

Polyphenols Content and Antimicrobial, Antioxidant and Hemolytic Activities of Essential Oils from Four Selected Medicinal Plants Growing in Algeria

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Abstract

The Saharan and steppe spontaneous plants are very characteristic because of their particular adaptation to the desert and extreme environment. Some species have pharmacological properties that give them a medicinal interest. The aim of the present work was to determine the polyphenol contents of essential oils obtained from four endemic plants growing in Algeria (*Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita*), and study its biological activity, including antimicrobial, antioxidant, and hemolytic. The antimicrobial activity was evaluated by the microdilution method against twelve strains. The antioxidant activity was carried out by two methods (DPPH radical scavenging and reducing power). However, the hemolytic effect has been evaluated against the red blood cells. *P. scoparius* and *M. piperita* showed yields of essential oils higher than 1%. All the strains showed sensitivity against the essential oils tested with the exception of the *C. albicans* treated by *R. officinalis* essential oils. The most sensitive strain was *C. albicans* treated by *P. scoparius* essential oils by MIC of 0.0781 mg/mL, it was the same plant that shows the highest polyphenol content (14.78 ± 0.72 g GAE/g DS). The antioxidant activity by the DPPH method was greater for all essential oils tested by IC₅₀ ranging from 0.69 ± 0.07 (*R. officinalis*) to 30.67 ± 2.12 mg/mL (*M. nivellei*). The *R. officinalis* essential oils reported more antioxidant power than the positive control (ascorbic acid). In reducing iron, it was the *R. officinalis* essential oils which were found to be the most active with an EC₅₀ concentration of 9.67 ± 1.36 mg/mL. After 120 min incubation, minimal haemolysis (10%) was obtained with essential oils of *R. officinalis* at a concentration of 0.39 mg/mL. We conclude that *P. scoparius* essential oils showed the high content of polyphenols and *R. officinalis* essential oils reported more antioxidant power than the positive control (ascorbic acid).

Keywords: Polyphenols; Antimicrobial; Antioxidant; Essential oils; Hemolytic; *Mentha piperita*; *Myrtus nivellei*; *Pituranthos scoparius*; *Rosmarinus officinalis*; Sahara.

INTRODUCTION

The total Algerian flora has about 16,000 plant species, of which more than 1,000 species with medicinal properties and 700 species are endemics. Algeria is the source of significant taxonomic, ecosystem and landscape diversity (APS, 2019). The spontaneous plants in Sahara area have many uses, traditionally practiced by the local population, in terms of pharmaceuticals, food and domestic use. These plants have the ability to synthesize many compounds called secondary metabolites and thus constitute an immense reservoir of compounds of great chemical diversity, possessing a wide range of biological activities (Seca and Pinto, 2019). This is the case, for example, of plant essential oils which are widely used in therapeutics. In recent years, studies of biological activities of medicinal

plants have increased remarkably because of their potential to be used as sources of drugs, food additives or active ingredients in cosmetics (Haddouchi et al., 2016). In this context, the aim of the present work was to determine the polyphenol contents of essential oils obtained from four endemic plants growing in Algeria (*Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita*), and study its biological activity, including antimicrobial, antioxidant, and hemolytic. *Pituranthos scoparius* and *Myrtus nivellei* are from Tassili n'Ajjer (Sahara), *Rosmarinus officinalis* and *Mentha piperita* are from El Bayadh and Tiffrit, respectively (steppe area).

MATERIAL AND METHODS

Plant Material and Essential Oil Extraction

For the four plants studied, the flowering aerial parts were used. The plant material was identified by Dr. Tayeb Si Tayeb (Laboratory of Biototoxicology, Pharmacognosy and Biological recovery of plants, University of Moulay-Tahar, Saida, Algeria). A voucher specimen was deposited at the Herbarium of the Laboratory under the following code numbers LBPBP-TS03-13. *Pituranthos scoparius* and *Myrtus nivellei* were collected in October 2018 and April 2018, respectively, from Tassili n'Ajjer in south-east Algeria (25°30'0" N and 9°0'0" E). *Rosmarinus officinalis* was collected in October 2018 from El Bayadh (33°40'49" N and 1°01'13" E). *Mentha piperita* was collected in July 2018 from Tiffrit (Wilaya of Saida) (34°54'0.01" N and 0°24'0" E).

Using a Clevenger-type apparatus, 400 g of dried plant materials were subjected to hydro-distillation in 4,000 mL of distilled water for 4 h. The obtained oil was dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4 °C prior to analysis (El Asbahani et al., 2015).

Total Polyphenols Content

The polyphenols are assayed according to the method described by Dewanto et al. (2002). A 100 µL quantity of each essential oil was mixed with 2 mL of a freshly prepared sodium carbonate solution (2%). After five minutes, 100 µL of the Folin-Ciocalteu reagent (1 N) was added to the mixture, the whole was left for 30 minutes at room temperature and the reading is performed against a blank using a spectrophotometer at 750 nm. A standard range based on gallic acid is also prepared. The total polyphenol contents of the essential oils are then expressed in milligrams gallic acid equivalent per gram of the dried sample (mg EAG/g DS) (Halla et al., 2019a).

Antibacterial and Antifungal Activity

The antimicrobial activity of the essential oils was evaluated using different strains; Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778; Gram-negative bacteria: *Escherichia coli* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Pasteurella multocida* ATCC 43137, *Salmonella typhimurium* ATCC 13311, *Salmonella enterica* ATCC 13312, *Campylobacter fetus* ATCC 27374, *Klebsiella pneumoniae* ATCC 700603 and *Enterobacter cloacae* ATCC 13047; and fungal microorganisms (yeasts): *Candida albicans* ATCC 10231 and *Candida albicans* IP 444. Bacteria were cultured at 37 °C for 18 h under aerobic conditions in Nutrient agar medium (Fluka, USA). Before experimental use, the cultures from different solid mediums were cultivated in liquid media, incubated, and used as the inoculum for each experiment. Mueller-

Hinton broth (bacteria) and RPMI-1640 (yeast) were used for antimicrobial tests (CLSI, 2008; CLSI, 2012).

The minimum inhibitory concentration (MIC) was determined based on the methods approved by the National Committee for Clinical Laboratory Standards (CLSI, 2008; CLSI, 2012), with slight modifications (Halla et al., 2019b). The Microtiter plates were inoculated within different concentration of essential oils and then incubated at 37 °C for Bacteria or 35 °C for *C. albicans* in a moist dark chamber. The MIC of each sample was recorded after 16-20 h of incubation for Bacteria and 24 h for *C. albicans*. End points were defined as the lowest concentration of antibacterial agent resulting in total inhibition of visual growth compared to the growth in the control wells containing no essential oil. The minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) were also determined. After determining the MIC, 50 µL (Bacteria) or 20 µL (*C. albicans*) samples were withdrawn from each well of the microtiter tray with a 96-pin replicator (Boeckel Scientific, Feasterville, PA, USA) and plated onto nutrient agar (Bacteria) or Sabouraud dextrose agar plates (*C. albicans*). Inoculated plates were incubated at 37°C for 24 h (Bacteria) or 35 °C for 48 h (*C. albicans*) before determining the MBC (or MFC), which was defined as the lowest concentration of essential oil that resulted in total inhibition of visible growth (Espinel-Ingroff and Cantón, 2007; Qaiyumi, 2007).

Antioxidant Activity

▪ DPPH Radical Scavenging Activity

The experimental protocol followed that of Prieto et al. (1999). Fifty microliters of essential oil at different concentrations were added to 1950 µL of a methanolic solution of DPPH at 6.34×10^{-5} M. A blank (negative) control was prepared by mixing 50 µL of methanol with 1950 µL of the methanolic solution of DPPH. After incubation in the dark for 30 min at room temperature, the reduction of DPPH was evidenced by the color change of the solution from violet to yellow. The absorbance of this solution was then determined at 515 nm using a spectrophotometer. The positive control used is Ascorbic acid.

The results were expressed as percent inhibition (PI), and this was calculated based on the reduction of the color intensity of the solution using the formula:

$$PI = (OD_{\text{control}} - OD_{\text{essential oils}} / OD_{\text{control}}) \times 100$$

Where OD_{control} is the absorbance of the negative control and $OD_{\text{essential oils}}$ is the absorbance with the essential oils. IC_{50} value is the concentration corresponds to 50% inhibition (Zeragui et al., 2019).

▪ Reducing Power Assays

The reducing power is determined according to the method described by Oyaizu (1986). A volume of 1 mL

of each essential oil at different concentrations was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide solution. The mixture obtained was incubated for 20 min at 50°C. After this period, 2.5 mL of 10 % trichloroacetic acid was added to stop the reaction. The resulting mixture was centrifuged at $650 \times g$ for 10 min at room temperature, and 2.5 mL of the resulting supernatant was added to 2.5 mL of distilled water and 0.5 mL of 0.1 % (w/v) iron chloride. The absorbance of this solution at 700 nm was compared to that of a blank solution and the activity of the essential oil was compared with that of the positive control, i.e., butylhydroxyanisole (BHA). The results allow calculation for effective concentration (EC_{50}), concentration of the corresponding essential oils with an absorbance equal to 0.5 (Halla et al., 2019a).

Hemolytic Assay

Red blood cells were isolated and suspended in PBS (100 mM at pH 7.4) at a rate of 4000 cells/mL. The erythrocyte suspension was incubated at 37 °C under continuous agitation for 120 min, from the addition of essential oil solution with DMSO (Dimethyl sulfoxide) at different final concentrations. Samples of 50 μ L from the reaction solution were made at regular intervals to which we added 2 ml of cold washing solution (150 mM of NaCl, 2 mM of $MgCl_2$). After centrifugation at 4000 rpm for 5 min, the absorption of the resulting supernatant was determined at 548 nm by photometric monitoring against a blank sample. Control samples of 0% lysis (in buffer) and 100% lysis (in double-distilled water) were employed in all experiments (Bolard, 1986; Halla et al., 2013; Silva et al., 2017).

Statistical Analysis

The yields obtained, polyphenols content and antioxidant activity tests were carried out in triplicate

and the results are expressed in mean values \pm standard deviation. All analyzes were performed in the Microsoft Excel for Windows program, 2007.

RESULTS AND DISCUSSION

Extraction Yield

In this study, we were interested in the study of different essential oils obtained by hydrodistillation of the aerial parts of four Algerian plants. The two studied plants, *Pituranthos scoparius* and *Myrtus nivellei*, were collected in the Tassili n'Ajjer region in the wilaya of Tamanrasset (south of Algeria). The Tassili National Park has been listed as a UNESCO World Heritage Site since 1982 and has been classified as a man and biosphere reserve since 1986. It is characterized by a contrasted landscape of rugged mountainous terrain and desert plateau of black rocks which form the Reg or white sands. The central barrier of 1500–2000 m in altitude extends over 800 km and covers 80,000 km² (Hammiche and Maiza, 2006). The two plants *Rosmarinus officinalis* and *Mentha piperita* were harvested in the steppe area (Saida and El Bayadh). In Algeria, the steppe constitutes a vast region which extends between the Tellian Atlas in the North and the Saharan Atlas in the South, extending over a land area of around 20 million hectares. The altitude ranges from 400 to 1,200 meters. The steppe is characterized by a strong climatic constraint (insufficient rainfall with an isohyet varying from 100 to 400 mm, strong and sometimes hot winds, etc.) and edaphic (vulnerable soils, thin and poor in organic matter) (Khaldi, 2014). The various yields obtained are reported in Table 1. *P. scoparius* and *M. piperita* showed yields higher than 1%, however, the yields of *M. nivellei* and *R. officinalis* were of the order of 0.81 and 0.76 %, respectively.

Table 1. Yields and total polyphenols content of essential oils of *Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita*.

	Yields (%)	Total polyphenols content mg GAE/g DS*
<i>Pituranthos scoparius</i>	1.081 \pm 0.061	14.78 \pm 0.72
<i>Myrtus nivellei</i>	0.81 \pm 0.02	0.611 \pm 0.056
<i>Rosmarinus officinalis</i>	0.76 \pm 0.42	0.279 \pm 0.087
<i>Mentha piperita</i>	1.10 \pm 0.13	0.104 \pm 0.012

* mg gallic acid equivalents per g dried sample

Pituranthos scoparius essential oil had a specific odour (odour of fennel) and was colorless. The yield of essential oils of *Pituranthos scoparius* was 1.081 \pm 0.061%, which is in agreement with that obtained by Lograda et al. (2013) from the same plant collected in Mechouneche (Biskra). However, the same authors found yields of 0.47%, 0.85% and 2.29% in different regions (T'Kout (Batna), Boussâada (M'sila), and ElKantra (Biskra), respectively) when the plant was

harvested in October. Gourine et al. (2011) obtained yields ranging from 0.6 up to 2.8%. Ksouri et al. (2017) studied the essential oil of *Pituranthos scoparius* harvested in the wilaya of Tamanrasset, they found a yield of 0.4% which is different than that obtained in our study. Our result was not correlated with that obtained by Vérité et al. (2004) (0.77% from the seeds and 0.50% from the stems of the plant harvested in April), Kalla et al. (2010) (0.25% and 0.3% of the aerial part of the plant

harvested in February and April, respectively), Abderrazak et al. (2013) (0.25% of the aerial part of the plant harvested in October) and Chikhoun et al. (2017) (0.502% of the aerial part of the plant harvested in April).

The essential oil of *M. nivellei* was pleasant with a light yellow colour. The yield was $0.81 \pm 0.02\%$. This result is supported by that obtained by Bouzabata et al. (2013) from the Hoggar zone, which was not the case with that harvested from Tassili n'Ajjer where these authors found yields ranging from 0.5 to 0.9%. Boukhalifa (2017) obtained that the yield was 1.6% of this plant harvested in the months of September and October in the Tagmart region in Tamanrasset. Unlike the abundant literature relating to the essential oil of *Myrtus comunis* in Algeria, studies on the essential oils of *M. nivellei* are counted on the fingers. Pereira et al. (2009) suggested that the highest yields of the essential oils of *Myrtus comunis* were obtained for the leaves in October. As well as, the samples taken in May and October produced very little oil. Altogether, September seems to be the month with the best yields for the different parts of the plant.

The yield of essential oils of *Rosmarinus officinalis* ($0.76 \pm 0.42\%$) is lower than those found by Jordán et al. (2013) of the various essential oils of *Rosmarinus officinalis* harvested in thermo Mediterranean, upper meso- and supra-Mediterranean areas in Spain, which are of the order of 1.74 ± 0.38 , 2.44 ± 1.26 and $2.58 \pm 0.75\%$, respectively. Zaouali et al. (2010) reported that the yields of essential oils of this plant harvested in Tunisia from three different zones (sub-humid, upper semi-arid and upper arid zone) are between 1.17 and 2.7%. A study by Djeddi et al. (2007) recorded a yield of 0.82%. This result seems very close to our result, may be due to the same origin of the two plants (Algeria).

The essential oil of *Mentha piperita* was pale yellow with a strong aromatic odour. The essential oil yield of *Mentha piperita* was $1.10 \pm 0.13\%$. Work carried out on the same species in Algeria (Ghardaïa) revealed a yield close (1.325%) to the value obtained by our study on the essential oil of *M. piperita* (Laghouiter et al., 2015). Another study in Morocco showed a yield around 1.02% (Derwich et al., 2010). On the other hand, yield values for essential oil of *M. piperita* have been recorded by several authors from different countries, and which are higher or lower than that obtained by our study. Singh et al. (2015) revealed a yield of about 0.64% from the leaves of *M. piperita* harvested in Libya. A yield of 2.29% was recorded by Gavahian et al. (2015) of the aerial part of the same plant harvested in Iran. Laghouiter et al. (2015) reported that the extraction yield was very low during the cold period (autumn and winter), however the best yields were obtained in Summer and Spring during the flowering period of the plant. Rohloff et al. (2005) studied the effect of the harvest time and the drying method on the essential oil

yield of *Mentha piperita*, and they concluded that the harvest of this plant should be carried out at full flowering to obtain the best yield.

This difference may be due to the geographical origin of the plant as well as to the harvest period. According to some authors, the harvest season can affect the variation in the yield of essential oils (Boukhalifa, 2017). Others explain this difference by the harvest period and the drying method (Rohloff et al., 2005). Other factors are likely to influence the difference in yield. Precipitation can particularly affect the yield of essential oils (Sangwanet al., 2001).

Plant ontogenesis can be one of the most important factors that influence the accumulation of essential oils in plants. Since many transformations and changes occur inside cells due to physiological processes, the harvest season and the organ used are often considered to be critical parameters that can affect the chemical compositions of essential oils (Lee and Ding, 2016).

Total Polyphenols Content

The total phenol content is determined from the equation of the linear regression of the gallic Acid calibration curve: $y = 0.005 x + 0.185$ ($R^2 = 0.996$) and the results are expressed in mg gallic acid equivalents per g of dried sample (mg GAE/g DS). Table 1 summarizes the results for essential oils of four species tested. From the results obtained, we observed a high content of polyphenols in the *P. scoparius* essential oils (14.78 ± 0.72 mg GAE/g DS), however, the essential oils of *M. nivellei*, *R. officinalis* and *M. piperita* contain 0.611 ± 0.056 , 0.279 ± 0.087 and 0.104 ± 0.012 mg GAE/g DS, respectively.

The total phenol contents are variable between the four plants. The total phenol content found by Wojdylo et al. (2007), in essential oils of *Rosmarinus officinalis* (1.71 ± 0.02 mg GAE/g DS) is greater than our value (0.279 ± 0.087 mg GAE/g DS).

For the essential oils of *M. piperita*, the total phenol content is lower than that of the same species previously studied by Zheng and Wang (2001); it is about 2.26 ± 0.16 mg of GAE/g of fresh weight.

Studies on the extract of *M. nivellei* leaves indicated that the hydromethanolic, ethyl acetate, butanolic and aqueous fraction had the highest values in phenolic content (222.98 ± 4.70 , 308.4 ± 7.40 , 414.96 ± 2.40 and 128.03 ± 1.87 mg GAE/g DS, respectively) (Ramdane et al., 2017). In another study on the aqueous extract of the leaves of *M. nivellei*, the level of total polyphenols was estimated around 242.68 ± 9.79 mg GAE/g DS (Rached et al., 2010).

Lograda et al. (2013) reported the variation in the composition of the essential oils of *Pituranthos scoparius* in Algeria and they recorded that these oils are very rich in alcoholic compounds such as terpinene-4-ol, p-cymèn-8-ol, β -eudesmol, Methyl-eugenol, spathulenol, linalool, γ cadinol and t-muurolol.

The value of the polyphenol content of *Mentha piperita* is not in agreement with those obtained by Sharafi et al. (2010), from the plant harvested in Iran, where the polyphenol level was 89.43 ± 0.58 , 40.43 ± 0.58 , 15.10 ± 1 and 9.43 ± 0.58 μg GAE/mg sample for oil concentrations of the order of 10, 5, 2.5 and 0.2 $\mu\text{g}/\text{mL}$. Atanassova et al. (2011) showed that the methanolic extract of *Mentha piperita* contains a polyphenol level of 0.4525 mg GAE/g DS where this plant was harvested in Bulgaria. The level of total polyphenols was estimated to be around 71.8 ± 5.1 and 55.1 ± 3.6 mg GAE/g DS of the methanol/chloroform extract and the aqueous extract from the leaves and stems of *Mentha piperita* harvested in Pakistan (Akhtar et al., 2018). Riachi and De Maria (2015) concluded that the composition of *Mentha piperita* oil is very sensitive to environmental variations where the maturity of the leaves plays an important role in the composition of the essential oil. Thus, mint plants harvested at the flowering stage usually have a higher concentration of menthol (phenol) than plants in the bud-forming process, which contain a high amount of menthone. Another factor, the exposure of the plant *Mentha piperita* to UV-A radiation in addition to white light, during the night, increased the phenol content (Maffei et al., 1999). In short, longer days, colder nights, older leaves, plants harvested at the flowering stage, and adequate hydration of plants should provide good quality peppermint essential oil (Riachi and De Maria, 2015).

By comparison with previous studies, it seems that the extraction of phenolic compounds is governed by several factors which directly influence the levels of

these molecules among these factors; increasing the extraction temperature, contact time of the plant material with water, and decreasing the particle size to increase the diffusion coefficient of the water.

Antibacterial and Antifungal Activity

The obtained results of the antibacterial and antifungal activity (Minimum inhibitory concentration 'MIC' and minimum bactericidal (fungicidal) concentration 'MBC' -'MFC') of the essential oils of the four plants are presented in Table 2. All the strains showed sensitivity against the essential oils tested with the exception of the *C. albicans* against *R. officinalis* essential oils. All essential oils tested are less active than the positive control (Gentamicin or Ketoconazole) with the exception of *Rosmarinus officinalis* essential oils against *Salmonella typhimurium* (MIC and MBC = 0.625 mg/mL), *Salmonella enterica* (MIC = 0.0825 mg/mL and MBC = 0.312 mg/mL) and *Klebsiella pneumonia* (MIC = 0.3125 mg/mL and MBC = 1.25 mg/mL), as well as essential oils of *Mentha piperita* against *Staphylococcus aureus* (MIC = 0.078 mg/mL). The strains treated by *P. scoparius* essential oils showed that the most sensitive strain was *C. albicans* ATCC 10231 by MIC of 0.0781 mg/mL. On the other hand, all bacteria tested against *P. scoparius* in this study revealed MICs of 5, 10 or higher than 10 mg/mL. *E. coli* was the most sensitive bacteria tested by *M. nivellei* essential oils with MIC around 2.5 mg/mL, while, the fungal strains showed good MIC (0.312 mg/mL) by comparing with those obtained against bacteria.

Table 2. Minimum inhibitory concentrations and minimum bactericidal (fungicidal) concentrations (mg/mL) of essential oils (*Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita*) and controls.

Test organisms	<i>Pituranthos scoparius</i>		<i>Myrtus nivellei</i>		<i>Rosmarinus officinalis</i>		<i>Mentha piperita</i>		Control ^d	
	MIC ^a	MBC ^b (MFC ^c)	MIC	MBC (MFC)	MIC	MBC (MFC)	MIC	MBC (MFC)	MIC	MBC
<i>Staphylococcus aureus</i> ATCC 25923	5	5	10	10	10	10	0.078	0.156	0.156	0.156
<i>Bacillus cereus</i> ATCC 11778	10	10	5	5	2.5	10	5	5	0.156	0.156
<i>Escherichia coli</i> ATCC 25933	10	10	2.5	2.5	1.25	5	0.312	0.625	0.312	0.312
<i>Pseudomonas aeruginosa</i> ATCC 27853	10	10	5	5	2.5	5	5	5	0.625	1.25
<i>Pasteurella multocida</i> ATCC 43137	10	10	10	10	2.5	10	0.156	1.25	0.039	0.039
<i>Salmonella typhimurium</i> ATCC 13311	> 10	>10	10	10	0.625	0.625	1.25	2.5	0.625	0.625
<i>Salmonella enterica</i> ATCC 13312	5	5	10	10	0.0825	0.312	10	10	0.625	0.625
<i>Campylobacter fetus</i> ATCC 27374	> 10	> 10	10	10	5	10	5	5	0.019	0.039
<i>Klebsiella pneumonia</i> ATCC 700603	10	10	10	10	0.3125	1.25	2.5	5	5	10
<i>Enterobacter cloacae</i> ATCC 13047	> 10	> 10	5	5	5	10	5	5	2.5	5
<i>Candida albicans</i> ATCC 10231	0.0781	0.0781	0.312	0.312	/	/	0.625	2.5	0.019	0.019
<i>Candida albicans</i> IP 444	0.039	0.039	0.312	1.25	/	/	5	5	0.019	0.019

^aMIC: Minimum Inhibitory Concentrations, ^bMBC: Minimum Bactericidal Concentrations, ^cMFC: Minimum Fungicidal Concentrations

^dControl: Gentamicin for Bacteria and Ketoconazole for *Candida albicans*

Concerning *R. officinalis*, *S. enterica* recorded the lowest MIC compared to the other tested bacteria (0.0825 mg/mL). By comparison, Gram negative

bacteria were more sensitive than Gram positive against *R. Officinalis* essential oils. Indeed, the registered MICs of *M. piperita* essential oils for *B. cereus*, *P. aeruginosa*,

S. enterica, *C. fetus*, *E. cloacae* and *C. albicans* IP 444 (from 5 to 10 mg/mL) were higher than those obtained for *S. aureus*, *E. coli*, *P. multocida*, *S. typhimurium*, *K. pneumoniae* and *C. albicans* ATCC 10231 (from 0.078 to 2.5 mg/mL).

The MICs for *Pituranthos scorparius* essential oil were similar to those reported by Boutaghane et al. (2004) for *Enterobacter*, *Klebsiella pneumoniae* and *Salmonella typhimurium* where they found MICs of the order of 256, 16 and 128 mg/mL, respectively. On the other hand, the MIC results obtained by these authors against *Escherichia coli* (256 mg/mL), *Pseudomonas aeruginosa* (1 mg/mL) and *Staphylococcus aureus* (256 mg/mL) are not in agreement with our results. Ksouri et al. (2017) found that no antibacterial activity of *Pituranthos scorparius* essential oil has been revealed against *Escherichia coli* and *Klebsiella pneumoniae*. However, the MICs obtained for *Staphylococcus aureus* and *Candida albicans* were of the order of 1 and 0.5 mg/mL. The MIC obtained by Ksouri et al. (2017) against *Pseudomonas aeruginosa* (greater than or equal to 2 mg/mL) is correlated with that of our result.

Bouzabata et al. (2013) studied the antifungal activity of the essential oil of *Myrtus nivellei* (same species in Algeria), they showed MICs and MFCs between 1.25 and 2.5 μ L/mL against *Candida albicans* ATCC 10231. These can be correlated with the CMF obtained against *Candida albicans* IP 444 (1.25 mg/mL).

A work by Kabouche et al. (2005) on essential oils of *Rosmarinus officinalis* grown in Algeria showed MICs greater than 0.128 mg/mL against both strains *E. coli* and *S. aureus*. The results of the CMI are in agreement with those obtained by Celiktas et al. (2007) from Turkey (Izmir, Çanakkale) of the essential oils of the plant harvested in September against the *Staphylococcus aureus* (10 mg/mL). However, a Tunisian study reported that essential oils of this species revealed MICs between 1.25-2.5 μ L/mL against *E. coli* and between 1.25-2.5 μ L/mL against *Bacillus cereus* (Zaouali et al., 2010). It is comparable to our results. According to Jordán et al. (2013), these essential oils have an MIC of 2-5 μ L/mL against *E. coli*.

İşcan et al. (2002) tested the essential oil of *M. piperita* from different origins (Turkey and India), they reported MICs between 1.25 and 2.5 mg/mL against *Escherichia coli*, between 0.625 and 2.5 mg/mL against *Staphylococcus aureus*, between 1.25 and 2.5 mg/mL against *Salmonella typhimurium*, of 2.5 mg/mL against *Klebsiella pneumoniae*, of 1.25 mg/mL against *Bacillus cereus* and between 0.312 and 0.625 mg/mL against *Candida albicans*. These results are correlated with our results, with the exception of those obtained for *Escherichia coli* and *Bacillus cereus*. The MIC values for essential oil of *M. piperita* against different bacterial strains, reported by Mahboubi and Kazempour (2014), range from 0.125 to a value greater than 64 μ L/mL. The

MICs are of the order of 1, 0.25, 1, 2, 16, 0.25 and 0.125 μ L/mL against *S. aureus*, *B. cereus*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans*, respectively. However, the MBCs are of the order of $2 \times$ 64, 1, 2, 16 0.5 and 0.125 μ L/mL against *S. aureus*, *B. cereus*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans*, respectively. Mohammadi et al. (2016) revealed percentages of 0.16%, 0.8% and 2% of MIC₅₀, MIC₉₀ and MBC, respectively, of the essential oil of *M. piperita* against *E. coli*. Compared with our result, it seems that the oil tested has a strong activity to that tested by Mohammadi and al. Tyagi and Malik (2011) recorded MIC and MBC (or MFC) of 1.13 to 2.25 mg/mL (MIC) and 2.25-9 mg/mL (MBC) for bacterial strains and 1.13 mg/mL (MIC) and 2.25 mg/mL (MFC) for yeasts. According to the study by Saharkhiz et al. (2012), the essential oils of *M. piperita* have an antifungal activity with an MIC of 1.5 μ L/mL against *C. albicans* strain. According to the study by Saharkhiz et al. (2012), the essential oils of *M. piperita* have an antifungal activity with a MIC of 1.5 μ L/mL against *C. albicans*. Samber et al. (2015) found that *M. piperita* essential oil is a bioactive fungicidal compound that has a strong effect on PM-ATPase (PM: Plasma Membrane) in *Candida* species. They suggested these essential oils enter the cell membrane and target the pathway for ergosterol biosynthesis, thereby compromising its biosynthesis. Simultaneously, they react with the membrane itself with their reactive hydroxyl moiety, and the extensive lesion on the membrane is a combined effect of the two events.

Antioxidant Activity

The evaluation of the antioxidant activity of the essential oils of the four plants was carried out by two conventional methods in order to test these essential oils by various reaction mechanisms involved in these antioxidant tests.

The DPPH radical is one of the most widely used substrates for the rapid and direct evaluation of antioxidant activity due to its stability in radical form and the simplicity of this analysis. The antioxidant power of the essential oils has been compared to that of the positive control used (Ascorbic acid). The values of the concentrations corresponding to the inhibition of 50% of the free radical DPPH (IC₅₀) are summarized in Table 3.

From the results obtained, we observed that the essential oils of the four plants showed an antioxidant activity by scavenging of the free radical DPPH. By comparing the antioxidant power of ascorbic acid (positive control) with that of the essential oils of *P. scorparius*, *M. nivellei* and *M. piperita*, we note that these essential oils were less active and showed a weak antioxidant activity relative to ascorbic acid. However, the *R. officinalis* essential oils (IC₅₀ = 0.69 \pm 0.07

mg/mL) reported more antioxidant power than the ascorbic acid ($IC_{50} = 0.81 \pm 0.14$ mg/mL). From the results obtained, the essential oils of *P. scoparius*, *M. nivellei* and *M. piperita* have IC_{50} values of 3.26 ± 0.65 , 30.67 ± 2.12 and 18.33 ± 1.84 mg/mL, respectively.

Table 3. IC_{50} values for reduction of radical DPPH and EC_{50} of reducing power of essential oils of *Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita* (mg/mL).

	IC_{50} DPPH	EC_{50} reducing power
Ascorbic acid	0.81 ± 0.14	/
BHA	/	0.42 ± 0.12
<i>Pituranthos scoparius</i>	3.26 ± 0.65	484.40 ± 5.14
<i>Myrtus nivellei</i>	30.67 ± 2.12	31.2 ± 2.59
<i>Rosmarinus officinalis</i>	0.69 ± 0.07	9.67 ± 1.36
<i>Mentha piperita</i>	18.33 ± 1.84	11.07 ± 0.07

The values of the optical densities obtained by reducing power assays allowed to drawing curves for each essential oil. In this test, the increase in absorbance means an increase in the reducing power of the essential oils tested. In order to compare the antioxidant activity of the essential oils tested by this method, we calculated the EC_{50} . The results obtained are illustrated in Table 3. We note that the essential oils of the *P. scoparius* showed a very low reducing power by comparing them with the positive control used (BHA) and the essential oils of the other plants (484.40 ± 5.14 mg/mL). The essential oils of *R. officinalis* reported an EC_{50} of 9.67 ± 1.36 mg/mL, this time their antioxidant activity have not powerful to those of the positive control BHA (0.42 ± 0.12 mg/mL). The EC_{50} of *M. nivellei* and *M. piperita* essential oils are 31.2 ± 2.59 and 11.07 ± 0.07 mg/mL, respectively.

The essential oils of *Pituranthos scoparius* have an IC_{50} value of 3.26 ± 0.65 mg/mL which results in a much higher antioxidant power than the value obtained by Ksouri et al. (2017) which is of the order of 11.21 ± 0.26 mg/mL of the same species.

To our best knowledge, there is no work done on the antioxidant activity of the essential oils of *M. nivellei*. Touaibia and Chaouch (2014) studied some extracts of this plant, they showed that the extracts studied all had a very good reducing activity, especially for the ethanol extract (EC_{50} equal to 0.59 mg/mL) by the DPPH method and methanol extract by the FRAP test (66.7%). Rached et al. (2010) evaluated the antioxidant activity of the fractions obtained from the raw extract of the leaves of *M. nivellei*. They found that the best activity was reported for the ethyl acetate and n-butanol fractions with IC_{50} values of 3.08 ± 0.40 μ g/mL and 4.40 ± 0.43 μ g/mL, respectively. In addition, Ramdane et al. (2017) reported IC_{50} values between 4.97 μ g/mL and 16.33 μ g/mL, for the different extracts obtained from *M.*

nivellei leaves (hydromethanol, ethyl acetate, butanolic and aqueous).

The antioxidant activity of essential oils of *Rosmarinus officinalis* is greater compared to that found by Zaouali et al. (2010) and Wojdylo et al. (2007), which revealed a low antioxidant power, by the two DPPH and FRAP methods.

For the essential oils of *M. piperita*, we compared our results with the work carried out by Singh et al. (2015), on the same species of Libya, which showed a higher antioxidant activity by scavenging the DPPH radical, with an IC_{50} value of 15.2 ± 0.9 μ g/mL. According to Sharafi et al. (2010), the *M. piperita* essential oil has shown a percentage inhibition of DPPH activity of $63.82 \pm 0.05\%$ at the concentration of 10 μ g/mL where the IC_{50} was around 3.9 μ g/mL. Laghouiter et al. (2015) recorded an EC_{50} of around 208.495 ± 4.247 μ g/mL of *M. piperita* essential oil. Gavahian et al. (2015) extracted the essential oil from *M. piperita* by four different methods (hydrodistillation; steam entrainment, hydrodistillation assisted by microwaves and hydrodistillation assisted by ohmic heating). They found that the antioxidant activity was almost similar for the essential oil obtained by these different methods whose EC_{50} values were between 9.6 ± 0.7 and 10.4 ± 0.6 μ g/mL. Our results, in most cases, are not in agreement with those obtained by previous work. This difference in the results is probably due to the diversity of the chemical composition and according to intrinsic and extrinsic factors, namely the harvest region and the evaluation method used.

Hemolytic Assay

The hemolysis test was evaluated because, even if a plant has potent antioxidant power or good antimicrobial activity, its use in traditional medicine and in pharmacological preparations will be impossible in the presence of their hemolytic effect, which is an indicator of cytotoxicity. When the plasma membrane of red blood cells is altered by the action of essential oil, it follows lysis resulting in the release of hemoglobin in the red blood cell, which is why we assayed the extracellular hemoglobin after the addition of different concentrations of essential oils. Final concentrations of essential oils were chosen according to the MIC founded in the first part. Figure 1 shows the effect of essential oils at different concentrations (0.39 , 0.781 , 1.56 and 3.125 mg/mL) on the release of hemoglobin from the red blood cells at 37 °C.

The results obtained show that the percentages of hemolytic effect are directly proportional to the increase in the concentrations of the essential oils of the four tested plants (Fig. 1). After 120 minutes of incubation and for all the concentrations tested, the hemolysis percentages are between 10 and 93%. Therefore, the hemolytic effect of the various essential oils tested, at the concentration of 3.125 mg/mL after 120 minutes of contact with human erythrocytes, can be classified as

follows: *P. scoparius* (93%) *M. piperita* (72.88 %) > *M. nivellei* (72.75%) > *R. officinalis* (43.26%). After 120 min incubation, minimal haemolysis (10%) is obtained with essential oils of *R. officinalis* at a concentration of 0.39 mg/mL, so these essential oils may be slightly

hemolytic at this concentration after two hours of incubation. On the other hand, the other essential oils have an important hemolytic effect against isolated erythrocytes, with a hemolysis rate that exceeds 41% at a concentration of 0.39 mg/ml.

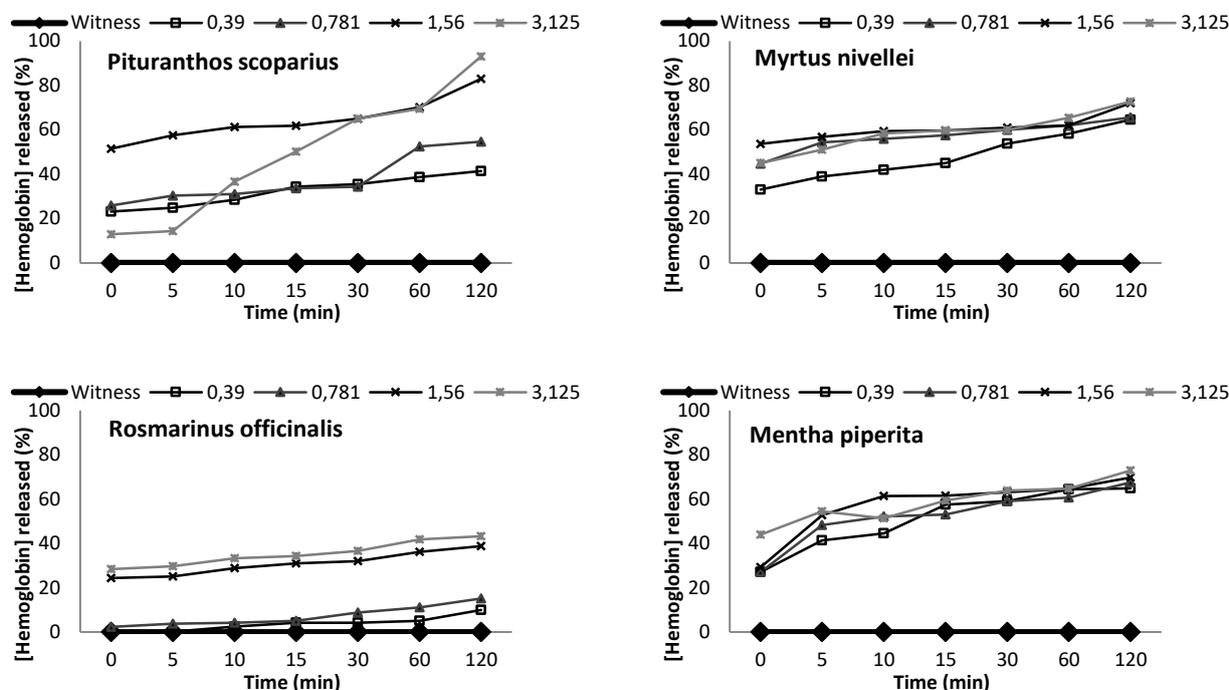


Figure 1. Release of hemoglobin by erythrocytes induced by different concentrations (mg/mL) of essential oils (*Pituranthos scoparius*, *Myrtus nivellei*, *Rosmarinus officinalis* and *Mentha piperita*).

Our results show that the essential oils of *Rosmarinus officinalis* have a very low toxic effect compared to isolated erythrocytes, with a haemolysis rate not exceeding 45% at a concentration of 3.1225 mg/mL, what characterizes it by the absence of risk of cytotoxicity. For other plants, they may be slightly hemolytic at high concentrations with respect to human erythrocytes.

Samber and his collaborators have shown that the rate of hemolysis of essential oil of *Mentha piperita* against human red blood cells does not exceed 6% at a concentration of 2 mg/mL after one hour of incubation (Samber et al., 2015). Mendanha et al. (2013) report that terpenes can compete with the intermolecular hydrogen bond between lipid molecules and thereby disrupt the network of hydrogen bonds in the lipid bilayer, which weakens the membrane. As well, Jain et al. (2002) found that terpenes, which have alcoholic 'OH' groups and which act as hydrogen bond donors, can disrupt the hydrogen bond network in the membrane bilayer.

According to some authors, the cytotoxicity of essential oils against red blood cells is due to their hydrophobic nature which is accentuated by the synergetic effect between their compounds (Sacchetti et

al., 2005). Silva et al. (2017) suggest that the constituents present in oils can interact with the components of the erythrocyte membrane, leading to destabilization of its structure and to a disordered influx of ions and water which leads to rupture of the membranes.

CONCLUSION

The plants studied were harvested from areas of specific climate in Algeria. *P. scoparius* and *M. piperita* showed yields higher than 1%. According to the antimicrobial activity results, all the strains showed sensitivity against the essential oils tested with the exception of the *C. albicans* against *R. officinalis* essential oils. The high content of polyphenols was reported in *P. scoparius* essential oils (14.78 ± 0.72 mg GAE/g DS). The antioxidant test shows that the essential oils of the four plants showed an antioxidant activity by scavenging of the free radical DPPH. Signally, the *R. officinalis* essential oils reported more antioxidant power than the positive control (ascorbic acid). The reducing power for all essential oils tested was by EC_{50} ranging from $9.67 \pm$

1.36 (*R. officinalis*) to 484.40 ± 5.14 mg/mL (*P. scoparius*). Our results show that the essential oils of *Rosmarinus officinalis* had a very low toxic effect compared to isolated erythrocytes (45% at 3.1225 mg/mL). From the results obtained, the geographical origin and period of harvest can influence the yield and the antioxidant activity of the essential oils of the studied plants.

Conflict of interest: The author declares that there are no conflicts of interest concerning the publication of this article.

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