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Original Article

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SIX ALGERIAN MEDICINAL PLANTS

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ABSTRACT

Objective: This work had two objectives: the first, to evaluate the total phenolic or flavonoid contents of six Algerian medicinal plants and second, to determine whether these compounds have antioxidant properties and antimicrobial potential.

Methods: The polyphenolic extractions of the dried powdered samples have been performed using 80 % methanol. Total phenols and flavonoids concentrations were analyzed using Folin-Ciocalteu's and aluminum chloride methods. The antioxidant activity was investigated with three different methods: the β-carotene bleaching (BCB) test, the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)free radical scavenging method and the ferric reducing activity power (FRAP) assay. Polyphenols from the medicinal plants were screened for their antibacterial activities against pathogenic microorganisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Esherichia coli*) using agar disc diffusion method and minimum inhibitory concentration.

Results: The total phenolic and flavonoids contents of the samples varied from (30.34±0.52) mg GAE/g extract to (602.71±2.01) mg GAE/g extract and from (16.61±0.04) mg QE/g extract to (21.91±0.31) mg QE/g extract, respectively. The antioxidant activity measurement, expressed as percentage inhibition of DPPH free radical, ranged from (44.00±0.06) % to (95.70±0.49) %. With further data analysis, it was found that there was a positive correlation between the total phenolic content of a given sample and its antioxidant activity (R^2= 0.8064). The results showed that a remarkable inhibition of the bacterial growth was shown against the tested microorganisms. The phytochemicals analyses of the plants were carried out. The microbial activity of the plants extracts was due to the presence of various active compounds.

Conclusion: These results suggest that phenolic compounds in these plants provide substantial antioxidant and antibacterial activities which vary to a great extent. Hence, these plants can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals research activities.

Keywords: Polyphenols, Antioxidant, Antibacterial, DPPH, FRAP, β-carotene.

INTRODUCTION

Food safety and organoleptic preservation property are a high topic for researcher and consumer. In a conventional way, chemical food additives, like preservatives and antioxidants, can be used. However, in the light of recent works, some of them as t-butyl hydroxytoluene (BHT) or 4-hydroxyanisole (BHA) used to prevent oxidative rancidity of fats, are a cause of concern. Several researchers reported the extensive use of these additives in the agro-alimentary industry. Restrictions on the use of these compounds are pronounced because of their carcinogenicity [1].

The natural antioxidants, more recently, have attracted the considerable attention of users and researchers. In this context, medicinal plants are being viewed as an easily available and potent source of antioxidants as they contain a mixture of different chemical compounds [2-5].

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced the number of new antibacterial in the last years, resistance to these drugs has increased and has now become a global concern. Due to the increase in resistance to antibiotics, there is a pressing need to develop new and innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated because they contain many bioactive compounds that can be of interest in therapeutic [6, 7].

Over the years and up to date, there have been numerous studies documenting the antibacterial, antifungal, antiviral, anticancer and anti-inflammatory properties of plant ingredients. Therefore, herbal-derived substances remain the basis for a large proportion of commercial medications used today in developing countries. When we refer to plants of medicinal value, we often list their active ingredients, which might include alkaloids, glycosides, essential oils, polyphenolic compounds and some other unusual substances [8].

Traditionally, olive tree leaves have been used as a folk remedy for combating fevers and other diseases, such as malaria. Previous investigations carried out on olive leaf extracts have demonstrated hypotensive, hypoglycemic, antimicrobial and antioxidant activities [9-13].

Aerial parts of *Pistacia lentiscus* have traditionally been used in the treatment of hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice. They possess stimulant and diuretic properties [14].

Celery (*Apium graveolens*) is an aromatic bitter tonic herb that reduces blood pressure, relieves indigestion, stimulates the uterus and is anti-inflammatory. It is also used as an effective remedy for various ailments such as bronchitis, liver and spleen disease, and arthritis pain [15].

Ziziphus lotus, also known as Jujube, is a medicinal plant largely found in the Mediterranean region including Algeria. Some parts of *Zizyphus* have been used by traditional and ancestral medicine for the treatment of much pathology including digestive disorders, weakness liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhea and insomnia [16].

Taraxacum officinalis (Family Asteraceae), commonly, known as dandelion is used for medicinal purposes because of its choleretic, diuretic, antioxidant, anti-inflammatory and hepatoprotective properties [17].

Hedera helix is a species of the family of Araliaceae. German Commission E approved *Hedera* leaves for the treatment of catarrhs of the respiratory tract and symptoms of chronic inflammatory

bronchial conditions. The leaves and berries were taken orally as an expectorant to treat cough and bronchitis [18, 19].

Several studies have already been realized on the bioactive compounds and antioxidant activity of *A. graveolens* [20], *H. helix* [21], *O. europaea* [22], *P. lentiscus* [23], *T. officinalis* [24] *and Z. lotus* [25]. However, there are no or not much information about the Algerian species. So far, to our knowledge, antioxidant compounds of these different plants were not investigated yet.

These justify the overwhelming interest in finding new naturally-occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants. Therefore, in recent years, considerable attention has been directed towards the identification of plant materials, with the antioxidant ability [26].

The aim of this study is to evaluate the antibacterial and antioxidant activities of *A. graveolens, H. helix, O. europaea, P. lentiscus, T. officinalis and Z. lotus*, plants growing in Chlef region, Algeria. In addition, assessing total phenolic contents of the plants aimed to investigate whether there is a relationship between phenolic content and antioxidant activity.

MATERIALS AND METHODS

Plant material

The plant material of the present study, fresh leaves of *A. graveolens O. europeae, P. lentiscus, Z. lotus, H. helix* and *T. officinalis*, were collected during the rainy season (between April and May, 2015) in Western Algeria (Chlef, 208 Km northwest of Algiers). The identification and authentication were done by Mr. Naji Omar, botanist at the Institute of agronomy, Hassiba Ben Bouali University, Chlef, Algeria, where voucher specimens were deposited. The plant raw material was cleaned of residual soil and air-dried at room temperature. Plants were ground to a fine powder using a laboratory mill, passed through a 24 mesh sieve to generate a homogeneous powder, stored at room temperature (22-23 °C), and protected from light until required for analyzes.

Preparation of extracts

The dried and coarsely powdered plant material (100g) was refluxed successively with methanol (80%) in a Soxhlet's extractor. Methanol extracts were defatted concentrated at 40 °C using a rotary evaporator under reduced pressure to give viscous mass [27]. The methanol extracts were weighed and stored at-4 °C before analyses.

DPPH• radical scavenging activity

The antioxidant activity of plants extracts and the standard was assessed by the method of Blois. [28]. A methanolic stock solution (50 µl) of the antioxidant (concentrations of stock solutions were 50, 100, 200, 400, 500, 1000, 2000, 3000 and 4000 µg/ml) was placed in a cuvette, and 2 mL of 6.0 $x10^{-5}$ mol/l methanolic solution of DPPH was added. The absorbance at 517 nm was determined by UV-visible spectrophotometer after 30 min for all samples. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e. the control, was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution.

The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh. [29]: % inhibition = $((AC_{(0)}-AA_{(t)})/AC_{(0)})$ x 100. Where $AC_{(0)}$ is the absorbance of the control at a t=0 min and $AA_{(t)}$ is the absorbance of the antioxidant at a t=30 min.

Ferric reducing antioxidant power (FRAP)

The FRAP procedure described by Benzie and Strain was followed [30]. The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 ml of a (10 mmol/l) TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mmol/l HCL plus 5 ml of $FeCl_3$ (20 mmol/l) and 50 ml of acetate buffer, (0.3 mol/l, pH=3.6), and was prepared freshly and warmed at 37ºC. Aliquots of 100 µL sample (concentrations of stock solutions were 50, 100, 200, 400, 500, 1000, 2000, 3000 and 4000 µg/ml) were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37ºC for 10 min. For construction of the calibration curve five concentrations of $FeSO_4.7H_2O$ (1000, 750, 500, 250, 125 mmol/l) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/l $FeSO_4.7H_2O$.

Beta-carotene bleaching (BCB) assay

Antioxidant activity of the leaves's extracts was determined according to a slightly modified version of the β-carotene bleaching method [31].

The β-carotene (2 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform. After evaporation to dryness, under vacuum at 50 °C by a rotary evaporator, oxygenated distilled water (50 ml) was added and the mixture was emulsified for 1 min in a Sonicator to form emulsion A. 200 µl of methanolic stock solution of each extract (concentrations of stock solutions were 50, 100, 200, 400, 500, 1000, 2000, 3000 and 4000 µg/ml) were mixed with 5 ml of emulsion A in open-capped cuvettes. A control was prepared by adding 200 µl of methanol instead of the extract.

A second emulsion (B) consisting of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 ml of oxygenated water was also prepared. Methanol (200 µl), to which 5 ml of emulsion B was added, was used to zero the spectrophotometer.

Readings of all samples were taken immediately (t=0) and at 30 min intervals for 120 min at 470 nm. The cuvettes were thermostated at 50 °C between measurements. Butylated hydroxytoluene (BHT) was used as the positive control. All determinations were made in triplicate. The percentage inhibition was calculated from the data with the formula:

$$\% \text{ inhibition} = [(A_{A(120)} - A_{C(120)})/(A_{C(0)} - A_{C(120)})] \times 100$$

Where $A_{A(120)}$ is the absorbance of the antioxidant at a t=120 min, $A_{C(120)}$ is the absorbance of the control at a t=120 min, and $A_{C(0)}$ is the absorbance of the control at a t=0 min.

Phytochemical screening

Freshmethanolic crude extracts were qualitatively screened for the following constituents: flavonoids, coumarins, hydrolysable tannins, alkaloids, terpenes, anthraquinones, and saponins [32]. The qualitative results of the methods have been rated from (+ve) for faint to (+++ve).

Total phenols

Total phenolic content of the leaves was calculated based on the Folin-Ciocalteu's method as modified by Singleton *et al.* [33]. Total phenols content was calculated assuming the calibration curve of gallic acid. Correlation analyses of antioxidant activity versus the total phenolic content were performed using the correlation and regression program in SPSS 20.0.

Total flavonoids

Total flavonoids content was measured by the aluminum chloride colorimetric assay [34]. Total flavonoids content was expressed as mg quercetin equivalents (mg QE)/g extract.

Test microorganisms

Clinical strains from nosocomial and community-acquired infections were isolated, identified and characterized by conventional biochemical methods.

Culture media

Mueller-Hinton Agar was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate to12 cm x 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use [35].

Antibacterial assay

Antimicrobial activity of each plant extract was determined using a modified Kirby-Bauer disc diffusion method [36].

Briefly, suspension of micro-organisms was made in sterile normal saline and adjusted to 0.5 McFarland standards (10^8 CFU/ml). Each plant extract was dissolved in Dimethyl Sulfoxide (80 mg/ml) and 100 µl of the bacterial suspension was spread onto nutrient agar plates. The extracts were tested using 6 mm sterilized filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4 °C for two hours before incubation with the bacterial test agents at 37 °C for 24 h. Following this incubation, the diameters of the inhibition zones were measured in millimeter.

All measurements were to the closest whole millimeter. Each antibacterial assay was conducted in triplicate. Mean values (±standard deviation) are reported in this study. Standard discs of Gentamycin (10 µg) served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of DMSO were used as a negative control.

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

The MIC value of the extract was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37 °C. For the determination of MBC, a portion of liquid (50 µl) from each plate well that exhibited no growth were taken and then incubated at 37 °C for 24 h. The lowest concentration that revealed no visible bacterial growth after sub-culturing was taken as MBC [37].

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA), and the differences between means were evaluated by Duncan's Multiple Range Test. SPSS statistic program (Version 20.0) was used for data analysis (*P<0.05).

RESULTS AND DISCUSSION

DPPH• Radical scavenging activity

Fig. 1 shows the antioxidant activity of the tested extracts and the positive control (Ascorbic acid), expressed as the percentage of deactivation of the DPPH free radicals.

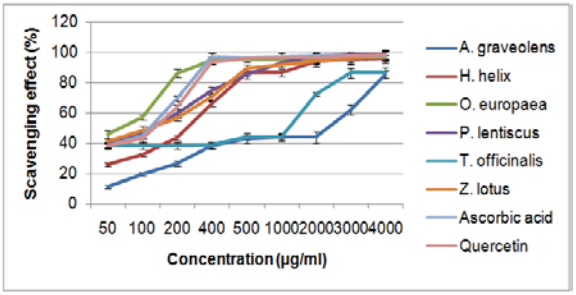

Fig. 1: Scavenging effect of different leaves extracts on DPPH-free radical. Bars represent the standard deviation from triplicate determinations

Table 1: Scavenging effect of different plants extracts on DPPH-free radical (%)

Concentration (µg/ml)	*A. graveolens*	*H. helix*	*O. europaea*	*P. lentiscus*	*T. officinalis*	*Z. lotus*	Ascorbic acid	Quercetin
50	11.46±2.45	25.95±1.02	45.79±2.40	38.84±1.89	38.21±1.59	41.27±1.79	38.20±1.59	38.30±2.48
100	19.56±2.59	32.68±1.26	57.25±1.87	46.92±1.23	38.34±1.89	48.62±2.49	44.98±1.89	43.22±2.54
200	26.67±2.25	43.58±1.89	86.25±2.59	59.75±2.98	38.45±1.48	56.84±2.12	69.68±1.59	63.99±1.45
400	38.77±1.48	66.23±2.02	94.68±2.36	74.80±2.73	38.54±1.45	70.43±2.48	97.26±2.46	93.78±2.01
500	42.98±3.45	86.78±2.58	95.18±1.28	86.00±3.48	44.25±2.58	89.71±2.48	96.63±1.27	96.23±1.22
1000	44.08±2.16	86.80±2.48	95.70±2.46	93.29±2.79	44.41±1.56	91.71±1.75	97.30±2.19	96.70±1.25
2000	44.11±2.45	94.47±3.85	95.79±2.45	98.12±1.48	72.53±2.58	94.05±1.25	97.72±2.45	97.15±1.75
3000	62.24±3.48	95.28±3.49	96.30±2.58	98.70±1.48	86.45±3.59	95.90±3.48	97.82±1.16	98.23±2.18
4000	85.47±2.17	95.95±2.58	97.24±2.89	98.76±3.48	86.72±2.89	97.72±3.49	97.97±2.75	98.75±2.49

Data are mean (n = 3)±SD.

O. europaea, *Z. lotus,* and *P. lentiscus* leave extracts exhibited the greatest activity, while *H. helix, T. officinalis* and *A. graveolens* had the lowest activity. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [38]. The reduction activity is usually proportional to the concentration [39, 40]. At a concentration of 500 µg/ml, *O. europaea, Z. lotus, H. helix and P. lentiscus,* leaves extracts expressed a percentage inhibition that is close to values observed for ascorbic acid: (95.18±1.28) %, (86.00±3.48) % and (89.71±2.48) % respectively. However, the scavenging effects of *A. graveolens,* and *T. officinalis* were (42.98±3.45) % and (44.25±2.58) % respectively (table 1). Excellent scavenging effects, (95.7±2.46) %, (93.29±2.79) % and (91.71±1.75) %, were observed with methanolic extracts from *O. europaea, P. lentiscus* and *Z. lotus* leaves at 1000 µg/ml, respectively. At 2000 µg/ml, the methanolic extracts from *O. europaea, P. lentiscus, Z. lotus* and *H. helix* leaves scavenged DPPH radical by (95.79±2.45) %, (98.12±1.48) %, (94.05±1.25) % and (94.47±3.85) %, respectively, whereas scavenging effects of methanolic extracts from other plants were (44.11±2.45) % and (72.53±2.85) % for *A. graveolens* and *T. officinalis* respectively. In addition, at 4000 µg/ml, methanolic extracts from *O. europaea* and *Z. lotus* scavenged DPPH radical by (97.24±2.89) % and (97.72±3.49) %, respectively, whereas those from *P. lentiscus* scavenged DPPH radical by (98.70±1.48) % at 3000 µg/ml and (98.76±3.48) % at 4000 µg/ml, respectively. Nevertheless, *A. graveolens* leaves extract was not so effective in scavenging DPPH radical (85.47 %±2.17 % at 4000 µg/ml).

It was described that the scavenging effects of *O. europaea* leaves methanolic extracts on DPPH radicals increased with the concentration increase and were high (87.10 % to 100 % at 5000 µg/ml), and comparable to the radical scavenging activity values obtained for the standards BHA (96% at 3.6 mg/ml) and α-tocopherol (95 % at 8.6 mg/ml) [41]. From the present results, it may be postulated that the scavenging effects of methanol extracts from medicinal plants on DPPH radical increased with the increased concentrations.

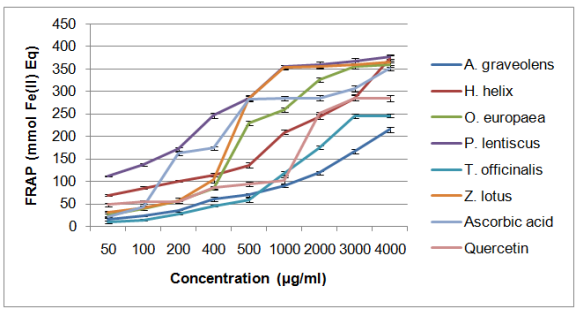

Fig. 2: Ferric reducing antioxidant power of different leaves extracts and ascorbic acid. Bars represent the standard deviation from triplicate determinations

Ferric reducing antioxidant power

Fig. 2 shows the reducing power of leaves methanolic extracts, as a function of their concentration.

The reducing power of the leaves methanolic extracts increased with concentration. Reducing powers of the different leaves were higher than 215.19 m moL Fe(II) Eq/g extract at 4000 µg/ml, and in the order of *P. lentiscus>H. helix>Z. lotus>O. europaea>T. officinalis>A. graveolens.*

Examining the FRAP results, *P. lentiscus, H. helix, Z. lotus* and *O. europaea* were the plants with the highest ferric reducing antioxidant power ((376.38±4.47) m moL Fe(II) Eq/g extract, (373.58±4.25) m moL Fe(II) Eq/g extract, (364.65±5.56) m moL Fe(II) Eq/g extract and (358.46±5.46) m moL Fe(II) Eq/g extract, respectively) at low concentrations (1000 µg/ml), followed by *T. officinalis* ((284.92±5.59) m moL Fe(II) Eq/g extract), whereas *A. graveolens* exhibited the lowest reducing power values for the FRAP assay ((215.19±3.25) m moL Fe(II) Eq/g extract) ($p<0.05$).

This result was in agreement with DPPH and BCB radical scavenging activities. However, FRAP of *A. graveolens* extracts was found to differ slightly from those found from DPPH and BCB assays.

There are many methods that differ in terms of their assay principles and experimental conditions, and particular antioxidants have varying contributions to total antioxidant potential [42]. In this study, we used the FRAP assay because it is quick and simple to perform to measure the antioxidant capacity.

In this assay, both extracts and standard compounds interfered with the formation of a ferrous complex with the TPTZ reagent, suggesting that it has chelation activity and capture the ferrous ion before TPTZ. The data obtained from fig. 2 reveal that the extracts demonstrate an effective capacity for iron binding, suggesting that its action as an antioxidant may be related to its iron-binding capacity. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds, which also related the presence of reductant agent. In addition, the number and position of the hydroxyl group of phenolic compounds also rule their antioxidant activity [43, 44].

The most commonly used antioxidant method is DPPH which characterized by excellent reproducibility under certain assay conditions. DPPH can only be dissolved in organic media, especially in ethanol, this being a major limitation when interpreting the role of hydrophilic antioxidants. However, the ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferroin analogue, the Fe^{3+}complex of tripyridyl triazine Fe $(TPTZ)^{3+}$to the intensely blue-coloured Fe^{2+}complex $Fe(TPTZ)^{2+}$by antioxidants in an acidic medium. However, the reducing capacity does not necessarily reflect antioxidant activity [45].

Beta-carotene bleaching (BCB) assay

The methanol extract of *Z. lotus* leaves inhibited linoleic acid oxidation to the greatest extent and had the finest DPPH antioxidant capacities relative to other extracts.

Statistically, BHT was found to possess the better degree of antioxidant activity than the extracts at each concentration point. It can be observed from fig. 3 that BHT presented an antioxidant activity of (96.10±4.59) % which is slightly higher than that of *Z. lotus, P. lentiscus and A. graveolens* which exhibited the highest antioxidant activity of the extracts tested, with (65.10±2.84) %, (73.50±2.95) % and (71.05±4.28) % at 1000 µg/ml and extended to (93.43±2.45) %, (90.13±3.26) % and (82.40±3.48) % at 4000 µg/ml. The other extracts namely, *H. helix* and *O. europaea* also showed strong activities of (75.85±2.59) % and (71.76±3.28) % at 4000 µg/ml (table 2). It was noted that the antioxidant activities of the tested samples were dependent on their concentrations.

Table 2: Antioxidant activity of the plants extracts as assessed with β-carotene bleaching method (%)

Concentration(µg/ml)	*A. graveolens*	*H. helix*	*O. europaea*	*P. lentiscus*	*T. officinalis*	*Z. lotus*	BHT
50	34.55±2.25	16.70±2.59	24.65±2.25	60.94±2.68	21.20±2.59	50.75±2.58	93.11±5.69
100	52.93±2.28	42.13±3.69	31.06±2.58	65.94±2.48	32.30±3.58	54.31±3.52	93.26±5.68
200	54.50±3.86	50.45±3.59	42.63±3.25	66.17±2.95	43.20±4.25	55.68±4.85	93.59±2.48
400	56.47±3.58	58.08±3.48	51.63±3.89	70.30±2.95	54.60±5.69	57.43±5.92	95.16±3.59
500	63.23±3.95	60.36±4.29	56.50±2.58	72.71±1.29	56.00±2.48	59.80±3.58	95.30±4.28
1000	71.05±4.28	61.03±5.96	59.08±3.56	73.50±2.95	60.12±3.86	65.10±2.84	96.10±4.59
2000	71.45±2.29	62.50±3.59	61.56±2.89	86.21±2.45	61.65±2.59	77.45±2.85	97.30±5.29
3000	81.40±3.75	63.61±2.24	68.83±3.58	87.40±2.57	63.26±3.59	87.35±3.69	98.10±3.59
4000	82.40±3.48	75.85±2.59	71.76±3.28	90.13±3.26	65.95±4.29	93.43±2.45	100.0±3.45

Data are mean (n = 3)±SD.

These results suggest that extracts may be potential antioxidants. In this model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The antioxidant activities of the extracts can be attributed to the mechanism: polyphenolic compounds associated in neutralizing the linoleate free radical and other free radicals formed in this model system, which oxidize unsaturated β-carotene molecules.

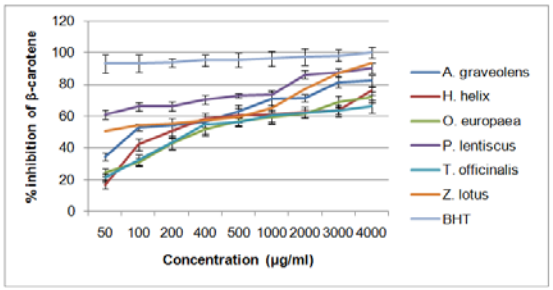

Fig. 3: Antioxidant activity of the leaves extracts as assessed with β-carotene bleaching method. Bars represent the standard deviation from triplicate determinations

At 1000 µg/ml, *P. lentiscus* ((73.50±2.95) %) and *A. graveolens* ((71.05±4.28) %) showed the highest antioxidant activity by the β-carotene bleaching method, while *O. europaea* ((59.08±3.56) %) presented the lowest result. One factor that may influence this analysis is the medium where the reaction occurs, characterized as an emulsion, with polar and non-polar regions simultaneously. Therefore, depending on the polarity of the sample, it can interact more or less intensely with the emulsion [46].

Carotenoids are also classified among the basic constituents of the antioxidative effect. Celery is rich in beta-carotene. It contains delta limonene, salience, and various sesquiterpene [47]. The results obtained by Mezni [48] highlight the richness of carotenoids, tocopherols and unsaturated fatty acids in *P. lentiscus* seed oil.

Among the methodologies used in this study to evaluate antioxidant activity, the results reveal the lack of consistency. This fact could be explained by the different characteristics and mechanisms of action of bioactive compounds present in the samples as well as the different principles used to detect antioxidant properties in each method.

Fig. 4 shows the decrease in absorbance of β-carotene in the presence of different leaves extracts. The control sample without addition of antioxidant oxidized most rapidly and descending bleaching rate were demonstrated in the presence of *T. officinalis>O.*

europaea>H. helix>A. graveolens>P. lentiscus>Z. lotus, indicating that the phytochemicals composition and concentration significantly influenced the antioxidant activity of the extract.

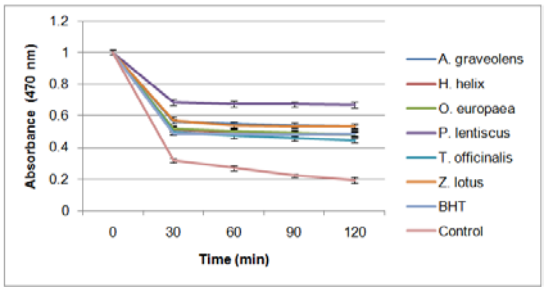

Fig. 4: Rate of β-carotene bleaching in control without antioxidant, with different plants extracts and with BHT. Bars represent the standard deviation of triplicate determinations

Similar results of BCB for different leaves extracts were generally observed compared with those of DPPH and FRAP radical scavenging activity. Even though, slight differences in activities determined by DPPH and BCB assays were observed for *A. graveolens* extract. The result suggested that those extracts might scavenge different radicals differently. Nevertheless, it was postulated that antioxidative compounds were most likely hydrophilic and could be extractable by water/methanol solution. The water-soluble fraction from different plants extracts could scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction [49, 50].

Phytochemicals screening

In the present study, leaves of these six species, tested negative for anthraquinones and coumarins, while positive for flavonoids (++ve), tannins (++ve) which was in parallel with polyphenolics content results (table 3), saponins(+++ve) with strong positive results indicating their richness of alkaloids (++ve) and strong for terpenes (+++ve only for *A. graveolens and P. lentiscus*).

Table 3: Phytochemical screening

Phytochemical compounds	*A. graveolens*	*H. helix*	*O. europaea*	*P. lentiscus*	*T. officinalis*	*Z. lotus*
Alkaloids	++	+++	+++	++	+++	+++
Saponins	+++	+++	+++	+++	+++	+++
Anthraquinones	-	-	-	-	-	-
Tannins	+++	++	+++	+++	+++	++
Terpenes	+++	++	++	+++	++	++
Flavonoids	++	++	++	++	++	++
Coumarines	-	-	-	-	-	-

Total phenols

Total phenols are reported as gallic acid equivalents (GAE) by reference to a standard curve ($y = 0.0032x + 0.0303$, $R^2 = 0.9903$). Among the six extracts, *O. europaea* extract had total phenols content, as measured by Folin-Ciocalteu reagent, of (602.71±2.01) mg GAE/g extract, followed by *P. lentiscus* ((223.21±1.52)mg GAE/g extract), *Z. lotus* ((207.52±1.92) mg GAE/g extract) *H. helix* ((131.25±1.54) mg GAE/g extract), *T. officinalis* ((58.38±0.95) mg GAE/g extract) and *A. graveolens* leaves extract ((30.34±0.52) mg GAE/g extract) (fig. 5).

Our quantitative analyses examined the correlation between antioxidant activity and phenolic compounds content in different extracts (table 4), with percentage of deactivation of the DPPH free radicals values taken to indicate antioxidant activity; correlation coefficients were calculated (R^2= 0.8064, $y = 0.0945x + 44.174$), and the correlations (antioxidant activity vs. total phenolics) are shown in fig. 6.

Table 4: Total phenolic (expressed as mg Gallic acid equivalents/g extract) and flavonoid contents (expressed as mg Quercetin equivalents/g extract) from different leaves extracts.

Plant	Total phenols[a]	Total flavonoids[a]
A. graveolens	30.34±0.52	16.61±0.04
H. helix	131.25±1.54	18.61±0.37
O. europaea	602.71±2.01	19.32±0.34
P. lentiscus	223.21±1.52	20.98±0.57
T. officinalis	58.38±0.95	19.62±0.64
Z. lotus	207.52±1.92	21.91±0.31

[a]mean±SD, n = 3.

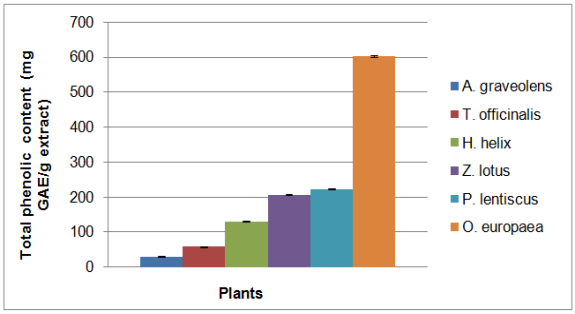

Fig. 5: Total phenolic content (mg GAE/g extract) in different plants extracts. Bars represent the standard deviation of triplicate determinations

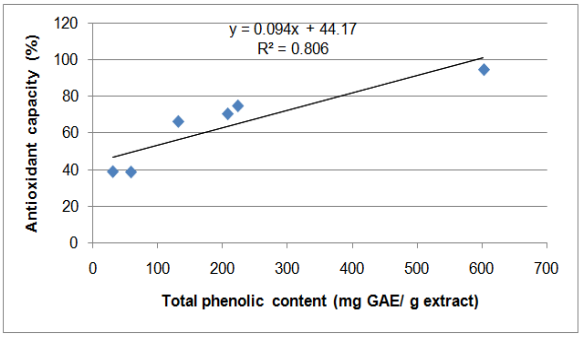

Fig. 6: Correlations of antioxidant capacity values with total phenolics in different extracts

Elevated antioxidant activity corresponded to high total phenolics in the tested extracts. This result suggests that the antioxidant activity of *O. europaea*, *P. lentiscus*, *Z. lotus* and *H. helix*, extracts is due to the contribution of phenolic compounds and that the antioxidant activity of these plant extracts is not limited to phenolic compounds but may also be related with the presence of other antioxidant secondary metabolites such as carotenoids and vitamins. The results of the present study showed that *O. europaea, P. lentiscus, Z. lotus* and *H. helix* leaves are rich in phenolic constituents and demonstrate good antioxidant activity measured by different methods.

The enhanced antioxidant activity of *P. lentiscus, O. europaea, Z. lotus* and *H. helix* could be attributed to its flavonoid content. Flavonoids act as scavengers of various oxidizing species i.e. superoxide anion, hydroxyl radical or peroxy radicals; they also act as quenchers of singlet oxygen [51]. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity [52]. The positive correlation between the polyphenolic content of plants extracts and their antioxidant activity is well documented [53]. Therefore, the content of total phenolic compounds in the extracts might explain their high antioxidant activities. In this study, the extracts exhibited a concentration-dependent antiradical activity by inhibiting DPPH radical. One of the possible mechanisms is poly phenolic associated compounds (the formation of a non-extractable complex between high molecular weight phenolics and compounds). Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxide.

Total flavonoids

Flavonoids concentrations are reported as quercetin equivalents by reference to a standard curve ($y = 0.0108x - 0.117$, $R^2 = 0.9732$). Results, as presented in table 4, revealed that the level of flavonoids in the leaves extracts was considerable. *A. graveolens* ((16.61±0.04) mg QE/g extract), *H. helix* ((18.61±0.37) mg QE/g extract), *O. europaea* ((19.32±0.34) mg QE/g extract) and *T. officinalis* ((19.62±0.64) mg QE/g extract) all contained less total flavonoids than *P. lentiscus*and *Z. lotus* leaves extracts ((20.98±0.57)mg QE/g extract and (21.91±0.31) mg QE/g extract, respectively).

Antibacterial assay

In this section, we have tested the methanolic extracts of six plants for their antimicrobial activity against gram-positive and gram-negative strains.

The antibacterial activity showed a significant reduction in bacterial growth in the term of the zone of inhibition, indicating that the plants exhibited antimicrobial activity against the microorganisms selected, and the zones of inhibition were recorded and presented in the tabulation drawn (table 5).

Table 5: Antibacterial activity of the leaves extracts using disc diffusion assay

	E. coli	***P. aeruginosa***	***S. aureus***
A. graveolens	0	0	0
H. helix	12.6±1.23	7.3±0.98	9.3±0.84
T. officinalis	0	0	0
P. lentiscus	12.5±0.63	13.9±1.51	12.2±0.42
Z. lotus	17.8±1.58	15.0±1.42	26.3±1.66
O. europaea	0	0	35.5±1.28
Gentamycin	32.4±0.58	28.1±0.73	38.6±1.16

Data are mean length of inhibition zones (mm) (n = 3)±SD.

Four of the six extracts tested showed antibacterial activity against one or more bacteria. Extracts (*H. helix, P. lentiscus, O. europaea* and *Z. lotus*) were versatile, being capable of inhibiting the growth of all three bacteria tested. Inhibition zones of the tested extracts were in the range from 7.3-35.5 mm.

Differences were observed between antibacterial activities of the extracts, *Z. lotus* and *O. europaea* extracts possessed slightly higher antimicrobial activity in comparison to the other tested samples. These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects, including antibacterial and antiviral properties [54].

The overall data from this study were in accordance with previous results. The antibacterial activity of *H. helix* is well known its methanol extract thus is the most active, showing activity against three selected Gram-positive and two Gram-negative bacterial strains [55].

Recent studies have reported the antibacterial activity of extracts from different plant species; Mezni [56] studied the antimicrobial activity of *P. lentiscus* edible oil and its phenolic extract. The phenolic extract had the largest spectrum of sensitive microorganisms. The minimum inhibitory concentration and minimum bactericidal concentration results showed that all strains were inhibited by both oil and extract.

Antimicrobial and antioxidant potentials *Z. lotus* was evaluated by Naili [57]. The crude methanolic leaves extract was appreciably active against Gram-positive species, associated with the weak anti-Gram-negative activity.

At low concentrations, olive leaves extracts showed an unusual combined antibacterial and antifungal action [58]. Also, Ahmed [59] investigated the antibacterial activity of crude *O. europaea* leaves extract depending on the decreased bacterial counts in samples of raw peeled undeveined shrimp kept in escalating concentrations of the plant leaves extract. Essentially, application of *O. europaea* leaves extract at 2% (w/v) concentration had a beneficial effect in controlling the microbial load of raw peeled undeveined shrimp.

Table 6: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of plants extracts

Bacterial cultures	***E. coli***		***P. aeruginosa***		***S. aureus***	
Plants extracts	**MIC[a]**	**MBC[b]**	**MIC[a]**	**MBC[b]**	**MIC[a]**	**MBC[b]**
A. Graveolens	nt	nt	nt	nt	nt	Nt
H. helix	97	200	781	1600	390	920
T. officinalis	nt	nt	nt	nt	nt	Nt
P. lentiscus	97	195	97	190	97	220
Z. lotus	48	95	48	102	6	15
O. europaea	nt	nt	Nt	nt	3	7

[a]Minimal inhibitory concentration (µg/ml of extract)., [b]Minimal bactericidal concentration (µg/ml of extract)., nt: Not tested

Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with proteins related polyamides polymers. The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes. Phenolic compounds notably proanthocyanidins (often called condensed tannins) is vulnerable to polymerization in air through oxidization reactions. Therefore, an important factor governing their toxicity is their polymerization size. Oxidized condensation of phenols may result in the toxification of microorganisms. On the other hand, polymerization can result in the detoxification of phenols [60].

This supports the fact that polyphenols may be responsible for the antimicrobial activities of extracts of the screened plants.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of plants extracts.

Minimal Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit the growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganisms to an antimicrobial agent and it monitors the activity of new antimicrobial agents.

MIC values against the tested gram-positive bacteria ranged from 3 to 390 µg/ml and against gram-negative bacteria from 48 to 1600 µg/ml. Antibacterial potential of plants extracts against these bacteria expressed in MIC indicated the plants extracts is more effective against gram-positive at a lower concentration than that against gram-negative bacteria.

Moreover, it was noted that most of the antibacterial properties in different plants extracts shows, MBC value that is almost two-fold higher than their corresponding MICs.

O. europaea and *Z. lotus* were proved to have the highest activity at 3 µg/ml and 6 µg/ml, against *S. aureus*, respectively. The relatively high activity of *Z. lotus* at 48 µg/ml was observed against E. coli and *P. aeruginosa*.

CONCLUSION

These plants, rich in flavonoids and phenolics could be a good source of natural antioxidant and antibacterial activities. Therefore, qualitative and quantitative analysis of major individual phenolics in these leaves could be useful for explaining the relationships between total antioxidant capacity and total phenolic contents in these plants.

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CONFLICT OF INTERESTS

All authors have none to declare.

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