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Composés bioactifs

Polyphenol analysis by HPLC-DAD, and antimicrobial and antioxidant activities of two species extracts of *Pelargonium*: *P. graveolens* and *P. zonale*

Analyse des polyphénols par HPLC-DAD et activités antioxydante et antimicrobienne d'extraits de deux espèces de *Pelargonium* : *P. graveolens* et *P. zonale*

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Abstract ***Introduction.*** *Pelargonium graveolens* is a well-known ethno-medicinal plant used in traditional medicine, whereas *Pelargonium zonale* has not been investigated thoroughly. ***Objective.*** This study was designed to quantify phenolic compounds in extracts of *Pelargonium graveolens* and *Pelargonium zonale*, evaluate their antioxidant activities, establish a method for assaying phenolic compounds by HPLC-DAD, and determine the antimicrobial activity of these extracts. ***Material and methods.*** Aerial parts polyphenols were extracted with solvents of different polarities. Total content of polyphenols, flavonoids, and condensed tannins were determined. Antioxidant activity was assessed by measuring free radical scavenging activity of 2,2-diphenyl-2-picrylhydrazyl (DPPH). Agilent C_{18} with a linear gradient elution program was performed for chromatographic separation, and disk diffusion method was used to determine antimicrobial activity. ***Results***. Total polyphenols content ranged from 159.4 to 164.2 mg GAE/g, and that of total flavonoids from 26.92 to 63.68 mg QE/g. DPPH values of ethanol extracts reached IC_{50} = 91.84±0.1 µg/mL. HPLC method permitted to analyze and separate twelve phenolic compounds (Acacetin, apigenin, myrecitin, quercetin, catechin, naringin, hesperetin, galengine, flavone, tannic acid, vanillic acid, and α-tocopherol). Extracts showed moderate to strong antimicrobial activity against five strains withminimum inhibitory concentration (MIC) ranging from 0.46 to 3.9 mg/mL. ***Conclusion.*** These results confirm richness of extracts by phenolic compounds, and are strongly correlated with DPPH values. In addition, the applied method is precise, and allows simultaneous determination of 12 phenolic compounds.

Key words: *Pelargonium, HPLC-DAD, Flavonoids, Polyphenols, Antioxidant activity, Antimicrobial activity*

Résumé ***Introduction.*** *Pelargonium graveolens* est une plante ethno-médicinale bien connue en médecine traditionnelle, alors que *Pelargonium zonale* n'a pas fait l'objet d'une enquête approfondie. ***Objectif.*** Cette étude a été conçue pour quantifier les composées phénoliques dans des extraits de *Pelargonium graveolens et Pelargonium zonale*, évaluer leurs activités antioxidantes, établir une méthode de dosage des composés phénoliques par HPLC-DAD et déterminer l'activité antimicrobienne de ces extraits. ***Matériel et méthodes.*** Les polyphénols des parties aériennes ont été extraits avec des solvants de polarités différentes. Les teneurs totales en polyphénols, en flavonoïdes et en tanins condensés ont été déterminées. L'activité antioxydante a été évaluée en mesurant l'activité de piégeage des radicaux libres du 2,2-diphényl-2-picrylhydrazyl. Un Agilent C_{18} avec un programme d'élution en gradient linéaire a été réalisé pour la séparation chromatographique et la méthode de diffusion sur disque a été utilisée pour déterminer l'activité antimicrobienne. ***Résultats.*** La teneur totale en polyphénols variait de 159,4 à 164,2 mg GAE/g et celle des flavonoïdes totaux de 26,92 à 63,68 mg QE/g. Les valeurs du DPPH des extraits éthanoliques ont atteint IC_{50} = 91.84 ± 0.1 μg/mL. La méthode HPLC a permis d'analyser et de séparer 12 composés phénoliques (Acacétine, apigénine, myrécitine, quercétine, catéchine, naringine, hespéretine, galengine, flavone, acide tannique, acide vanillique et α-tocophérol). Les extraits ont montré une activité antimicrobienne modérée à forte contre cinq souches avec une concentration minimum inhibitrice (CMI) allant de 0,46 à 3,9 mg/mL. ***Conclusion.*** Les résultats obtenus confirment la richesse des extraits en composés phénoliques. Ces résultats sont fortement corrélés aux valeurs de DPPH. Par ailleurs, la méthode appliquée est précise et permet de déterminer simultanément 12 composés phénoliques.

Mots clés : *Pelargonium*, *HPLC-DAD, Flavonoïdes, Polyphénols, Activité antioxidante, Activité antimicrobienne*

Introduction

The genus *Pelargonium* belongs to the *Geraniaceae* subfamily [1]. In the chemical taxonomic survey of 57 *Pelargonium* species, flavonoids in leaf secretions were detected in 35% of samples [2]. They are also a rich source of sesquiterpenes, coumarins, tannins, phenolic acids, cinnamic acids, and flavonols derivatives [3]. Previous phytochemical studies have also shown presence of three alkaloids, two of which could be fully characterized [4]. The most studied species is *P. graveolens*, which is known to exhibit antitumor, antiviral [5], antibacterial, anti-inflammatory, antithrombotic, and antidiabetic activities [6]. Other species of *Pelargonium* have also been investigated ; those include *P. sidoides, P. reniforme* [7], and *P. hortorum* [8].

To obtain more data related to the antibacterial activity of other species, a further comprehensive evaluation was imperative.

The increased discoveries in herbs and spices as sources of natural antioxidants have initiated researchers to look for natural antioxidants with low cytotoxicity [9].

Traditionally, *Pelargonium* species have been used in medicine for different purposes since ancient times by native African tribes to treat malaria, diarrhea, respiratory tract infections, abdo-minal, and uterine disorders. In Germany, ethanol extracts of *P. graveolens* roots have been used as herbal medicine under the name Umckaloabo (Spitzner Arzneimittel) to cure chronic infections, especially those of respiratory tract [10].

Although data exist on polyphenols antioxidant activity including references on many fruits and vegetables [11], little was known about *P. zonale* composition, which was a waste product that could be a valuable raw material for antioxidants preparation.

This current study established a quality control method by HPLC-DAD, which could separate and quantify phenolic and flavonoid components from *P. graveolens* and P. *zonale* extracts. Moreover, their antimicrobial activities were evaluated against tested microorganisms, the various solvent polarity extracts of *P. graveolens* and *P. zonale* were investigated as potential antioxidant agent.

Material and methods

Plant collection and authentication

Fresh aerial parts of *P. graveolens* and *P. zonale* were collected during the period of February to April 2021 from three localities in Algeria (Ain-Defla, Blida and Relizane). Leaves were separated manuallyfrom stems, and washed with tap water several times to remove mucilages and stem leftover. Plants were authenticated at University of Khemis-Miliana by the institution botanist Dr Moussa Kouache at the Botanical Department.

Preparation of different extracts

Aerial parts of both species were air-dried in shade at room temperature for 21 days. Dried leaves were grounded, sieved (<0.80 mm particle-sized), and extracted with ethanol 95%, by a ratio of 12.5 g of ground plant material into 100 mL of extracting solvent. Extraction was done for 3 hours. Obtained extract was filtered, and concentrated to dryness under reduced pressure using a rotary evaporator (Rotavapor R-200, Buchi, Switzerland). Samples were stored directly at - 4°C until further analysis.

Extraction yield was characterized as follows:

$$[R\ (\%) = (\frac{m^{residue}}{m}) \times 100]$$

$m_{residue}$: weight of residue (g); m: weight of plant material (g).

Table 1 summaries the extraction yields of different extracts.

Determination of total phenolic, flavonoids, and condensed tannins contents

Sample preparation

About 2–10 mg of extract were dissolved in 5 mL ethanol, and stirred for 30 min at 35°C followed by centrifugation at 1,000×g for 16 min. Clear supernatant was collected, and stored in test tube for further analysis.

Total phenolic content (TPC)

The amount of phenolic compounds in *P. graveolens* and *P. zonale* extracts was measured with the method of Singleton and Rossi [12], described later by Bahorun *et al.*, [13] with some modifications. In short, 500 μL of Folin-Ciocalteu phenol reagent (Na_2WO_4/Na_2MoO_4) (10%) were added to test tube with 0.125 mL of each extract. After incubation for 5 min at room temperature (20–27°C), 0.5mL of sodium carbonate solution (Na_2CO_3) was added to reaction mixture, and finally, mixture was diluted with distilled water. After 90 min incubation at room temperature, the absorbance was measured with a UV–Vis. spectrophotometer (UV-1600PC, USA) at 765 nm. TPC was determined from standard gallic acid curve equation $y = (0.0135x + 0.0005)$, and the results were expressed as mg gallic acid equivalents per gram of extract (mgGAE/g extract). The external calibration was done using different concentrations of gallic acid *i.e*. 0.25, 0.50, 0.75, and 0.99 mL.

Total flavonoids content (TFC)

Total flavonoids in *P. zonale and P. graveolens* were quantified using the method of Arjamand *et al.,* [14]. Briefly, 0.25 mL of 2% $AlCl_3$ in ethanol (w/v) was added to 0.25 mL of sample solution. After incubation for 60 min at room temperature, (20–27°C), absorbance was measured with a UV–Vis spectrophotometer at 420 nm. TFC was determined from standard quercetin curve equation:

y = (0.0415x + 0.0407), and results were expressed as mg of quercetin equivalent (mg QE/g).

Total condensed tannin content (CTC)

CTC was evaluated using vanillin assay method [15] with some modifications. 1.5 mL of vanillin/ethanol solution (4%, w/v) and 0.75 mL of concentrated hydrochloric acid were added to 10 µL of extract, and allowed to react at room temperature for 25 min. Absorbance was measured at 500 nm.

Table 1. Summarization of extraction yield R (%) of various parts of two *Pelargonium* species

	Collecting part	Extracts weight (g)		Extraction yield R (%)
P. *zonale*	Leaves	Ethanol	4.12	32.96
		Petroleum ether	0.78	6.24
		Chloroform	1.04	8.32
	Flowers	Ethanol	2.82	22.56
		Chloroform	0.67	5.36
P. *graveolens*	Leaves	Ethanol	4.20	33.6
		Butanol	3.89	31.12
		Petroleum ether	0.90	7.20
		Chloroform	1.10	8.80

TCT was determined from standard catechin curve equation (y = 0.0319x + 0.0052), and results were mg catechin equivalents per gram of extract (mg CE/g extract). External calibration was done using different concentrations of catechin *i.e*. 0.20, 0.60, 0.80, and 1 mL.

Antioxidant activity of extracts

Antioxidant activity of ethanolic extracts from aerial part of the two species was assessed by measuring their scavenging abilities of 2,2'-diphenyl-1-picrylhydrazyl stable radicals following the method of Shimada *et al*., [16]. DPPH assay was performed as described elsewhere [17]. In succinct terms, 4 mL of various extracts concentrations were separately added to 1 mL solution of 0.004% DPPH ethanol solution. After 30 min incubation period at room temperature, absorbance was read against a blank at 517 nm with a spectrophotometer UV. DPPH free radical inhibition percentage [I (%)] was calculated as follows:

$[I\ (\%) = (A_{test} - A_{sample}) / A_{test} \times 100\ ]$

A_{test} : absorbance of control ; A_{sample} = absorbance of test compound.

Test compound concentration providing 50% inhibition (IC_{50}, expressed in $\mu g.mL^{-1}$) was calculated from the graph plotted inhibition percentage against extract concentration. Synthetic antioxidant reagent Ascorbic acid was used as the positive control.

HPLC method validation

Limit of detection (LOD), limit of quantification (LOQ), and linearity

The linearity of calibration curves, obtained for 12 phenolic compounds (**Table 2**), was assessed using regression coefficients (R^2) estimated for five-point curves constructed using reference compound solutions prepared in MeOH at mean working concentrations ranging from 0.11 to 86.2 µg/mL Detection limits were determined following standard deviations at minimum concentrations, and slopes values of each analyzed compound.

Apparatus and chromatographic conditions

HPLC analysis of polyphenolic compoundsand flavonoids was conducted using an Agilent YL 9100 HPLC Series system equipped with an autosampler YL9150 coupled with an HP 1260 Diode Array Detector, and automatic sample injection from Agilent Hp-Waldbronn. The column was a Agilent XDB ECLIPSE C_{18}, 4,6×250 mm, 5 µm; 20µL loop; 1 mL/min flow; the mobile phase was prepared from acetic acid added to 1% water (A) and methanol (B) (v/v) was used as follows: 0 min/95% (PA), 5% (PB); 55 min/5% (PA), 95% (PB); 60 min/95% (PA), 5% (PB). The posting time of 10 min was applied for the column equilibration. The flow rate was 1.0 mL. min^{-1}.

Temperature of thermostated column compartment was maintained at 35°C during each chromatographic separation, and the injection volume was 10 µL. All solvents are filtered through 0.5 µm filter (Sartorius). Chromatographic data were processed using YL-CLARITY software, model 195_7016_02. The acquisition was set at 254 and 280nm (spectral acquisition in the range 200-400 nm). By comparing retention time and UV spectrum (collected in 190 to 400 nm range) with retention time and UV spectrum of reference compound, chromatogram peaks can be identified.

Measurement of extracts antimicrobial activity

Bacterial strains

Antibacterial activity of *P. zonale and P. graveolens* extracts was evaluated against three Gram-negative bacteria, including *Escherichia coli* ATCC 25922, *Salmonella typhimurium,* ATCC 14028, and *Pseudomonas aeruginosa* ATCC 15442, and two Gram –positive bacteria, including *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 13932. These strains were obtained from the Medical Analysis Laboratory of Dr Zibouche (Ain-Defla), and the Medical analysis laboratory of Dr Djendli (Relizane). Before conducting experiments, bacterial cultures were inoculated on Tryptone Soya Agar (TSA) from frozen stock and incubated at 37°C during 24 h.

Disc diffusion assay

Antimicrobial activity of all extracts was tested using diffusion method of Ingolfsdottir *et al.,* [18]. Activity was determined by disk diffusion method on agar plates for bacterial strains. Bacterial suspensions of strains tested, at turbidity comparable to that of 0.5 McFarland standard, were diluted in saline (1:10) to obtain a final inoculum of 10^7 CFU/mL. Then, suspensions were spread uniformly on agar plates using sterile swabs. *Pelargonium* extract was dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 100 mg/mL. Afterward, a 6 mm diameter filter disc was placed aseptically on agar plates, 15 min later, a 10 µL aliquot of DMSO-dissolved extract was added to disks, shaken, and incubated at 37°C for 24h0.1% DMSO was used as a negative control, and ciprofloxacin was used as a positive control. Results were recorded by calculating area zone (mm) of growth inhibition around disks.

Experiments were performed in triplicate, and results were the average of three independent tests.

Evaluation of minimum inhibitory and minimum bactericidal concentrations (MIC, MBC)

MIC is defined as the lowest concentration in extract where there is no visible microorganism growth. Extracts MIC was carried out by using the broth micro-dilution method [19]. MBC is defined as concentration that does not show colony growth compared to the culture of the same strain initial inoculum. The lower concentration that did not show any increase on the Tryptic Soy Agar (TSA) is considered to be MBC.If the ratio of MBC/MIC is ≤ 2, the plant extract is considered to be bactericidal, otherwise, it is considered to be bacteriostatic. A ratio ≥16, hints at the ineffectiveness of the extract.

Statistical analysis

Microsoft Excel (New York, USA) was used for data analysis. Experiments were performed in triplicate, and results were presented as means ±standard error mean (SEM). *P* value less than 0.05 was considered statistically significant using *t*-student test.

Results

Determination of TPC, TFC, and CTC

TPC, TFC, and CTC of extracts from aerial parts of *P. zonale* and *P. graveolens* are shown in **Table 3**. Ethanolic extract of *P. zonale* flowers showed the highest TPC (164.2±3.4 mg GAE/g), and TFC (63.6±4.1mg QE/g).

Determination of antioxidant activity

Table 4 illustrates the antioxidant tests performed in this study reported as the IC_{50}. *P. graveolens* extract presented a higher antioxidant power (IC_{50} = 173.11 ± 0.1 µg/mL).

HPLC analysis of extracts

Qualitative methods

For each chromatogram, and based on the sample retention value method, add-peak method and DAD spectra of the standard compound analyzed under the same conditions, many phenolic compounds were identified (**Fig. 1)**. In total, 12 phenolic compounds were detected and identified in all extracts through their relative retention time. In this analysis, it was demonstrated that no less than 86 phenolic components were present in leaves extract of *P. graveolens*, 64 and 45 in flowers and leaves of *P. zonale*, respectively. Other phenols were not identified. Their UV spectra were recorded in online mode during the chromatographic process. According to the spectral features, unidentified phenols were classified as flavanones (λmax = 270-280 nm), and flavanols (λmax = 254-260 nm).

Table 3. Total phenolic, total flavonoids and condensed tannin concentrations of *P .zonale* and *P. Graveolens* extracts

	EtOH (*P.zonale*)		EtOH (*P.graveolens*)
	Leaves	**Flowers**	
TPC (mg GAE/g)	160.0±2.4 *p*-value = 0.00259	164.2±3.4 *p*-value = 0.00581	159.4±4.9 *p*-value = 0.000074
TFC (mg QE/g)	41.6±1.0 *p*-value = 0.000783	63.6±4.1 *p*-value = 0.000939	26.9±2.2 *p*-value = 0.000592
CTC (mg CE/g)	10.2±1.8 *p*-value = 0.000179	2.5±1.9 *p*-value = 0.00113	41.2±5.1 *p*-value = 0.000694

Data are presented as mean ± SEM (n=3) and $t_{0.0.5}$ with a ddl of 3 =5.064. TPC: Total phenolic concentration; TFC: Total flavonoids concentration; TCT: Condensed tannin concentration; GAE: Gallic acid equivalent; CE: Catechin equivalents; QE: Quercetin equivalents.

Quantitative methods

Based on calibration curves, and considering the dilutions made, 7 phenolics contentsfor each sample were established. Relative standard deviation (RSD) values of retention time and peak area of the 12 indexes were in the range of 0.09%-0.14% and 0.01%-0.64%, respectively, indicating that instrument precision was good. RSD values were less than 0.38% and 2.84%, respectively, indicating that sample solution was stable within 48 hours. Therefore, it showed that the method had a good reproducibility. Hence, the developed method was accurate, precise, rapid, and sensitive enough for the simultaneous quantitative determination of 12 compounds in our

Table 4. Antioxidant activities of *P. graveoloens* and *P. zonale* aerial extracts

Sample	ELZ	PLZ	CLZ	EFZ	CFZ	EG	BG	PG	CG
IC_{50}/µg cm^{-3}	134.38 ±0.3	1807.65 ±0.1	4727.1 ±0.4	91.84 ±0.1	199 ±0.5	173.11 ± 0.1	125.68 ± 0.1	7043 ± 0.5	9155.4 ± 0.4

Data are presented as mean±SEM, n=3). IC_{50}/µg cm^{-3}. ELZ: Ethanol Leaves- P.zonale, PLZ: Petroleumether Leaves-P.zonale, CLZ: Chloroform Leaves-P.zonale, EFZ: Ethanol Flower-P.zonale, CFZ: Chloroform Flower-P.zonale, EG: Ethanol P.graveolens), BG: Butanol P.graveolens, PG: Petroleumether P.graveolens, CG: Chloroform P.graveolens.

extracts.

Additionally, the HPLC-DAD results (**Table 5**) were strongly correlated with the results found by the assays, and confirmed the extract richness by the phenolic compounds. To summarize, overall almost all extracts prepared using EtOH extraction solvents tended to produce an excellent antioxidant activity.

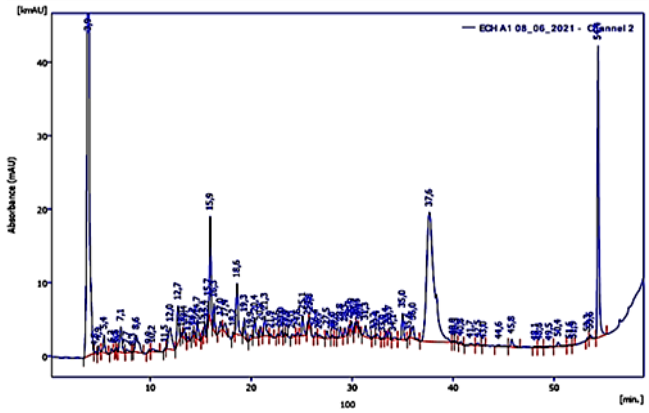

Fig. 1. HPLC chromatogram of polyphenols in *P. graveolens* extract

Antimicrobial activity of extracts

Inhibition of bacteria growth by *P. graveolens* and *P. zonale* areal parts extracts is presented in **Table 6**. Results of disc diffusion assay showed that extracts created inhibition diameter ranging from 24.6±0.2 to 7.1±0.1 mm against Gram-positive bacteria, and from 23.5±0.5 to 7.5±0.1 mm against Gram-negative bacteria. Results showed that ethanolic extract had high activity, but the highest was from *P. zonale* leaf against *Listeria monocytogenes* (24.6±0.2mm). The highest activity against *Pseudomonas aeruginosa* was exhibited by *P. graveolens* ethanolic extract (20.5±0.4 mm).

Based on inhibition zone diameters, MIC and MBC values (**Table 7)** showed that *L. monocytogenes,* and *S. aureus* were more sensitive to different extracts than other Gram-positive bacteria with MIC and MBC values varying from 0.94-1.18 and 1.91-2.6 mg/mL, respectively. The present results confirmed the antimicrobial potential of *P. zonale* leaves against *L. monocytogenes*, with MIC of 0.46 mg/mL for ethanolic extract, and of 1.03 for its flowers extract. Goodantimicrobial potential of *P. Graveolens* was observed against *P. aeruginosa* with MIC of 0.53 mg/mL.

Discussion

P. graveolens is one of the most widely cultivated in the world, leaves are a rich source of polyphenolic compounds with a multiple biological activities confirmed by several studies [6]. However, the pharmacological properties of *P. zonale* have not been investigated thoroughly. The present research was designed to investigate the phenolic contents of these species, and establish an HPLC method permitted to analyze and separate twelveof them from extracts with solvents of different polarities. The difference in the extracts amounts might be related to the availability of the extractable components.

Table 5. Retention time, relative retention time, peak area, and relative peak area of 12 characteristic peaks in *P. zonale* and *P.graveolens*

Component	EtOH *P. zonale*								EtOH *P. graveolens*			
	Leaves				Flowers							
	tr min	RRT	PA mV. S	RPA	tr min	RRT	PA mV. S	RPA	tr min	RRT	PA mV. S	RPA
Acacetine	49.5	0.11	4975	0.864	49.6	0.11	241.7	0.042	49.5	0.11	73.63	0.012
Apigenin	25.2	0.09	234	0.011	25.2	0.09	765.7	0.083	---	---	---	---
Myricetin	33.5	0.10	602.4	0.659	33.9	0.09	15.30	0.052	33.8	0.09	19.4	0.066
Quercetin	---	---	---	---	35.7	0.09	1212.7	0.108	---	---	---	---
Catechin	---	---	---	---	---	---	---	---	17.8	0.01	3.79	2.58
Naringin	32.1	0.09	207.5	0.183	---	---	---	---	32.4	0.10	1250	0.036
hesperetin	---	---	---	---	30.7	0.11	40.88		---	---	---	---
Galangin	47.5	0.12	4569	0.091	---	---	---	---	47.2	0.11	34.68	0.03
Flavone	25.4	0.14	62.70	0.063	25.5	0.14	408.8	0.440	24.9	0.13	363.5	0.025
Tannic Acid	---	---	---	---	6.62	0.10	731.7	0.645	---	---	---	---
Vanillic Acid	24.7	0.09	154.5	0.001	---	---	---	---	24.7	0.09	13.23	0.030
α- tocopherol	--	---	---	---	---	---	---	---	11.4	1.02	76.4	0.237

t_R : Retention time, RRT : Relative retention time, PA : Peak area, RPA : Relative peak area.

Table 6. Inhibition zone diameter of *P. zonale* and *P. graveolens* extracts on bacterial strains

Extracts	Inhibition zone diameter (mm)				
	Gram-negative bacteria (-)			Gram-positive bacteria (+)	
	E. coli	*S. typhimurium*	*P. aeruginosa*	*S. aureus*	*L. monocytogenes*
ELZ	18.2±0.1	23.5±0.5	15.7±0.1	22.9±0.5	24.6±0.2
PLZ	---	---	---	8.9±0.1	10.9±0.1
CLZ	---	---	---	---	---
EFZ	15.9±0.1	19.6±0.1	13.0±0	17.6±0.1	20.9±0.1
CFZ	---	---	---	---	---
EG	15.9±0.1	20.1±1.2	20.5±0.5	19.2±0.2	23.1±0.2
BG	13.1±0.1	14.2±0.1	11.9±0.2	15.0±0.1	19.2±1.2
PG	9.5±0	7.5±0.1	---	7.1±0.1	---
CG	---	---	---	7.9±0.2	---

Data are presented as mean±SEM, (n=3). ELZ: Ethanol Leaves-P.zonale, PLZ: Petroleum ether Leaves-P. zonale, CLZ: Chloroform Leaves-P. zonale, EFZ: Ethanol Flower-P.zonale, CFZ: Chloroform Flower-P.zonale, EG: Ethanol P. graveolens, BG: Butanol P. graveolens, PG: Petroleum ether P. graveolens, CG: Chloroform P. graveolens.

Therefore, the choice of a suitable solvent was crucial in the isolation of natural extracts or compounds. Cavar*et al*., had illustrated that different parts of *P. Graveolens,* such as leaves, flowers and stems contained a rich amount of phenols and flavonoids [23]. Other study carried out by Riahi*et al*., showed that the highest total phenol content in the aqueous extracts from *P. graveolens* under semi-controlled conditions was in the order of 105.6 mg GAE/g with an increasing rate of 67.35 % [24]. Similarly, condensed tannins content showed higher amounts in Algerian extract of *P. graveolens* (41.2±5.1 mg CE/g extract) than Tunisian extract (15.6±1.5 mg CE/g extract) [20], whereas, TFC was almost the same. This could be explained by the difference in ecological conditions, such as soil type, microclimatic conditions, geographic position, sampling site, vegetative stage of plants.

Data on total phenolic, flavonoid and condensed tannins contents of *Pelargonium zonale* has not been previously reported. Thus, in this experiment, TPC, TFC, and TCT of *P. zonale* will be reported for the first time. Statistically, all extracts revealed a good antioxidant activity. Previous studies have invest-tigated DPPH free radical scavenging in *P.graveolens* flowers and leaves, and content was very high compared to these results. Our results are in agreement with those of Ben Hsouna [22], but are higher compared with the Bulgarian extract (IC_{50} = 198.9±10.7 μg/mL) [21]. This difference was explained by the study conditions (extraction method, distilled water, temperature, use of other solvents), and relationship used to calculate the content. However, no data was found about antioxidant activity of *P.zonale*.

Antioxidant activity determined by DPPH assays were almost in accordance with TPC results. Therefore, other compounds might contribute to this extract activity. These findings suggested that ethanolic extract of *P. zonale* could serve as a potential antioxidant pharmaceutical source, and our validated method would be useful for the extracts quality control.

So far, some previous studies have reported the antimicrobial activity of *P. graveolens* extracts, but there was no data about *P. zonale* extracts. In a study performed previously in Tunisia, Hamdi *et al*., showed that leave extracts of *P. graveolens* had a great antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* [23]. Also, Ben-El Hadj *et al.,* studied antimicrobial activity of *P.graveolens* extracts prepared from different parts in Tunisia with different solvents against various strains of bacteria and fungi [20]. Ethanol extract exhibited good to moderate activity against almost all strains tested, whereas, butanolic extract were inactive (MIC>1.5 mg/mL), except L. *monocytogenes*. The presence of hydroxyl groups (-OH) in the structure of polyphenols is believed to be directly related to their antimicrobial activity, as they possess the ability to interact with the cell membrane of bacteria. In addition, our results demonstrated that *P. zonale* leaves extracts had a bactericidal effect because of a ratio MBC/MIC ≤ 2 against two strains, *S. aureus* and *S. typhimurium*. Interestingly, chloroform extracts with relatively low antibacterial activity were active against *Staphylococcus aureus*. This could be due to the difference in the cell wall structure of the tested bacteria. Indeed, the susceptibility of Gram-positive bacteria to *P. Zonale* petroleum ether extract in comparison with Gram-negative bacteria might be explained by the higher susceptibility of its cell wall to the extract chemical components. Nevertheless, the lower sensitivity of Gram negative-bacteria could be explained by the distinction in the structure of

peptidoglycan, presence of outer membrane, receptors or lipids, cross-linking, and the activity of autolytic enzymes that determine the penetration, binding, and action of the compounds [25]. The obtained results could be used for the production of dietary supplements with strong antioxidant activity.

Conclusion

This study reveals that the chemical profile and bioactivities of *P. graveolens* and *P. Zonale* support their traditional use, these species grown in Algeria show a high product yield and comparable antioxidant properties with other famous species. Moreover, this is the first study investigating phenolic compounds in *P. zonale* using HPLC-DAD method. The quantitative DPPH assay indicates that *P. Zonale* ethanolic extract has potent antioxidant activity which can be an excellent option for biological analysis, and can be further subjected for the isolation of the therapeutically active compounds. These preliminary results should be supplemented by further investigations to explore whether these polyphenols compounds could work synergistically to achieve much improved activities than each single component. Therefore, the above results could serve as a reference for the utilization of *P. graveolens* and *P. Zonale* extracts to exploit the different pharmacological activities.

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Conflict of interests

The authors declare that they have no conflict of interests in this research.

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