streaked onto MH agar plates using a sterile swab. A sterile filter disk (diameter 6 mm, Whatman paper N°3) were impregnated with 10 µl of each extract placed on the MH agar medium. Gentamicin (10 µg/disk) was used in this study as positive controls.

The antibiotic susceptibility was determined by using the Kirby-Bauer method and Mueller Hinton agar plates. After incubation at 37°C for 18 to 24 h, the diameter of the inhibition zone was measured with 1 mm flat rule and the diameters were interpreted according to the Comité de la Société Française de l'Antibiogramme (Cavallo et al., 2006). The anti-Candida spp. activity was achieved by the agar-well diffusion method. All Candida strains were inoculated onto Sabouraud dextrose agar and incubated for 18 h at 37°C. The yeast cultures were harvested and then suspended in sterile saline (0.8 % NaCl) and the cell density was adjusted to 10⁷ cells/ml (OD₅₄₀= 0.5).

For the antifungal activity of the plants extracts used in this study, three sterile 6 mm paper disks (Whatman paper N°3), impregnated with 30 mg of extract (10 µl/disk) at a final concentration of 300 mg/ml were placed on the inoculated surface. Plates were then incubated at 37°C for 18 to 24 h. The ATCC and CECT strains were used as a quality control strains. The diameter of the zones of inhibition around each disk were examined after 24 h, measured and recorded as the mean diameter (mm) of complete growth-inhibition. As a positive control, 10 µg of amphotericin B

(Fungizone, BioBasic INC) was used. Tests were done in triplicate and results given as mean average (Table 1). The dishes were incubated at 37°C for 18 to 24 h for microbial strains. The diameter of the zones of inhibition around each of the disks was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Phenolic compounds analysis

Evaluation of total antioxidant capacity

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

Total phenolic content

Phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified (Dewanto et al., 2002). An aliquot (0.125 ml) of appropriately diluted sample extract was added to 0.5 ml of distilled water and 0.125 ml of the Folin-Ciocalteu reagent. After 3 min, 1.25 ml of Na₂CO₃ solution (7g/100 ml) were added and the final volume was made up to 3 ml with distilled water. The absorbance was measured at 760 nm, after incubation for 90 min at 23°C in dark. Total phenolic content of leaves was expressed as mg gallic acid equivalents per gram of dry

weight (mg GAE.g⁻¹ DW) through the calibration curve with gallic acid. The calibration curve range was 0 to 400 µg.ml⁻¹. Triplicate measurements were taken for all samples.

Total condensed tannins

Proanthocyanidins were measured using the modified vanillin assay described by Sun et al. (1998). To 50 µl of suitably diluted samples were added 3 ml of methanol vanillin solution and 1.5 ml H₂SO₄ respectively. The mixture was allowed to stand for 15 min at room temperature, and the absorption was measured at 500 nm against solvent as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin g⁻¹ DW.

Antioxidant activities

DPPH radical-scavenging activity

The antioxidant activity of different solvent extracts was measured in term of hydrogen donating or radical scavenging ability using the stable DPPH method (Hanato et al., 1998). The sample was diluted in pure solvent of extraction at different concentrations (10, 20, 100 and 200 µg.ml⁻¹), then 1 ml of each diluted plant extract was added to 0.25 ml of a 0.2 mmol/l DPPH methanolic solution. The mixture of different extract concentration and DPPH were placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then read at 517 nm. The antiradical activity was expressed as IC₅₀ (µg.ml⁻¹). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}^{\cdot} \text{ scavenging effect (\%)} = ((A_0 - A_1)/A_0) * 100 \quad (1)$$

Where A₀ is the absorbance of the control at 30 min, and A₁ is the absorbance of the sample at 30 min.

All samples were analyzed in triplicate.

Determination of reducing power

The ability of the extracts to reduce Fe³⁺ was assayed by the method of Oyaizu (1986). Briefly, 1 ml of *S. persica* and *J. regia* extracts was mixed with 2.5 ml of phosphate buffer (0.2 mol/l, pH 6.6) and 2.5 ml of K₃Fe(CN)₆ (1g/100 ml). After incubation at 50°C for 25 min, 2.5 ml of trichloroacetic acid (10g/100ml) was added and the mixture was centrifuged at 650xg for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of aqueous FeCl₃ (0.1g/100 ml). The absorbance was measured at 700 nm. The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power. EC₅₀ value (mg.ml⁻¹) is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as positive control.

Superoxide anion radical-scavenging activity

Superoxide scavenging capacity was assessed using the method described by Duh and Yen (1999). The reaction mixture contained 0.2 ml of different leaf extracts at varying concentrations, 0.2 ml of 60 µmol/l PMS, 0.2 ml of 677 µmol/l NADH, and 0.2 ml of 144 µmol/l NBT, all in phosphate buffer (0.1 mol/l, pH 7.4). After 5 min of incubation at room temperature, the absorbance was read at 560 nm against blank. Evaluating the antioxidant activity in leaf extract was based on IC₅₀. The IC₅₀ index value was defined as the amount of antioxidant necessary to reduce the generation of superoxide

radical anions by 50%. The IC₅₀ values (3 replicates) were expressed as mg.ml⁻¹. The inhibition percentage of superoxide anion generation was calculated using the following formula:

$$\text{Superoxide quenching (\%)} = ((A_0 - A_1)/A_0) * 100$$

Where A₀ is the absorbance of the control, and A₁ is the absorbance of the sample.

All samples were analyzed in triplicate.

B-carotene-linoleic acid model system (B-CLAMS)

The B-CLAMS method by the peroxides generated during the oxidation of linoleic acid at elevated temperature (Koleva et al., 2002). In this study the B-CLAMS was modified for the 96-well micro-plate reader. In brief, the B-carotene was dissolved in 2ml of CHCl₃, to which 20 mg of linoleic acid and 200 mg of tween 40 were added. CHCl₃ was removed using rotary evaporator. Oxygenated water (100 ml) was added, and the flask was shaken vigorously until all material dissolved.

This test mixture was prepared fresh and using immediately. To each well, 250 µl of the reagent mixture and 35 µl sample or standard solution were added. The plate was incubated at 45°C. Readings were taken at 490 nm using visible/UV microplate kinetics reader (EL x 808, Bio-Tek instruments) All samples were prepared and analyzed in triplicate.

RESULTS AND DISCUSSION

Several studies were performed around the world with the aim of studying the antimicrobial (Gram-negative and Gram-positive bacteria), antifungal and antioxidant power of a large list of herbs and plants (Bakkali et al., 2008; Busatta et al., 2008; Al-Bayati and Sulaiman, 2008). Various chemicals such as alkaloids, tannins, saponins, cyanoglycosides, terpenoids, oleic and stearic acids which are naturally present in plants have been implicated in the conferment of antimicrobial activities on the plant containing them. In this study, we report the biological activities of two plants largely used in Tunisia for oral hygiene.

Chemical composition of *S. persica*

In this study, we succeed to evaluate only the chemical composition of *S. persica* despite the low yield of extraction obtained (0.1%: 20 µl /200g of stems). The qualitative and quantitative compositions of the oil are given in Table 2, where the components were listed according to their elution on the Innowax column. The different constituents of the samples were identified and quantified by GC and GC-MS. Fifteen compounds were identified. The essential oil obtained from the stems of *S. persica* was rich on benzyl isothiocyanate (52.5%), benzyl nitrile (38.3%), carvacrol (3.3%), benzaldehyde (2.5%), aniline (0.7%) and naphthalene (0.6%). In 2003, Alali and Al-Lafi reported that the GC-MS analysis of the volatile oil extracted from *S. persica* L. leaves from

Table 2. Chemical composition of the essential oil obtained from the stems of *S. persica*.

Strains	Inhibition zone in diameter (mm ± SD) around the discs impregnated with 10 µl of plant extract						Antibiotic (Gent)
	<i>Salvadora persica</i>			<i>Juglans regia</i> L.			
	1	2	3	1	2	3	
Gram + bacteria							
<i>S. epidermidis</i> CIP 106510	9±0	9±0	8±0	12±0	20.33±0.57	10.66±0.57	21.33±0.58
<i>S. aureus</i> ATCC 25923	9.66±0.57	9±0	10±0	13.66±0.57	19.66±0.57	13.33±0.57	32.67±0.58
<i>M. luteus</i> NCIMB 8166	9±0	9.66±0.57	9.66±0.57	15 ±0	17.66±0.57	14 ±0	27.67±1.53
Gram- bacteria							
<i>S. typhimurium</i> LT2	9±0	9.33±0.57	10±0	10.33±0.57	11.33±0.57	9.33 ±0.57	21±1
<i>P. aeruginosa</i> ATCC 27853	10±0	8±0	9.33±0.57	10.33±0.57	13±0	10 ±0	30.33±0.58
<i>P. aeruginosa</i> (Oral cavity)	7±0	8±0	7±0	8±0	10±0	7±0	*

1: Methanol; 2: ethyl acetate; 3: Diluted acetone. Gent = Gentamicin (10 µg/disc) were used as positive reference standards antibiotic discs. *: not tested. SD: Standard deviation of three replicates.

Jordan reveal seven major components (Benzyl nitrile 53.96%; Isotymol 15.39%; thymol 11.37%; eugenol 10.49%; β-caryophyllene 4.72%; eucalyptol, 79% and isoterpinolene <0.5%). In 2004, Alali and collaborators studied the chemical composition of the essential oil of the stem of the toothbrush tree *S. persica* L. grown in Jordan by gas chromatography-mass spectrometry (GC-MS). The oil obtained by hydrodistillation (yield: 0.6% w/w) was determined as a mixture of monoterpenes hydrocarbons (11%), oxygenated monoterpenes (54%), and sesquiterpene hydrocarbons (21%). Sixteen components were identified and the major components identified were 1,8-cineole (46%), α-caryophyllene (13.4%), β-pinene (6.3%) and 9-epi-(E)-caryophyllene (6.3%).

Antibacterial activity of *S. persica* and *J. regia* extracts

The antimicrobial activity of methanol, ethyl

acetate and diluted acetone extracts of *S. persica* dry stems and *J. regia* leaves included in this work was conducted by disk diffusion method. The results obtained for the zones of growth inhibition (mm) scored in Mueller-Hinton agar were summarized in Table 3. The results obtained demonstrate that *J. regia* extracts were most effective against Gram positive and Gram negative bacteria than the extracts of *S. persica*. In fact, the ethyl acetate of *J. regia* was more effective than diluted acetone and methanol extracts. The highest zones of growth inhibition were obtained for Gram positive bacteria strains with a diameter of inhibition ranging from 17.66 mm against *M. luteus* NCIMB 8166 to 20.33 mm when *S. epidermidis* CIP 106510 was tested. For Gram negative bacteria, *S. typhimurium* LT2 was the most susceptible bacteria tested with a diameter of inhibition about 11.33 mm. Comparatively to the antimicrobial agent used in the present work as the most active antibiotics on these bacteria (Cavallo et al., 2006), ethyl acetate extract of *J. regia* exhibits a high level of antibacterial activity followed by methanol

and diluted acetone extract. These results were in accordance with those founded by Pereira and colleagues (2008). In fact, these authors studied the antimicrobial of six walnuts (*J. regia* L.) cultivars capacity against Gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*).

The authors showed that Gram positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*) were inhibited by cv. Lara aqueous extract at very low concentrations, presenting MICs of 1 mg/mL for *B. cereus*, and 0.1 mg/mL for *B. subtilis* and *S. aureus*. *S. aureus* was also inhibited for cv. Franquette but at higher extract concentration (100 mg/mL). While Gram negative bacteria (*P. aeruginosa*, *E. coli* and *K. pneumoniae*) were also sensible for some walnut extracts. The cultivar Lara inhibited the growth of *K. pneumoniae* (MIC of 100 mg/mL), cv. Mayette inhibited the development of *P. aeruginosa* and *E. coli* with MICs of 50 and 10 mg/mL, respectively, and cv. Mellanise

Table 3. Antifungal activity of diluted acetone extract of *S. persica* and ethyl acetate extract of *J. regia* against several *Candida* species.

Strains	Number of strains	Mean diameter of the inhibition zone (mm ± SD)		Amphotericin B (10 µg/ml)
		<i>S. persica</i> (Diluted acetone extract)	<i>J. regia</i> (Ethyl acetate extract)	
<i>C. albicans</i>	25	9.98±0.26	13.39±0.64	10.85±0.7
<i>C. glabrata</i>	4	8.99±0.28	10.66±0.57	11.99±0.68
<i>C. parapsilosis</i>	3	8.11±0.19	9.55±0.38	10.912±0.42
<i>C. kefyr</i>	4	10.91±0.42	12.32±0.71	10.007±0.86
<i>C. sake</i>	2	8.33±0.57	14.16±0.78	11.5±1.36
<i>C. atlantica</i> CECT 11860	1	7±0	13.66±0.57	11±0
<i>C. dubliniensis</i> CECT 11455	1	7±0	14.66±0.57	11.33±0.57
<i>C. famata</i> CECT 11957	1	7±0	11.33±0.57	12.33±0.57
<i>C. holmii</i>	1	11.33±0.57	10.66±0.57	11.33±0.52
<i>C. intermedia</i> CECT 11869	1	7±0	14.66±1.15	12±0
<i>C. krusei</i> ATCC 6258	1	6±0	15±0	12±0
<i>C. lusitaniae</i> CECT 11458	1	7±0	10.66±0.57	12.33±1.15
<i>C. maritima</i> CECT 1435	1	7±0	13±1	12.66±1.15
<i>Pichia guilliermondii</i> CECT 1456	1	7±0	14±1	12.33±0.57
<i>Pichia jadinii</i> CECT 1060	1	7±0	14.33±0.57	11.66±1.15

SD: Standard deviation of three replicates.

inhibited the growth of *E. coli* and *K. pneumoniae* at concentration of 100 mg/mL. Almas et al. (1997) demonstrated that aqueous and methanol extracts of *S. persica* inhibited most bacterial growth, but their effectiveness varied. Previous studies have reported that *S. persica* extracts were effective against *Streptococcus mutans* (Salehi and Momeni, 2006) and *Streptococcus faecalis*, even using low extract concentrations. In 2000, Almas and Stakiw (2000) reported that the aqueous extract (50% v/v) of the chewing sticks *S. persica* inhibit the growth of *Streptococcus faecalis* with 2 mm as a diameter of inhibition zone. Recently, Al-Bayati and Sulaiman (2008) tested the activity of aqueous and methanol extracts of Iraqi *S. persica* against seven isolated oral pathogens. The strongest antibacterial activity was observed using the aqueous extract

against *S. faecalis* (Zone of inhibition: 22.3 mm; MIC: 0.781 mg/ml).

Interestingly, the only *P. aeruginosa* strain isolated from oral cavity from a patient suffering from denture stomatitis was the most resistant bacteria to all extracts tested obtained from the two plants comparatively to the type strain *P. aeruginosa* ATCC 27853. These results were in accordance with those reported by Al-Bayati and Soliman (2008). In fact, these authors founded that aqueous extract of Iraqi *S. persica* (200 mg/ml) exhibits the weakest activity against oral *P. aeruginosa* with a diameter of inhibition zone about 10.8 mm and this bacteria was also resistant to all concentration of methanol extracts tested (12.5 to 200 mg/ml). It was found that the essential oils of the leaves have a considerable effect on several different oral aerobic bacteria

with comparable results to known antibiotics (Almas and Stakiw, 2000). In 2004, Alali and colleagues reported that the volatile oil of Jordanian *S. persica* stems exhibits potent antibacterial activity against both Gram-positive and Gram-negative bacteria. In fact, the diameter of zones of growth inhibition was about 13 mm for *E. coli*, 12 mm for *S. aureus*, 3 mm for *B. subtilis* and 3.8 mm for *P. aeruginosa* strain. Moreover, the volatile oil exhibits significant activity against resistant strains of *P. aeruginosa* and *S. aureus* with a diameter of zones of growth inhibition about 2.9 and 3 mm respectively. The anticandidal activities of ethyl acetate of *J. regia* leaves and diluted acetone extract of *S. persica* stems were tested against *C. albicans* (n = 25), *C. dubliniensis* (n = 1), *C. Glabrata* (n = 4), *C. parapsilosis* (n = 3), *C. krusei* (n = 1), *C. famata* (n = 1), *C. kefyr*

(n = 4), *C. sake* (n = 2), *C. holmii* (n = 1), *C. lusitaniae* (n = 1), *C. intermedia* (n = 1), *C. atlantica* (n = 1), *C. maritima* (n = 1), *P. guillermondii* (n = 1) and *Pichia jadinii* (n = 1).

The results obtained using the disk diffusion method recorded in sabouraud dextrose agar were represented in Table 3. In the present study, the ethyl acetate solvent of *J. regia* L. leaves was most active against all *Candida* strains than the diluted acetone extract of *S. persica* stems. Comparatively to the standard antifungal drug, amphotericin B, it seems that *J. regia* ethyl acetate extract was most active against all *Candida* sp. strains tested with a diameter of inhibition zone ranging from 9.55 to 15 mm. In a previous study, Pereira and colleagues (2008) studied the anticandidal (*C. albicans* and *Cryptococcus neoformans*) potency of six walnuts (*J. regia* L.) cultivars. They founded that all the walnut varieties exhibited antifungal activity against the tested species. *C. albicans* was susceptible to five walnut varieties, only being resistant to cv. Mellanaise extract. The higher inhibition was observed with cv. Lara extract (MIC of 1 mg/mL). While *C. neoformans* was resistant to cv. Mellanaise extract at the tested concentrations (maximum of 100 mg/mL) and was very sensitive to cv. Franquette extract (MIC of 0.1 mg/mL). As expected, the standards ampiciline (antibacterial) and cycloheximide (antifungal) presented lower MICs than the walnut extracts. In the other hand, Alali et al. (2004) founded that Jordanian essential oil of *S. persica* stems revealed significant inhibition against *C. albicans* (16 mm) and *T. cutaneum* (12 mm). Water extract showed weak activity against all tested microorganisms except for *C. albicans* and the alcohol extract of the plant showed good activity against gram-negative bacteria and *C. albicans*.

Al-Bayati and Sulaiman (2008) founded that both aqueous and methanol extracts of *S. persica* were active against oral *C. albicans* strain with a MIC value 6.25 mg/ml. These antimicrobial and cleaning properties of *S. persica* may be attributed to various chemicals contained in the extracts such as sodium chloride, potassium chloride, vitamin C, salvadoura, salvadorine, saponins, silica sulfated compounds, isothiocyanate, tannins, tannic acid, and benzyl isothiocyanate and resin (Darout et al., 2000), cyanogenic or lignin glycosides (Kamel et al., 1992) and alkaloids, terpenoids, and oleic, linoleic and stearic acids.

While fluoride and calcium salts are quite effective in preventing dental caries (. In fact, Darout and co-workers (2000) reported potential antimicrobial anionic components in Saoudian *S. persica* root and stem water extracts. They founded that *S. persica* root and stem extracts contained chloride, sulphate, thiocyanate and nitrate in the following concentrations (w/w %) in stem and root extracts, respectively: 6.84 and 4.64%, 20.1 and 19.85%, 0.38 and 0.28%, and 0.05 and 0.05%. Moreover, the silica and calcium salts in the plant act as grinder and detergent (Al-Otaibi et al., 2003).

Antioxidant activities of *S. persica* and *J. regia* extracts

Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. Exogenous and endogenous whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. On the basis of all antioxidant tests used in his study, *J. regia* ethyl acetate extract exhibited high antioxidant activities comparatively to the diluted acetone extract of *S. persica*. In fact, the global antioxidant activity of the two plants extracts was expressed as the number of gallic acid equivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The study reveals that the antioxidant activity of walnut extract was 623 folds higher than that of arak extract (329 mg and 0.528 mg GAE.g⁻¹ DW; respectively). This strong antioxidant activity of walnut bark might be attributed to the presence of phytochemicals such as phenolic compounds. Interestingly, the total phenolic content was higher in the ethyl acetate extract of *J. regia* bark (34.833 mg GAE.g⁻¹ DW), while only 0.443 mg GAE.g⁻¹ DW was found in the diluted acetone extract of *S. persica* stems. Similarly, total condensed tannin content in walnut ethyl acetate extract was 41.4 folds higher than which found in arak diluted acetone extract (16.167 and 0.39 mg EC.g⁻¹ DW, respectively).

Comparing our results with the results obtained by Kornsteiner et al. (2006), that registered 1025 mg GAE/100 g, we concluded that Tunisian *J. regia* (bark) exhibited higher amounts of phenols in a ratio of 3.4 folds more. However, these authors also observed that walnut fruits showed the highest total phenolic contents when 10 nuts extracted the phenolic fraction with a solution of 75% acetone and 25% of 526 µmol/L sodium metabisulfite (almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans, pines, pistachios and walnuts) were studied. Probably, the differences in the results could be explained by the different extraction methodologies. In fact, Pereira et al. (2008) reported that the study of phenolic content present in different walnut cultivars aqueous extracts revealed values between 58.78 mg GAES/g in cv. Lara and 95.06 mg GAES/g in cv. Mayette. Scavenging activity on DPPH radicals assay provides basic information about the antiradical activity of extracts, and is expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. In fact, the determination of the concentration corresponding to 50% of inhibition of radical DPPH showed that the antiradical activity of ethyl acetate extract of *J. regia* (IC₅₀, 3 µg/ml) is very significant comparing to BHT as a synthetic

Table 4. Antioxidant activities of diluted acetone extract of *S. persica* and ethyl acetate extract of *J. regia*.

	<i>S. persica</i> (Diluted acetone extract)	<i>J. regia</i> (Ethyl acetate extract)	BHT
TAA: (mg GAE.g ⁻¹ DW)	0.528	329	-
Total Polyphenols: (mg GAE.g ⁻¹ DW)	0.443	34.833	-
Tannins : (mg EC.g ⁻¹ DW)	0.39	16.167	-
DPPH: IC ₅₀ (μg.ml ⁻¹)	75	3	11.5
β- carotenes: IC ₅₀ (μg.ml ⁻¹)	460	280	75
RP: EC ₅₀ (μg.ml ⁻¹)	1940	99	75
O ₂ ⁻ : IC ₅₀ (μg.ml ⁻¹)	*	70	1.5

TAA: Total Antioxidant Activity is expressed as mg gallic acid equivalents per gram of dry weight; Total Polyphenols is expressed as mg gallic acid equivalents per gram of dry weight; Tannins is expressed as mg (+)-catechin/g DW; DPPH radical-scavenging activity is expressed as IC₅₀ values (μg/ml); β-Carotens bleaching test is expressed as IC₅₀ values (μg.ml⁻¹); RP: reducing power was expressed as EC₅₀ values (μg/ml); O₂⁻: Superoxide anion radical-scavenging activity is expressed as IC₅₀ values (μg/ml).

antioxidant used in this study (IC₅₀, 11.5 μg/ml) (Table 4). While the value of IC₅₀ of the diluted acetone extract of *S. persica* was 75 μg/ml.

Souri et al. (2008) investigated the antioxidant activity against linoleic peroxidation and free radical scavenging activity on DPPH of 13 medicinal plants traditionally used in Iran for various disorders. These authors founded that methanol extract of *S. persica* have a weak free radical DPPH scavenging activity with an IC₅₀ value of 37.19±6 comparatively to Trolox (IC₅₀, 8.64±0.03) and Quercetin (IC₅₀, 5.22±0.13). On linoleic peroxidation, out of 13 plants tested, seven showed IC₅₀ values lower (20 to 30 times) than Quercetin (IC₅₀, 40.83±3 ng/ml) and all plant materials showed IC₅₀ values smaller than Trolox (IC₅₀, 97.34±0.66 ng/ml), while methanol extract of *S. persica* showed IC₅₀ value about 52.35±1.28 ng/ml). In the same year, 2008, Pereira and colleagues (2008) studied the chemical composition, antioxidant potential and antimicrobial activity of six walnuts (*J. regia* L.) cultivars (cv. Franquette, Lara, Marbot, Mayette, Mellanaise and Parisienne) produced in Portugal. They founded that all walnut extracts exhibited antioxidant capacity in a concentration-dependent manner being the lowest EC₅₀ values obtained with extracts of cv. Parisienne (0.16 mg/mL), and cv. Lara the one with the highest EC₅₀ value (0.26 mg/mL). Recently, Zhang et al. (2009) identified seven phenolic compounds in *J. regia* by spectroscopic methods which are pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid and 3,4,8,9,10-pentahydroxydibenzo(b,d)pyran-6-one, containing significant antioxidant activities. In fact, the relative order of DPPH- scavenging capacity for the isolated phenolic compounds was found to be: 3,4,8,9,10-pentahydroxydibenzo(b,d)pyran-6-one > gallic acid, Pethyl gallate and Ppyrogallol > TroloxP protocatechuic acid > vanillic acid > p-hydroxybenzoic acid.

The compounds 3,4,8,9,10-pentahydroxydibenzo(b,d)pyran-6-one, gallic acid, ethyl gallate and pyrogallol showed significant free radical scavenging capacities with IC₅₀ values of 0.007, 0.011, 0.013 and 0.015 mM,

respectively, all of which were much more active than Trolox (0.026±0.001). In the reducing power assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (that is antioxidants) causes the conversion of the Fe³⁺/ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe²⁺ concentration; a higher absorbance at 700 nm indicates a higher reducing power. Our results showed that the EC₅₀ (EC₅₀, 99 μg.ml⁻¹) of walnut ethyl acetate extract is 19.6 folds more potent than the EC₅₀ of *S. persica* diluted acetone extract (EC₅₀, 1940 μg.ml⁻¹) and closely related to the reducing power of the standard BHT (EC₅₀, 75 μg.ml⁻¹). Pereira et al. (2008) reported that walnut's kernel showed high reducing power, even at concentrations below 1 mg/mL, being even more potent than BHA (3.6 mg/mL) and α-tocopherol (8.6 mg/mL) standards.

The antioxidant activity of arak and walnut extracts measured by the bleaching of β-carotene is shown in Table 4. The linoleic acid free radical attacks the highly unsaturated β-carotene models.

The best result was obtained with ethyl acetate *J. regia* extract (IC₅₀, 280 μg.ml⁻¹) comparatively to the IC₅₀ value (IC₅₀, 460 μg.ml⁻¹) of diluted acetone *S. persica* extract. Studying the antioxidant activities of to six *J. regia* cultivars, Pereira et al. (2008) founded that the cultivar Parisienne presented the lowest EC₂₅ value (EC₂₅, 1.56 mg/ml). As seen in Table 4, the walnut ethyl acetate extract exhibit a considerable O₂⁻ scavenging activity (IC₅₀, 70 μg.ml⁻¹) but 46.6 folds less than BHT (IC₅₀, 1.5 μg.ml⁻¹).

Conclusion

Comparatively to *S. persica*, Tunisian walnut bark, which is largely used in our country to prevent oral hygiene, seems to be more efficient against several *Candida* strains and human pathogenic bacteria. These activities

may be correlated to the high phenolic content and tannins in the bark of *J. regia*. Moreover, the findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases.

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