



EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS OBTAINED FROM TWO ALGERIAN MEDICINAL PLANTS:

Mentha rotundifolia AND *Satureja calamintha*

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In this work phytochemical characterization of two medicinal plants from Lamiaceae family, *Mentha rotundifolia* and *Satureja calamintha*, has been carried out. Extracts obtained with different solvents were screened for different plant secondary metabolites and were biologically characterized by defining their antiradical and antibacterial activities. Phytochemical screening of *M. rotundifolia* and *S. calamintha* confirmed their richness in different secondary metabolites. The determination of phenolic compounds revealed high polyphenols contents in water: methanol (30:70) extracts with concentrations of 20.64 ± 1.74 mg EAG/g DW and 13.45 ± 0.91 mg EAG/g DW for *M. rotundifolia* and *S. calamintha*, respectively. These extracts were also characterized by high concentrations of flavonoids (*Mentha rotundifolia* 12.33 ± 1.58 mg EQ/g DW, *Satureja calamintha* 7.11 ± 0.02 mg EQ/g DW). Furthermore, the water: methanol (30:70) extract of *M. rotundifolia* was the most effective in inhibiting free radicals. Recorded inhibition diameters for both plant samples and tested microbial strains ranged from 6.66 mm to 13.66 mm. Presented results confirmed that tested indigenous Algerian plants are favorable sources of polyphenols with antioxidant and antimicrobial properties.

Keywords: *Mentha rotundifolia*, *Satureja calamintha*, phytochemical screening, extraction, phenolic compounds, antioxidant activity, antibacterial activity.

INTRODUCTION

Medicinal plants have been used since ancient times in folk medicine to prevent or treat different diseases. In fact, their therapeutic properties are due to the presence of hundreds or even thousands of natural bioactive compounds, most often plant secondary metabolites (1). Algeria has an important plant heritage characterised by its richness and diversity. In coastal areas, mountain ranges, high plateaus, steppes and Saharian oases,

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more than 3000 plant species have been identified. Among these natural resources, aromatic and medicinal plants, such as *Mentha rotundifolia* and *Satureja calamintha*, occupy large space (2).

Mentha rotundifolia or mint, has a special place in herbal medicine owing to its tonic, aromatic and digestive properties used to relieve colic, nausea, diarrhea and Crohn's disease (3). Bioactive compounds from mint demonstrate also hypotensive and vasodilating activities, as well as effects on sympathetic nerve centers (relaxing, stimulating, depressing). It's antibacterial, antifungal and antimalarial properties, have also been reported (4).

The genus *Satureja*, belonging to the Lamiaceae family, comprises about 30 species distributed in Tropical Africa, Europe and North America (5). *Satureja calamintha* subsp. *nepeta* (L.) Briq enjoys great popularity in Algeria as a traditional cure for coughs, impaired digestion and mild respiratory infections. In addition to acting as expectorant, stomachic and tonic, the plant has antiseptic, antispasmodic and carminative properties (6). *Satureja* species are also used as potent disinfectants and odoriferous agents in perfumes (7).

In this work, two plants used in traditional Algerian medicine, namely *Mentha rotundifolia* and *Satureja calamintha* were studied. Their secondary metabolites were extracted using different solvents (methanol, water:methanol (30:70) and water). Total polyphenols, flavonoids and tannins were quantified and their contents were related to antioxidant and antibacterial activities of the extracts. Antioxidant properties of extracts were characterised by DPPH and ABTS radical scavenging tests. Antibacterial activity was screened for different bacterial strains. The extracts were also subjected to phytochemical screening to reveal major plant constituents responsible for the reported activities.

EXPERIMENTAL

Samples

In this work aerial parts of *S. calamintha* and *M. rotundifolia* were studied. *M. rotundifolia* was harvested in Jijel and Bejaia regions (North East of Algeria) during the period from February to April 2018, while *S. calamintha* was harvested in the Jijel area during the same period. The stems and leaves of the samples were air dried at room temperature during 15 days and were powdered and stored at 4 °C for further analysis.

Chemicals and reagents

Gallic acid, butylhydroxytoluene (BHT), Folin-Ciocalteu, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Methanol, acetic and hydrochloric acids, ammonia, ferric trichloride, dimethyl sulfoxide (DMSO) were obtained from Merck (Germany), Rectapur, Cheminova (France) and Fluka. Mueller-Hinton agar and broth were obtained from Pasteur Institute (Algeria).



Phytochemical screening

Phytochemical screening was performed to detect different chemical classes in decoctions and infusions of the samples, using the method as described by Bruneton (8). In general, the method relies on specific chemical reaction between specific chemical class and reagents.

Polyphenols

Free flavonoids

A few drops of concentrated HCl and a few milligrams of magnesium were added to 1 ml of the infusion. The reaction gives an orange-red coloration in the presence of flavonoids.

Anthocyanins

The identification of anthocyanins was done by adding 10 drops of ammonia to 5 ml of the plant infusion. The reaction gives a greenish-blue coloration.

Leuco-anthocyanins

Powdered plant material (2 g) was introduced into 20 ml of a propanol/chloric acid (v/v) mixture. The solution thus obtained was then placed in a water bath (100°C) for a few minutes. In the presence of leucoanthocyanins a red coloration develops.

Tannins

Total tannins

A few drops of ferric chloride solution (5%) were added to 5 ml of the infusion. The positive reaction gives a black or greenish blue coloration in the presence of tannins.

Gallic tannins

Sample infusion (5 ml) was introduced into a vial using a graduated pipette and then supplemented with 2 g of sodium acetate and few drops of ferric chloride solution (1%). After stirring, a dark blue coloration appears in the presence of gallic tannins.

Condensed tannins

Five milliliters of the sample infusion were added to 2 g of ammonium acetate and three drops of ferric chloride solution (1%) after what the mixture was shaken. The appearance of a blue-black coloration indicated the presence of catechetical tannins.



Free quinones

Powder plant material (2 g) was moistened with 2 ml of hydrochloric acid (1 M) which was in contact with 20 ml of chloroform during three hours. The mixture was filtered and then stirred with 5 ml of ammonia. The appearance of red coloration indicated the presence of free quinones.

Reducing sugars (Fehling's test)

Twenty drops of the Fehling reagent were added to 1 ml of infusion and 2 ml of distilled water. A positive reaction was characterized by the appearance of a brick red precipitate.

Cardiac glycosides

Two milliliters of the infusion were mixed with 2 ml of chloroform and concentrated sulfuric acid was carefully added. The formation of a dark reddish brown layer at the interface indicated the presence of cardiac glycosides.

Alkaloids

A mixture of 5 ml of infusion, 2 ml of HCl and 1 ml of Dragendroff reagent gives a red or orange precipitate in the presence of alkaloids.

Coumarins

The presence of coumarin was revealed after mixing 5 ml of the infusion and 0.5 ml of ammonia (25%). An observation of the fluorescence under an ultra violet lamp at 365 nm indicated the presence of coumarin.

Saponosides

Hydrochloric acid (5 ml, 0.1 M) and 5 ml of NaOH (0.1 M) were introduced separately into two test tubes. The tubes were shaken after addition of few drops of the infusion. Foam formation indicated the presence of saponosides.

Heteroglycosides

O-heterosides

Distilled water (5 ml) and 0.5 ml of HCl were mixed with powdered plant material previously put in contact with CHCl_3 . Then, the mixture was heated for 15 minutes. After cooling and filtration, 2.5 ml of CHCl_3 were added and the organic phase thus formed was separated. The appearance of a brown color after addition of 0.5 ml of diluted ammonia indicated the presence of O-heterosides.



C-heterosides

Distilled water (10 ml) and 1 ml of ferric chloride solution (10%) were added to the aqueous phase previously obtained for O-heterosides. The mixture was heated for 30 minutes in a water bath and then cooled. The organic phase was separated after mixing with 5 ml of diluted NH_4OH (50%). The appearance of, more or less intense red coloration, indicated the presence of C-heterosides.

Sterols and triterpenes

Five milliliters of the infusion were added to 5 ml of acetate anhydride. After that 1 ml of H_2SO_4 was added to the bottom of the tube without stirring. The formation of a brownish red ring at the contact area of the two liquids and a purple coloration of the supernatant layer indicated the presence of sterols and triterpenes.

Extraction of phenolic compounds

Twenty grams of powdered stems and leaves of the samples were macerated separately with 250 ml of different solvents (pure methanol, water-methanol (30/70) and water) for 24 h. The obtained extracts were filtered through Wattman paper and solvents were evaporated under reduced pressure at 40° C using rotary vacuum evaporator (RV 8 IKA, Belgium). After evaporation, the residues were dissolved in small volume of the adequate solvent and kept in sterile vials at 4°C until use.

Determination of total polyphenols

Sample (100 μl) was dissolved in 500 μl of Folin-Ciocalteu reagent (diluted 1/10) and 1 ml of distilled water was added. The solution was mixed and incubated at room temperature for 1 min. After that 1500 μl of 20% sodium carbonate solution was added. The mixture was shaken and incubated for 2 hours in the dark at room temperature. The absorbance of the samples was measured at 760 nm using spectrophotometer (Varian 50 Tablet, USA) and the results were expressed in milligrams of gallic acid equivalent (GAE) per gram of dry plant matter. A calibration curve was defined using gallic acid as a standard (9).

Determination of flavonoids

Total flavonoids were determined by mixing 500 μl of the extract with 2 ml of distilled water and 0.15 ml of sodium nitrite solution (15% w/v). After 6 minutes at room temperature, 0.15 ml of aluminum chloride solution (10% w/v) was added. After 6 minutes of incubation at room temperature, 2 ml of sodium hydroxide solution (4 % w/v) were added to the mixture adjusting the volume to 5 ml with distilled water. The mixture was agitated and incubated for 15 min and the absorbance was measured at 510 nm (10). The results were expressed in milligrams of quercetin equivalent (EQ) per gram of dry plant matter.



Determination of condensed tannins

Condensed tannins were determined using the vanillin method in an acidic medium. A crude extract (50 μ l) was added to 1500 μ l of the vanillin/methanol solution (4%, m/v) and then mixed using a vortex. Then, 750 μ l of the concentrated hydrochloric acid were added and allowed to react at room temperature for 20 minutes. Absorbance at 550 nm was measured against a blank (11). The concentration of tannins was expressed in milligram of catechin equivalents per gram (g) of dry matter (mg EC/g MS) from the calibration curve defined by catechin.

Biological activity

Antioxidant activity

DPPH assay

The antioxidant activity of the extracts was defined *in vitro* by DPPH radical scavenging and ABTS assays. The results were compared against a positive control containing butylated hydroxytoluene (BHT). The DPPH (1,1-diphenyl-2-picrylhydrazyl ($C_{18}H_{12}N_5O_6$)) is a stable free radical, which gives a dark violet color in solution. When it is reduced, the color becomes pale yellow. The DPPH assay was performed following method as described by Sanchez-moreno *et al.* (12). Different concentrations of the extracts (500 μ L) were added to 375 μ l of absolute methanol and 125 μ l of DPPH methanolic solution (60 mM) used as a free radical source. The obtained mixture was covered with parafilm and incubated in dark for one hour. The absorbance was measured at 517 nm using a spectrophotometer (T70 UV-visible) and compared to BHT calibration curve. DPPH solution was used as a blank. The antioxidant activity was expressed as percentage of inhibition of DPPH free radicals (A%) and calculated using the following formula:

$$A\% = [(A_0 - A_s) / A_0] 100$$

where A_0 is the absorbance of the blank and A the absorbance of the sample.

Moreover, the concentration for 50% inhibition (IC_{50}) was calculated by linear regression equation of the corresponding scavenging effect vs. concentration dependence.

ABTS assay

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging activity was measured according to the method of Re *et al* (13) with small modifications. First, ABTS and potassium persulfate were dissolved in distilled water to obtain final concentrations of 7 mM and 2.45 mM, respectively. The obtained solutions were allowed to stand in the dark at room temperature (25 $^{\circ}$ C) for 12 to 16 h before use in order to generate ABTS radical. Finally, The $ABTS^{\bullet+}$ solution was diluted with ethanol, to adjust the absorbance to 0.70 ± 0.02 at 734 nm. Trolox (2.5 mM) solution was prepared in methanol for use as a stock standard.



The scavenging ability of the sample was expressed on percentage using the following equation:

$$I_{\text{ABTS}} \% = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where:

A_{blank} is the absorbance of the blank containing only ABTS^{•+} solution,

A_{sample} is the absorbance of the ABTS^{•+} solution after the addition of the sample.

Antibacterial activity

The antibacterial activity of the extracts was determined by the disc-diffusion method that used impregnated discs. Nutrient agar medium was prepared by pouring 15 ml of Muller Hinton agar into Petri dishes. After cooling and solidification, 100 µl of bacterial suspension was spread on the surface of agar medium using a Pasteur pipet. Then, with sterile forceps, filter paper discs 6 mm in diameter impregnated with 15 µl of the tested extract were placed on the agar (14, 15). Each assay was done in three replicates. The Petri dishes were closed with parafilm and stored at 4°C for 2 hours, after what they were turned and incubated at 37°C for 18-24 hours. Chloramphenicol antibiotic (1 mg/ml) was used as a standard. The antibiotic disc was deposited on the surface of the agar medium, previously seeded with a culture of the studied strain. The sensitivity of bacteria to antibiotic was assessed according to the same protocol as with paper discs impregnated with the extract.

Statistical analysis

All experiments were performed in triplicates and the results were presented as mean ± standard deviation. Analysis of variance was conducted using ANOVA with two factors in the software STATISTICA 5.5 and differences were considered to be significant at probability less than 0.05 ($P < 0.05$).

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical screening was conducted to detect different chemical classes in infusions and decoctions of *M. rotundifolia* and *S. calamintha*. The results are presented in Table 1.

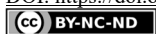


Table 1. Identified chemical classes in *M. rotundifolia* and *S. calamintha* extracts

Metabolites	Sample		
	<i>M. rotundifolia</i> (Jijel)	<i>M. rotundifolia</i> (Bejaia)	<i>S. calamintha</i>
Flavonoids	+++	+++	++
Anthocyanes	+++	+++	+
Leucoanthocyanes	-	-	-
Total tanins	+++	+++	+++
Glycosides anthraquinones	-	-	-
Cardiac glycosides	-	-	-
Quinones	-	-	-
Reducing sugars	++	+++	+++
Alcaloids	++	+++	+++
Coumarins	+++	++	+
Saponines	+++	+++	+++
O-heterosides	+++	++	+++
C-heterosides	+++	++	+
Sterols and triterpenes	++	+++	++

(-): Absence, (+): Presence in low concentrations, (++) : Presence in average concentrations, (+++): Presence in high concentrations.

In both studied plants same classes of compounds were identified. Six classes of compounds were identified in the extracts of *M. rotundifolia* in high concentrations, more specifically flavonoids, anthocyanins, total tannins, gallic tannins, reducing compounds and saponins. The concentrations of reducing sugars, alkaloids, coumarins, O-heterosides, C-heterosides, sterols and triterpenes showed moderate concentration variation in *M. rotundifolia* species depending on the geographical region.

Leucoanthocyanins, anthraquinone, cardiac glycosides and quinines were not detected in either of the studied plant extracts. Presented results were in agreement with the results obtained by Seladji (16) who reported numerous secondary metabolites in *M. rotundifolia*. In addition, here obtained results concerning both gallic and catechetal tannins were also in concordance with those found by Bounihi (17). Concerning the *S. calamintha*, the results of phytochemical screening revealed also the presence of numerous secondary metabolites. The plant showed to be high in total and gallic tannins, reducing compounds, alkaloids, saponins and heterosides. Reported results were in agreement with numerous other studies that dealt with chemical characterisation of the plant (18, 19, 20).

Extraction yield

The extraction yields for three different solvents (pure methanol, methanol:water (70:30) and water) was compared (Figure 1).

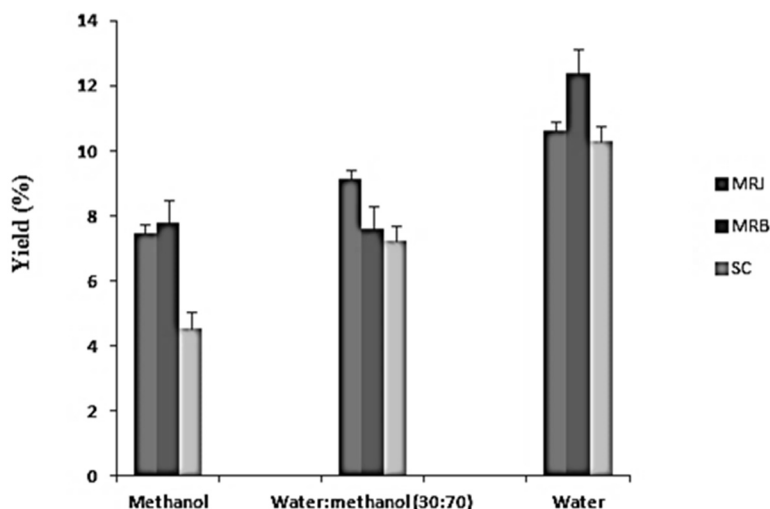


Figure 1. Extraction yield for three different solvents (%)

MRJ: *Mentha rotundifolia* harvested in the Jijel region; MRB: *Mentha rotundifolia* harvested in the Bejaia region; SC: *Satureja calamintha*.

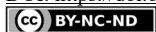
The highest extraction yields for all studied samples was seen for the aqueous extracts, being in agreement with the results reported by Majhenic *et al* (21), Seladji (16) and Bougendoura (22). This can be explained by simple fact that water is a polar solvent, solvating wide range of molecules including significant amount of non-phenolic compounds such as carbohydrates and proteins, which in plants, are present in higher concentrations than secondary plant metabolites (23).

Total polyphenols content

Phenolic compounds are secondary plant metabolites synthesized by many plants. Numerous biological activities have been reported for these chemical classes (24). The total polyphenols were determined in all extracts and were expressed in milligrams of gallic acid equivalents per gram of dry matter (mg EAG/g DW). The results obtained are presented in Table 2.

Table 2. The content of total polyphenols in extracts

Sample	Total polyphenols (mg EAG/g DW)		
	Methanol	Water : methanol (30:70)	Water
MRJ	16.02±2.63	20.52±5.34	7.06±0.45
MRB	13.16±1.34	20.64±1.74	9.74±1.25
SC	9.31±1.18	13.45±0.91	12.83±1.83



The highest content of total polyphenols was determined in water: methanol extract of *Mentha rotundifolia* collected from Béjaia region (20.64 mg EAG/g DW) whereas the water extracts of the three studied plants had the lowest total polyphenols content varying from 7.06 to 12.83 mg EAG/g DW. Less polar phenolic substances, mostly phenolic acids, were not quantitatively extracted with pure water leading to lower recoveries and explaining the lowest contents of total phenols when using pure water, in comparison to other two solvents (25). According to Lapornic et al (26), the use of water in combination with organic solvent contributes to the creation of a moderately polar medium which ensures the extraction of different classes of phenolic compounds.

The results on total polyphenols contents in methanolic extracts were in agreement with the results of Seladji (2015) who analysed methanolic extract of *Mentha rotundifolia*. Contents determined in this work, however, were superior to those reported by Benabdallah et al (27) who analysed water:methanol (20:80) extracts of *Mentha rotundifolia* (15.10±0.60 mg EAG /g MS) collected in the East of Algeria. Here determined contents of total polyphenols in methanolic extracts of *Satureja calamentha* were ~3 fold greater of those reported by Bougendoura (22). It should be, however, taken into consideration that the content of plants metabolites is dependent on numerous factors, such as geographical region, climate, soil composition, stress etc.

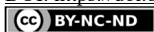
Flavonoids content

Determined flavonoid contents for all analysed extracts are presented in Table 3.

Table 3. Flavonoids contents in extracts

Sample	Flavonoids content (mg EAG/g DW)		
	Methanol	Water : methanol (30 :70)	Water
MRJ	12.01±0.50	13.55±2.53	6.49±0.76
MRB	11.47±0.80	12.33±1.58	11.28±0.73
SC	6.88±1.04	7.11±0.02	5.61±0.82

Similarly as for total phenols contents, the highest flavonoids contents were obtained in water:methanol extracts, but didn't differ substantially from the contents seen in methanolic extracts. For three tested solvents the contents of flavonoids ranged from 5.61 to 13.55 (mg EQ/g DW), with water extracts presenting the lowest concentrations. The contents of flavonoids in *M. rotundifolia* extracts determined in this work were substantially higher of those reported by Seladji (16) who reported flavonoids contents of 2.19±0.08 mg EC/g DW and 1.97±0.04 mg EC/g DW for methanolic and aqueous extracts, respectively.



The content of condensed tannins

The contents of condensed tannins in analysed samples are illustrated in table 4.

Table 4. The contents of condensed tannins

Plant	Condensed tannins content (mg EAG /g DW)		
	Methanol	Water:methanol (30 :70)	Water
MRJ	4.50±2,59	0.52±0.05	3.64±0.03
MRB	3.01±0.13	0.75±0.05	3.76±1.19
SC	3.00±0.85	0.57±0.14	4.45±0.20

On oppose to other phenols classes, the contents of condensed tannins were the lowest in water:methanol extracts, whereas methanolic and aqueous extracts presented comparable contents. In methanolic extracts the contents varied from 3 to 4.5 mg EAG /g DW, whereas in aqueous extracts the contents ranged 3.64-4.45 mg EAG /g DW. Here obtained results were comparable with those reported by Seladji (22) who determined the concentration of tannins in *M. rotundifolia* between 0.6 and 1.2 mg EAG /g DW for aqueous extract, and between 1.1 and 1.5 EAG /g DW for methanolic extracts.

Biological activity

Antioxidant activity

Free radicals are known to be a major factor in biological damages. The DPPH radical scavenging and the ABTS assay are commonly used to evaluate the ability of plant extracts to scavenge free radicals. (13). The results of antioxidant activity of the extracts obtained are shown in table 5.

Table 5. DPPH scavenging activity of the extracts

Sample	Pure methanol			Water-methanol (30:70)			Water		
	MRJ	MRB	SC	MRJ	MRB	SC	MRJ	MRB	SC
Concentration (mg/ml)	0.56±0.20	0.97±0.15	0.93±0.01	0.90±0.23	0.94±0.13	1.14±0.07	ND	ND	ND
% of inhibition of DPPH	76.80	59.81	68.33	80.13	78.90	78.46	ND	ND	ND

ND: Not determined

Table 5 indicates that water-methanol and methanolic extracts had a good ability to scavenge DPPH radicals, while aqueous extracts were inactive, which was in collision with the previously determined contents of polyphenols in aqueous extracts. Namely, previous results confirmed the presence of total polyphenols, flavonoids and condensed tannins in the aqueous extracts of all three plant samples. The content of these natural antioxidants was lower in comparison to methanol extracts, however owing to well-known DPPH-scavenging activity of these chemical classes, it was reasonable to expect



radical-scavenging activity. Water-methanol and methanolic extracts of *M. rotundifolia* (Jijel) at concentrations of 0.90 mg/ml and 0.56 mg/ml inhibited around 80.13% and 76.80% of DPPH radicals, respectively. Water-methanol and methanolic extracts of the *S. calamintha* plant inhibited around 78.46% and 68.33% of DPPH radical, respectively, corresponding to concentrations of 1.14 and 0.93 mg/ml respectively. Furthermore, at a concentration of 0.9 mg/ml, the water-methanol and methanol extracts of *M. rotundifolia* (Bejaia) presented the inhibition percentages of 78.93% and 59.81%. High antioxidant activity could be related to high polyphenols in the samples.

Since water:methanol extracts showed to be the most efficient in inhibiting DPPH radicals, their IC₅₀ values were calculated and compared to standard antioxidants BHT (butylhydroxytoluen). Calculated IC₅₀ were low, reflecting significant antiradical activity (Table 6).

Table 6. Calculated IC₅₀ values for water: methanol extracts

Extract	IC ₅₀ (mg/ml)	IC ₅₀ (mM Eq Trolox/g DW)
MRJ	1.41	17.91
MRB	1.27	18.85
SC	1.51	31.06
BHT	0.53	-
Trolox	-	1.27

Calculated IC₅₀ values for *M. rotundifolia* (Jijel and Bejaia) and *S. calamintha* were 1.41, 1.27 and 1.51 mg/ml, respectively, being around 2.4-2.8 higher in comparison to the standard. Benabdallah et al (27) reported better DPPH scavenging activity for *M. rotundifolia* and the same solvent, whereas for *S. calamintha* the authors found lesser activity.

Examined extracts demonstrated weaker inhibition effect for ABTS radicals in comparison to DPPH radicals, which could be attributed to different redox potentials, reaction stoichiometry or/and steric effects of the two radicals (28). Calculated IC₅₀ values for *M. rotundifolia* from the two regions Jijel and Bejaia) were very close corresponding to 17.91 and 18.85 mM Eq Trolox/g DW, respectively and much lower in comparison to *S. calamintha* (31.06 mM Eq Trolox/g DW). Here presented results were in concordance with those cited by Nickavar et al. (28) who calculated IC₅₀ (DPPH*) = 21.71 µg/ml and IC₅₀(ABTS*⁺) = 158.90 µg/ml for *M. rotundifolia* collected in Teheran, Iran.

The Fig 2 represents the spider diagram which is used to better visualize the relationship between total polyphenols, flavonoids, tannin and antioxidant activity of the most active water : methanol extracts. Water : methanol extract of *Mentha rotundifolia* from Bejaia had the highest content of total polyphenols and demonstrated the most pronounced antioxidant activity, followed by *Mentha rotundifolia* from Jijel.

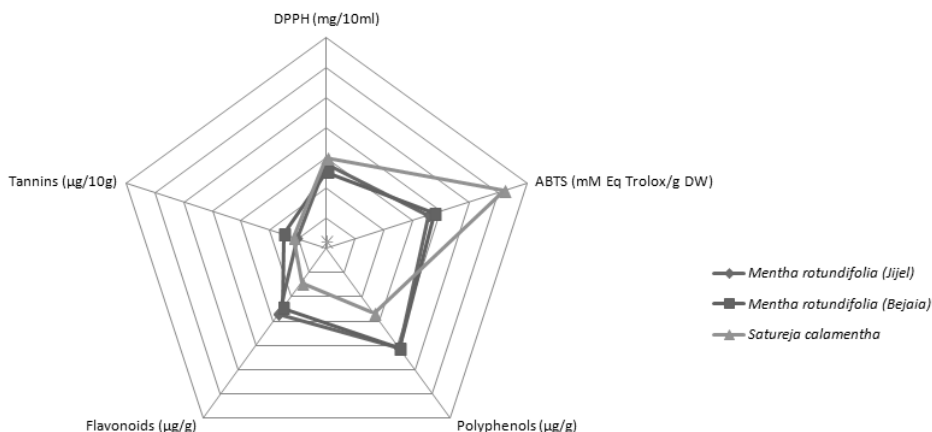


Figure 2. Antioxidant activities and total polyphenols content of the tested plants

Antibacterial activity

The antimicrobial activity of water-methanol extracts of the studied plants was performed using the disk diffusion method (Table 7).

Table 7. Antibacterial activity of water-methanol extracts

Bacteria strains	Inhibition zone (mm)			
	MRJ	MRB	SC	Chloramphénicol
<i>Enterococcus faecalis</i>	10.66±0.94	10.33±0.94	7.00±0.81**	10±00
<i>Staphylococcus aureus</i>	8.66±1.88*	10.33±2.49	11.33±2.86	14±00
<i>SARM</i>	10.33±0.47*	13.66±0.94	11.66±0.94	18.33±0.5
<i>Escherichia coli</i>	7.33±0.94	10.66±0.94***	8.66±0.47***	30±00
<i>Pseudomonas aeruginosa</i>	8.66±1.24	10.66±0.47***	7.00±0.47***	22.50±0.50
<i>Micrococcus luteus</i>	7.66±0.47*	9.33±0.47*	6.66±0.94*	17.50±2.50

It could be noted that antibacterial activity of *M. rotundifolia* was close to standard antibiotic chloramphenicol for *Enterococcus faecalis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *SARM*. The inhibition zone of the extracts of both plants was inferior in comparison to chloramphenicol.

The inhibitory effect of the *M. rotundifolia* (Bejaia) extract against six bacterial strains could be described by inhibition zone ranging from 9.33 to 13.66 mm. This extract demonstrated statistically significant results against *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. The extract of the same plant *M. rotundifolia* but col-



lected from different region (Jijel) had lower antibacterial activity against all tested strains. Inhibition zones for this extract ranged from 7.33 to 10.66 mm. These results were statistically significant for *Staphylococcus aureus*, *SARM* and *Micrococcus luteus*. Statistically significant results for methanolic extract (70%) of *S. calamintha* were noted for *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. Similar results were reported by Seladji (16) who studied antibacterial activity of the methanolic extract of the *M. rotundifolia* harvested in West of Algeria against three common strains (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*). Labiod, (19) reported the absence of antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* for the methanolic extract of *S. calamintha* harvested growing in East of Algeria. On the other hand, a very effective inhibitory effect on bacterial growth of this plant extract was reported by Benkhedimallah and Kismoun (18) who determined inhibition diameters ranging from 15.75 to 25.50 mm.

CONCLUSION

The results of the phytochemical screening of *M. rotundifolia* and *S. calamintha* revealed the presence of different secondary metabolites, namely flavonoids, tannins, reducing compounds, alkaloids and saponins. The determination of phenolic compounds revealed high polyphenols contents in water : methanol (30:70) extracts with the concentrations range of 20.64 ± 1.74 mg EAG/g DW and 13.45 ± 0.91 mg EAG/g DW for *M. rotundifolia* and *S. calamintha*, respectively. The extracts were also characterized by high concentrations of flavonoids, more specifically for the two plants the contents were 13.55 ± 2.53 mg EQ/g DW and 7.11 ± 0.02 mg EQ/g DW, respectively. The evaluation of antioxidant activity by the DPPH test showed that the water : methanol (30:70) extract of *M. rotundifolia* was the most effective in inhibiting radicals. Furthermore, the study of antibacterial activity revealed that the water : methanol (30:70) extracts obtained from the three plants had significant inhibitory activity against different pathogenic strains of bacteria, in some cases superior to standard antibiotic chloramphenicol. These findings suggest that these extracts could be used as potential natural antimicrobial agents.

Acknowledgements

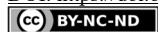
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