Phenological stage effect on phenolic composition, antioxidant, and antibacterial activity of *Lavandula stoechas* extract

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Received: September 25, 2021
Accepted: February 7, 2022

Phenolic contents of *Lavandula stoechas* aerial parts and their antioxidant effects were significantly dependent on the maturity stage of the plant. The maximum polyphenol content was reached during the vegetative stage in methanol extract with an amount of 232.77 mg GAE/g DW. At flowering stage, ethanol and aqueous extracts showed better phenol content. Luteolin 7-O glucoside was the dominant flavonoid in the methanol extract during flowering stage. Methanol extracts of fructification and flowering stages have the strongest antiradical activity than those of the vegetative stage. These extracts showed a higher content of polyphenols and flavonoids than ethanolic and aqueous extracts. Following a screening, methanol of *Lavandula stoechas* was selected for its strong antioxidant (IC_{50} = 28 µg/ml) and antibacterial (*Methicillin-resistant Staphylococcus aureus,* IZ = 25 mm) potentials in flowering stage. Inhibitor effect of the extracts (methanolic and ethanolic) of three stages on bacteria development give them an important role in the fields of food industry as an additive in food packaging, and the cosmetic and pharmaceutical industries.
Keywords: *Lavandula stoechas*, extract, solvent, antioxidant activity, antibacterial activity.

1. INTRODUCTION

Tunisian is located in the Mediterranean region characterised as one of the world's highest area of flora biodiversity. In this region, aromatic and medicinal plants are commonly used in traditional medicine as well as modern pharmaceutical, cosmetics, perfume and agro industries. In the context of the enhancement of the Tunisian flora, attention was paid to the Lamiaceae family. The plant we chose is a "*Lavandula stoechas*" used in traditional medicine. The genus *Lavandula* is an important member of the Labiateae family (Lamiaceae) and consists of about 28 species, which are mostly of Mediterranean (2004) origin. In Tunisia, *L. stoechas* has a fairly limited distribution area in the northwest and northeast, in continental regions or near the northern coast [1]. For lavender, the area has stagnated at about 10 ha since 2004. This stagnation does not reflect the potential demand on the world market. Rather, it can be explained by a still timid strategy for developing exports of this high-potential product.
The lavender species are of great market value due to their pleasant aroma. The vegetable material and its essential oil are mainly used in perfumery, cosmetic and food industry. The medicinal importance of the plant is well documented, and extracts prepared from this plant are energised in many pharmacopoeias [2]. The extracts of lavender have been reported as having many biological activities such as antibacterial, antifungal, carminative, antiflatulence, antiholic, antispasmodic, anticonvulsant, sedative anti-inflammatory, antioxidant, antihyperglycemic and antidepressive [2-6].
The lavender extract flowers and leaves are extensively used in cosmetics,

hygiene products, food industry, perfumery and pharmaceutical preparations with high industrial value [7,8]. *L. stoechas* subsp. luisieri and *L. pedunculata* can be useful as a new potential source of natural antioxidants and antimycotic agents and can be directly applied on the composition of dermocosmetic formulations as antiaging products or, as a raw material for the isolation of bioactive chemical compounds [9].
Nevertheless, no research has been cited concerning the effect of the phenological stage of this species according to the solvent of extraction from Tunisia. The objective of this study was to assess the potential impact of phenological stages on polyphenol, flavonoid and proanthocyandin contents and their antioxidant activity of *L. stoechas* according to the nature of solvent of extraction and to determine the antibacterial activity.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

Aerial parts of *Lavandula stoechas* were collected from El-Mida region (Nabeul Governorate) at vegetative (ST1, 17 November 2018), flowering (ST2, 5 January 2019) and fructification (ST3, 25 Mars 2019) stages. The plant material was identified by Professor Abderrazek Smaoui (Centre of Biotechnology of Borj-Cedria) according to the Tunisian flora.

2.2 POLYPHENOL EXTRACTION

The extraction was done according to the method of Ceylan et al. [10]. Triplicate samples of 2 g of dry matter were extracted by mixing with 50 ml of selected solvent (ethanol, methanol and aqueous). The mixture was stirred for 6 h and was then kept for 24 hours at 4°C in darkness. Finally, this mixture was filtered with a Whatman filter paper (N°4) and concentrated under rotary vacuum evaporator at 40°C. Finally, the crude extracts obtained were stored at 4°C until further analysis.

2.3 DETERMINATION OF TOTAL POLYPHENOLS, FLAVONOIDS AND PROTHOCYANIDIN CONTENTS

The determination of total polyphenols (TPC) was described by Chaouche et al. [11]. To do this, 100 µl of the three extracts (ethanol, methanol and aqueous) was mixed with 2 ml of a sodium carbonate (2%) solution were freshly prepared. After vigorous stirring, the mixture was left to rest for 5 min, and then a 100 µL of the Folin-Ciocalteu diluted reagent (1/20) was added, the mixture was incubated in total darkness for 30 min at room temperature. The absorbance reading was taken at a wavelength of 760 nm. TPH was expressed as mg gallic acid equivalent per gram of dry matter (mg EAG / g DW) through the calibration curve with gallic acid.
Total flavonoids (TF) were determined by a colorimetric method according to Mechraoui et al. [12]. A 125 µL intake of the three extracts (ethanol, methanol and aqueous) were mixed with 75 µl $NaNO_2$ (5%). After a rest of 6 min in the dark, 150 µl of freshly prepared $Alcl_3$ (10%) were added, 500 µl NaOH (1M) were added to the mixture 5 min later. Finally, the mixture was adjusted to 2.5 ml with distilled water. The standard range was prepared with quercetin at increasing concentrations, allowing a calibration curve to be drawn after reading the absorbance at 510 nm. TF levels are expressed in mg quercetin equivalent per gram of dry matter (mg EC/g DW).
The protocol followed in the extraction of proanthocyanidin content was that recommended by Salar and Purewal [13]. This reaction solution consists of preparing 1.5 mL of vanillin (4%) reagent, added into 100 µL of extracts followed by addition of 750 µL of concentrated hydrochloric acid. The resulting mixture was vortexed and then allowed to stand at room temperature. After 20 min of rest, the absorbance was done at 500 nm and the contents of condensed tannins were expressed in mg of catechin equivalent per gram of dry matter (mg CE/g DW).

2.4 ANALYSIS OF PHENOLIC PROFIL BY RP-HPLC

Twenty µL of pennyroyal extracts were analysed on a HPLC system (Agilent 1260, Agilent technologies, Germany). This method consisted of a vacuum degasser, an autosampler and a binary pump with a maximum pressure of 400 bar. The phenolic compounds were separated on a reversed phase C18 analytical column (Zorbax Eclipse XDB, 4.6 mm × 100 mm, 3.5 µm particle size) at a temperature set at 25°C. The chromatograms obtained at 280, 320 and 550 nm (DAD detector, Germany) were compared with those obtained on known phenolic compound standards. The separation gradient was formed by two mobile phases: A, methanol, and B, 99.9% H_2O and 0.1% formic acid. The optimised gradient elution was illustrated as follows: 0-5 min, 10%-20% A; 5-10 min, 20%-30% A; 10-15 min, 30%-50% A; 15-20 min, 50%-70% A; 20-25 min, 70%-90% A; 25- 30 min, 90%-50% A; 30-35 min. Phenolic compound contents were expressed in mg/g DW.

2.5 ANTIOXIDANT EVALUATION OF EXTRACTS

2.5.1 Antiradical scavenging potential

The estimation of this anti-radical activity was measured according to the method of Ceylan et al. [10]. In microtubes, 0.5 ml of extracts at different concentrations was mixed with 3 ml of a DPPH solution (0.004% in methanol). The mixture was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm. Inhibition (I%) of the DPPH free radical was expressed using the following equation:

$$I\% = [(A_b - A_S)/A_b] \times 100$$

Where:
A_b: Absorbance of the blank,
A_s: absorbance of the sample.

The concentration of the extract that could scavenge 50% of the DPPH radicals (IC_{50}) was calculated. Butylatedhydroxytoluene (BHT) was used as standard antioxidant.

2.5.2 Ferric-reducing power (FRAP) assay

This activity was measured using the method described by Ferreira et al. [14]. This method consists of mixing of extract (1000 µl) at different concentrations with phosphate buffer (1250 µl, 0.2 mol/l, pH 6.6) and potassium ferricyanide (1250 µl, 1%) ($K_3Fe\ (CN)_6$). The resulting mixture was incubated for 20 minutes at 50°C. Then, 1250 µl of trichloroacetic acid (TCA, 10%) was added to stop the reaction. Then, the tubes were centrifuged at 3000 rpm for 10 minutes. Finally, a volume of 1250 µl of the supernatant was added to 1250 µl of distilled water and 250 µl of a 0.1% solution of ferric chloride ($FeCl_3,6H_2O$). Absorbance was measured at 700 nm, referring to a positive control which was ascorbic acid. The results are expressed in effective concentration (EC_{50}, µg/ml), which was the concentration of the extract corresponding to an absorbance equal to 0.5.

2.5.3 Ferrous ion chelating activity

The chelating capacity was measured according to Zhao et al. [15]. An aliquot of 0.1 mL of samples at different concentrations was added to 0.05 mL of $FeCl_2$ (2 mM). After 5 min of incubation, 0.1 mL of ferrozine (5 mM) and 2.75 mL of distilled water were added. The mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The scavenging activity to chelate ferrous ion was calculated as follows:

$$\text{Chelating effect (\%)} = [100 \times (A_c - A_s)/A_c]$$

Where:

A_c is the absorbance of the control reaction

A_s is the absorbance of the tested sample.

The results were expressed as IC_{50}, which was the efficient concentration of the extract corresponding to 50% ferrous iron chelating. EDTA was used as a positive control.

2.6 ANTIBACTERIAL ASSAY

The antibacterial activity of *L. stoechas* extracts was performed using a well diffusion method. Bacterial strains were grown respectively on Luria-Bertani (LB) broth at 37°C for 18-24 h and were inoculated into Mueller-Hinton (MH) agar (Beef infusion 2.0 g/l, casein hydrolysate 17.5 g/l, starch 1.5 g/l and agar-agar 17.0 157 g/l). Petri dishes containing WB medium were aseptically inoculated with a suspension of 10^8 CFU (colonies forming unit)/mL of *Candida albicans*. The turbidity was adjusted to 0.5 McFarland to yield approximately 10^8 colony-forming units (CFU)/mL with a Densimat (BioMérieux). After incubation at 37°C for 18-24 h, the antibacterial activity was determined by measuring the zone of growth inhibition (IZ) around wells. Gentamicin (10 µg/disc) was used as microbial standards.

The minimal inhibitory concentration (MIC) was determined by microdilution method as described by De Lima Marques et al. [16]. Subsequent serial decimal dilutions were performed on sterile 96-well microplates. Each EO dilution was placed in contact with a microbial inoculum in exponential growth phase in MH or WB medium. The initial microbial concentration was adjusted to 5 × 105 CFU/ml for bacterial strains and 5 × 104 CFU/ml for yeasts with sterile saline. We defined the MIC in current study as the lowest concentration of sample dilutions from half to half which leads to inhibition of any microbial growth after 24h of incubation. We considered that a higher value showing a multiplication of the initial microbial population is apt to cause a visible disorder of the culture medium as it was possible to observe in the

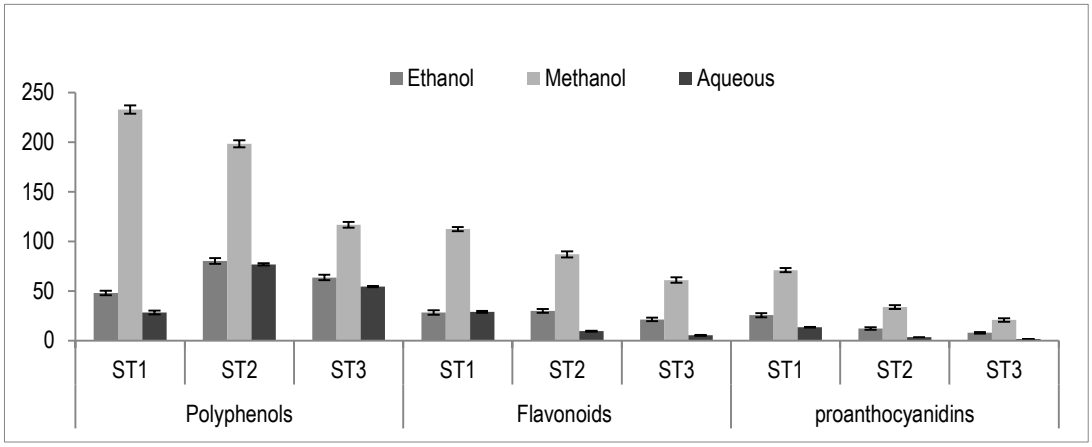

Figure 1 - Total polyphenol, flavonoid and tannin contents of different solvents of *Lavandula stoechas*

Total polyphenol and proanthocyanidins contents were expressed by mg GAE/g DW and total flavonoid contents were expressed by mg CE/g DW. Values are represented as mean ± standard deviation of triplicates.The data marked with the different lower case letters in the histograms of each phenolic category share significant differences at $p < 0.05$ (ANOVA test).

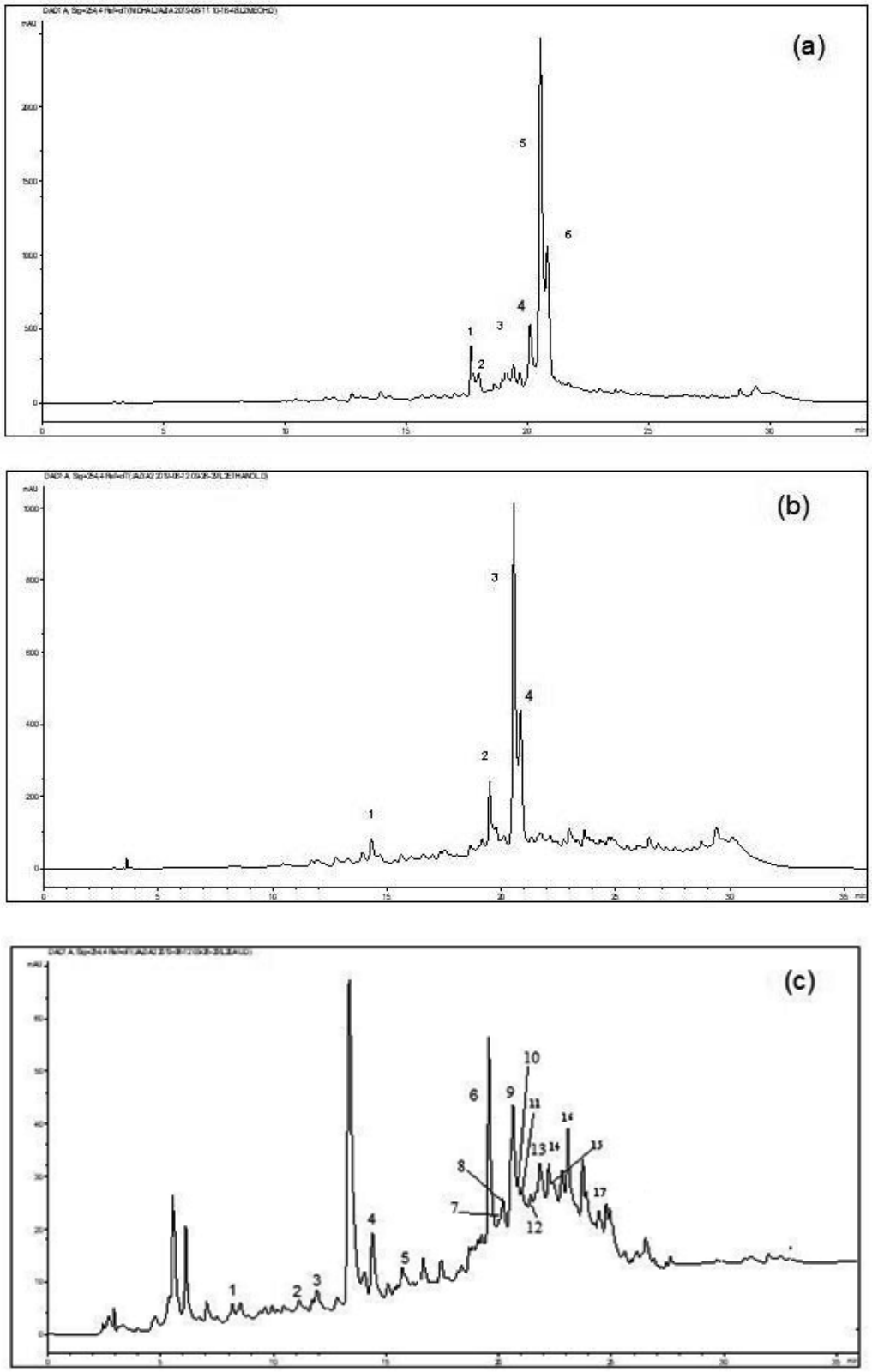

Figure 2 - RP-HPLC chromatogramtype of lavendermethanol (a), ethanol (c) and aqueous (c) extracts. Signalwascollectedat 280 nm. Peaksnumberscorresponding to:(a) 1. Cafeic acid, 2. Synergic acid, 3. *p*-Coumarique acid, 4. Ferrulic acid, 5. Luteolin 7-O glucoside,6. Coumarin, (b) 1. Catechinhydrate, 2. *p*-Coumarique acid, 3. Ferrulic acid, 4. Luteolin 7-O glucoside, (c) 1. Gallic acid, 2. Catechol, 3. Catechine hydrate, 4. Chlorogenic acid, 5. *p*-Coumarique acid, 6. Sinapic acid, 7. Ferulic acid, 8. Luteolin 7-O glucoside, 9. Coumarin, 10. Isorhamnetin 3-O glucoside, 11. Isoquercitrin, 12. Rosmarinic acid, 13. Ellagic acid, 14. Myricetin, 15. Isorhamnetin 3-O rutinoside, 16. Naringenine, 17. Luteolin

medium. A growth control without antimicrobial standard and a sterility control were performed for each strain. The plates were incubated at 36°C for 24h. To determine the MIC value, two methods were used: visual determination of the microbial growth or optical density of each well at a wavelength of 600nm in a reader plate (EL × 800, BioTeck, USA).

2.7 DATA ANALYSIS

All analyses were performed in triplicate and the results were expressed as means values ± standard deviations (SD). An analysis of variance (ANOVA) was carried using SPSS 23.0 (SPSS IBM2017). A Duncan test was used to determine the statistical difference of the mean value at 95%.

The Spearman's rank correlation coefficient was used to analyse the relationship between the total phenolic contents, flavonoid contents, and antioxidant activities.

3. RESULTS AND DISCUSSION

3.1 PHENOLIC QUANTIFICATION

Figure 1 showed that the levels of polyphenols, flavonoids and proanthocyanidins varied significantly depending on the harvest period and solvent of extraction. The highest contents were obtained in the methanol extract at the ST1 of 232.77 mg GAE/g DW for polyphenols, 112.43 mg CE/g DW for flavonoids and 70.98 mg CE/g DW for proanthocyanidins. Our results were higher than that found by Messaoud et al. [17]. These authors noted that the polyphenols contents of methanolic extract of *L. stoechas* harvested during the vegetative stage was 25.2 mg GAE/g DW. These same authors reported also that the total flavonoid content was 10.1 mg EC/g DW in methanol extract. Ceylana et al. [10] mentioned that the content of polyphenols in *L. stoechas* was 105.5 mg GAE/g DW. Concerning the fructification stage, ethanol presented the best solvent for extracting the polyphenols and flavonoids. However, no data is available regarding the effect of the harvest period on the polyphenols, flavonoids and proanthocyanidins contents of *L. stoechas* originating in Tunisia. In the same way, Ezzoubi et al. [3] noted that the total polyphenols in the hydro-ethanolic extract of *L. stoechas* revealed a value of 130.15 mg of GAE/g.

3.2 PHENOLIC PROFILS

The analysis of different chromatographic profiles of the main phenolic compounds obtained from the extracts with methanol, ethanol and aqueous from the vegetative and pre-flowering stages of *L. stoechas* enabled us to identify 20 phenolic compounds including 8 phenolic acids (Figure 2): *p*-coumaric acid which was the major compound in ethanol (10.75% of ST2) and aqueous (5.68% of ST1) extracts (Table II). In methanol extract, the major phenolic acid was syringic acid (8.76%) at ST1, while in the pre-flowering

Table I - Antioxidant activity of *Lavandula stoechas* extracts at different phenological stages

	DPPH (IC_{50}, µg/ml)			Reducing power(EC_{50}, µg/ml)			Chelating power(IC_{50}, mg/ml)		
Solvents	***ST1***	***ST2***	***ST3***	***ST1***	***ST2***	***ST3***	***ST1***	***ST2***	***ST3***
Ethanol	70 ± 2.33^{Ba}	55 ± 1.05^{Bb}	47 ± 1.32^{Bc}	1060 ± 3.02^{Aa}	690 ± 3.11^{Ab}	574 ± 2.75^{Ac}	7.12 ± 0.22^{Aa}	5.5 ± 0.12^{Ab}	4.27 ± 0.34^{Ac}
Methanol	50 ± 1.08^{Ca}	28 ± 0.86^{Cb}	22 ± 0.87^{Cc}	250 ± 2.22^{Cb}	360 ± 2.07^{Ca}	259 ± 2.76^{Cb}	6.42 ± 0.12^{Aa}	2.4 ± 0.05^{Cb}	1.28 ± 0.03^{Cc}
Aqueous	2600 ± 3.12^{Aa}	920 ± 2.14^{Ab}	729 ± 2.63^{Ac}	280 ± 3.13^{Bc}	430 ± 3.21^{Ba}	330 ± 1.47^{Bb}	5.09 ± 0.04^{Ba}	4.3 ± 0.04^{Bb}	2.61 ± 0.06^{Bc}
Synthetic antioxidant									
BHT	46.6±0.08			-			-		
Ascorbic acid	-			68±0.06			-		
EDTA	-			-			0.03±0.01		

ST1: vegetative stage, ST2: flowering stage, ST3: fructification stage. Each value is expressed as mean SD (n=3). Values with the letters (in capital letters for solvent extracts and in lowercase for phenological stage) are significantly different at p≤0.05.

stage, caffeic acid was the predominant compound (7.27%). The rest of the identified compounds constituted of the flavonoids where luteolin 7-O glucoside was more the abundant compound in the ethanol at the pre-flowering stage (58%), followed by coumarin (Figure 2). These results were identical to those of Celep et al. [4] that found that luteolin 7-O glucoside was the major constitutes of *L. stoechas* in Turkey. On the other hand, the comparative analysis of the chromatographic profiles of three extracts showed the differences in the contents of the phenolic compounds relative to the solvent and the harvest period (Table II). The phenolic composition of *L. stoechas* have not been studied in literature except by Ceylan et al. [10] who noted a different appearance than that found in this study. These authors showed that the major compound was rosmarinic acid, followed by caffeic acid in methanolic extracts. They also reported the presence of other phenolic acids: *p*-coumaric and ferulic acids and the flavonoids: quercetin, rutin and eriodictyol. In other hand, Celep et al. [4] noted that the chromatography analysis of *L. stoechas* presented the two phenolic acids (rosmarinic acid and chlorogenic acid) and two flavone glycosides (apigenin 7-glucoside and luteolin-7-O-β-glucoside). Recent studies showed that natural antioxidants, such as polyphenols, were incorporated in the foods to ensure their stability and protect them from oxidation [18]. Indeed, phenolic acids represented almost a third of dietary phenols and were involved in the organoleptic, nutritional and antioxidant properties of food [19].

3.3 ANTIOXIDANT POTENTIAL

Both methanolic and ethanolic extracts of *Lavandula stoechas* showed in vitro antioxidant activity during the DPPH assay (Table I). However, the methanolic extract recorded a significantly higher antioxidant activity with IC_{50} was 22 µg/ml at ST3 which is higher than that recorded for BHT with IC_{50} = 46 µg/ml. Similar results were found by Messaoud et al. [17] with a IC_{50} of 34.2 µg/ml for the methanolic extracts of lavender. Our result was higher to those found by Karabagias et al. [20] which indicated that methanol and an aqueous extract exhibited the higher activities with IC_{50} and was 7.05 mg/ml and 1.78 mg/ml, respectively. In the same way, ethanol extract exhibited a high activity against the DPPH free radical with IC_{50} was 47 µg/ml and 55 µg/ml at ST3 and ST2, respectively. At the fructification stage (ST3), the capacity anti-free radical evaluated in the same way as that of the flowering stage (ST2) where methanol exhibits the highest capacity to reduce the DPPH radical followed by ethanol and aqueous extracts. Our results are more important than those mentioned by Ceylan et al. [10] and Bayrak et al. [21] that found lower activities of methanol extract from *L. stoechas* with an IC_{50} equal to 300 µg/ml and 200 mg/ml respectively. Phenolic acids and flavonoids in *L. stoechas* in methanol extracts such as rutin and caffeic acid can be attributed the higher antioxidant activity [10].

The *Lavandula* extracts genre from different region showed a considerable antioxidant activity against the DPPH free radical, as reported in literature Spiridon et al. [22]. The extracts of methanol and aqueous from the vegetative stage exhibited the best activity with the lowest EC_{50} value (250 and 280 µg/ml, respectively). Ethanol was more efficient in reducing Fe^{3+} ions at the fructification stage than that in the vegetative stage with EC_{50} equal to 574 µg/ml.

The results presented in Table I showed that all extracts have a lower chelating power by compared with that of EDTA (IC_{50} = 0.03 mg/ml). However, the methanolic extract presented the greatest chelating capacity with an IC_{50} of 2.5 mg/ml. Our results were more important than those mentioned by Messaoud et al. [17] who found a lower activity obtained by methanolic extract with an IC_{50} equal to 3.5 mg/ml. Ethanol and aqueous extracts presented less chelating power with an IC_{50} of 4.27 and 5.5 mg/ml, in ST3 and ST2, respectively. In general, phenolic compounds are the major class to contribute the potential antioxidants in plants. A hypothesis that has been confirmed by several studies [23] which have shown the existence of the close relationship between the content of phenolic compounds in a plant matrix and the biological power they exert as major antioxidants of plants.

3.4 RELATIONSHIP BETWEEN TOTAL PHENOLS AND ANTIOXIDANT ACTIVITY

Highly significant positive correlations were obtained between polyphenols and flavonoids in the three phenological stages with r = 0.99 for ST1, r = 0.94 for ST2 and r = 0.98 for ST3 to $p < 0.05$ (Table IV). The contents of polyphenols correlate positively with those of the proanthocyanidins (r = 0.95 for ST1 and r = 0.93 for ST2). However, highly significant negative correlations ($P<0.05$) were determined between flavonoid contents and DPPH radical activity (r = -0.49 for ST1 and r = -0.72 for ST2), proanthocyanidins and DPPH (r = -0.66 for ST1, r = -0.73 for stage 2 and r = -0.77 for ST3).The statistical analysis shows highly significant negative correlations for ST2 ($p<0.05$) between polyphenols and chelating power (r = - 0.83), flavonoids and chelating power (r = - 0.75) and proanthocyanidins and chelating power (r = - 0.75) (Table IV). The levels of the different phenolic categories (tannins, flavonoids and proanthocyanidins) as well as those of total polyphenols are maximum during the vegetative period while decreasing during the flowering and fruiting stages.

In fact, a positive correlation between polyphenols extracted from *Sidastrum micranthum* and *Wissadula periplo cifolia* crude extracts and their antioxidant abilities was shown by De Oliveira et al [24]. In addition, a high correlation was found between the DPPH scavenging potency and total phenolic contents of

Table II - Analysis of variance (ANOVA) of phenolic quantity (mg/g Extract) of different solvents extracts of *Lavandula stoechas*

	Ethanol				Methanol				Aqueous			
	ST1		ST2		ST1		ST2		ST1		ST2	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
Phenolic acids												
Cafeic acid	-	-	-	-	0.47	0.25^{b}	23.12	7.88^{a}	-	-	-	-
Gallic acid	-	-	-	-	-	nd	-	nd	-	-	0.029	3.05
Syringic acid	-	-	-	-	1.14	9.84^{a}	0.69	3.77^{b}	-	-	-	-
p-coumarique acid	-	-	0.92	10.75a	0.1	0.80^{b}	0.36	1,84^{a}	-	-	0.18	27.90
Sinapic acid	-	-	-	-	-	-	-	-	-	-	0.00	0,15
Rosaminic acid	-	-	-	-	-	-	-	-	-	-	0.12	11.05
Ferrulic acid	5.48	51.65	8.34	58.02^{a}	2.07	8.72^{a}	2.9	7.70^{a}	-	-	0.048	4.44
Ellagic acid	-	-	-	-	-	-		-	-		0.078	5.15
Chlorogenic acid	-	-	-	-	-	-		-	-	-	0.14	5.63
Flavonoids												
luteolin 7-O glucoside	14.13	48.35b	10.49	26.62^{b}	38.27	58.63^{a}	57.8	55.67^{a}	-	-		5.27
Coumarin	-	-	-	-	3.08	21.76^{b}	5.21	23.15^{a}	-	-	0.18	3.16
Catechin hydrate		-	3.71	4.61	-	-	-	-	-	-	0.76	12.83
Isorhamnetin 3 O glucoside	-	-	-	-	-	-	-	-	-	-	0.06	3.87
isoquercitrin	-	-	-	-	-	-	-	-	-	-		0.28
Myricetin	-	-	-	-	-	-	-	-	-	-	0.024	4.33
Isorhamnetin 3 O rutinoside	-	-	-	-	-	-	-	-	-	-	0.12	5.37
Catechol	-	-	-	-	-	-	-	-	-	-	0.29	4.87
Naringenin	-	-	-	-	-	-	-	-	-	-	0.055	3.65
Luteolin	-	-	-	-	-	-	-	-	-	-	0.007	4.70

Quantities and percentages of phenolic compounds represent the average of three replicates (n=3), nd: not deteted; Capital letters (A-D) and small letters (a-d) in the same line indicate significant differences at $P < 0.05$.

Table III - In vitro antimicrobial activities of *Lavandula stoechas* extracts at two phenological stages

		Bacteria Gram(+)		
Stadephenological	**Solvents**	***Methicillin-resistant Staphylococcus aureus (MRSA)***	***Staphyloccocusaureus* ATCC 6835**	***Enterococcus faecalis ATCC 29212***
ST1	Ethanol	15^{Ab}	10^{Ab}	10^{Bc}
	Methanol	20^{Ba}	12^{Ba}	15^{Bb}
	Aqueous	15^{Ab}	10^{Ab}	17^{Aa}
ST2	Ethanol	15^{Ab}	12^{Ab}	16^{Ab}
	Methanol	25^{Aa}	18^{Aa}	19^{Aa}
	Aqueous	16^{Ab}	10^{Ac}	12^{Bc}
ST3	Ethanol	8^{Bc}	4^{Bb}	4^{Cc}
	Methanol	12^{Ca}	8^{Ca}	10^{Cb}
	Aqueous	10^{Bb}	8^{Ba}	12^{Ba}
Standard antimicrobial	Gentamicin (10µg/disc)	25 (S)	22 (R)	15 (R)

ST1: vegetative stage, ST2: flowering stage, ST3: fructification stage. IZ: inhibition zone. S: sensitive. R: resistant. Values were given as mean ± SD (n=3) and were the average of three replicates. One-way ANOVA followed by Ducan's multiple range test was used. Values with different superscripts (a-c) were significantly different at $P < 0.05$.

traditional plant species such as *Allium sativum* and *Pistacia lentiscus* [25].

3.5 ANTIBACTERIAL ACTIVITY

The antibacterial activity of the three extracts of *L. stoechas* was tested against nine strains. Only three bacterial strains showed a variability of different extracts (Table III). The highest inhibition diameter was obtained against MRSA with IZ of 25 mm in methanol extract at ST2. *Enterococcus faecalis* and *Staphylococcus aureus* strains also showed significant sensitivity with IZ of 19 and 18 mm, respectively. The results obtained showed that the antibacterial activity was greater only against Gram positive bacteria. These results agreed with those mentioned in literature [26]. These authors mentioned that *L. stoechas* was effective against Gram (+) bacteria and Gram (-) bacteria were weakly effective. Various studies have already shown that Gram positive bacteria were more susceptible towards plant extracts as compared to Gram negative bacteria. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram-negative cell wall is multi-layered structure [26]. Gören et al. [27] reported that antimicrobial activities of extracts of *L. stoechas* had a high effect on bacteria. Oskay et al. [28] proved sensitivity towards methicillin resistant *S. aureus (MRSA)* with methanol and ethanol extracts compared to aqueous fraction. Khosravi and Malecan [29] added that alcoholic extract of *L. stoechas* has significantly inhibitory effect on the growth of *S. aureus*. These results are similar to our study. In brief, the antibacterial activity of the extracts appears to be related to the nature of the solvent used in maceration. Indeed, the inhibitory effect on microorganisms is in high levels of polyphenols and flavonoids in the extract that are reported to be very active. In addition, significant differences in the inhibitory effects of extracts from the two stages appear to be due to existing minority volatile compounds. In this context, Shan et al. [30] demonstrated that polyphenols, like tannins and flavonoids like epigallocatechin, catechin, myricetin and quercetin are powerful antibacterial substances. These molecules have a broad spectrum of physiological activities, like anti-inflammatory, anti-allergic, anti-microbial, anti-carcinogen, anti-thrombotic, an-

Table IV -Relationship between total phenols and antioxidant activity ST1: vegetative stage. ST2: flowering stage, ST3: fructification stage

Variable	Correlation Matrix Stage 1					
	PPT	**FT**	**TCT**	**DPPH**	**RP**	**CP**
PPT	1.00	**0.99**	**0.95**	-0.56	-0.44	0.10
FT	**0.99**	1.00	**0.96**	-0.49	-0.53	0.01
TCT	**0.95**	**0.96**	1.00	-0.66	-0.34	0.15
DPPH	-0.56	-0.49	-0.66	1.00	-0.47	**-0.74**
RP	-0.44	-0.53	-0.34	-0.47	1.00	**0.69**
CP	0.10	0.01	0.15	**-0.74**	**0.69**	1.00

Marked correlations are significant at p < .05000
N=9 (Casewise deletion of missing data)

Variable	Correlation Matrix Stage 2					
	PPT	**FT**	**TCT**	**DPPH**	**RP**	**CP**
PPT	1.00	0.94	0.93	-0.54	-0.64	**-0.83**
FT	**0.94**	1.00	**0.99**	**-0.72**	-0.45	**-0.75**
TCT	**0.93**	**0.99**	1.00	**-0.73**	-0.42	**-0.75**
DPPH	-0.54	**-0.72**	**-0.73**	1.00	-0.29	0.13
RP	-0.64	-0.45	-0.42	-0.29	1.00	**0.87**
CP	**-0.83**	**-0.75**	**-0.75**	0.13	**0.87**	1.00

Marked correlations are significant at p < .05000
N=9 (Casewise deletion of missing data)

Variable	Correlation Matrix Stage 3					
	PPT	**FT**	**TCT**	**DPPH**	**RP**	**CP**
PPT	1.00	**0.98**	**0.98**	-0.63	-0.56	**-0.75**
FT	**0.98**	1.00	**0.99**	**-0.74**	-0.44	-0.64
TCT	**0.98**	**0.99**	1.00	**-0.77**	-0.39	-0.60
DPPH	-0.63	**-0.74**	**-0.77**	1.00	-0.27	-0.03
RP	-0.56	-0.44	-0.39	-0.27	1.00	**0.97**
CP	**-0.75**	-0.64	-0.60	-0.03	**0.97**	1.00

Marked correlations are significant at p < .05000
N=9 (Casewise deletion of missing data)

ti-viral and preventive actions against cardiovascular diseases [31]. The methanolic extract of *L. stoechas*, should be beneficial with an antioxidant ability against oxidative damage and can be proved as a protection system for the human body. This activity can be attributed to the flavonoids with potential antioxidant substances with the capacity to trap free radicals. *L. stoechas* can be used on development research and may be a source of natural antioxidants for potential exploitation in food, and the cosmetic and pharmaceutical industries.

4. CONCLUSION

This study revealed the ripening stage, as well as the extraction solvent significantly affected total polyphenol, flavonoids, prothocyanidins, antioxidant and antibacterial activities in *L. stoechas*. Methanol and ethanol of the three stages of ripening contained high amounts of phenolic compounds and exhibited strong antioxidant and antibacterial activities. This result is of a high important significance in the food industry for the creation of strategies, provenance selections and solvents with high polyphenols, flavonoids and prothocyanidins contents, and high antioxidant potential for producing specific health-promoting antioxidants.

Declaration of Interest

The authors declare they have no knowledge of financial interests or personal relationships that could have influenced the work reported in this paper

Ethical approval

All authors mentioned in this paper have agreed on the authorship, and have read, and approved the paper, and given their consent for the submission and subsequent publication of the paper.

REFERENCES

[1] H. Chograni, C. Messaoud, M. Boussaid. Genetic diversity and population structure in Tunisian *Lavandula stoechas* L. and *Lavandula multifida* L. (Lamiaceae). BiochemSystEcol 36, 349-359, (2008).

[2] X. Guo, P. Wang, Aroma characteristics of lavender extract and essential oil from *Lavandulaangustifolia* Mill. Molecules 25, 5541, (2020).

[3] Y. Ezzoubi, D. Bousta, A. Farah, A phytopharmacological review of a Mediterranean plant: *Lavandula stoechas* L. Clin Phytosci, 6-9, (2020).

[4] E. Celep, S. Akyüz, Y. İnan, E.Yesilada, Assessment of potential bioavailability of major phenolic compounds in *Lavandula stoechas* L. ssp. Ind Crops Prod 118, 111-117,(2018).

[5] S.S. Kulabas, H. Ipek, A.R. Tufekci, S. Arslan, Ameliorative potential of *Lavandula stoechas* in metabolic syndrome via multitarget interactions. J Ethnopharmacol 223, 88-98, (2018).

[6] S. Bint Mustafa, M. Akram, H.M. Asif, I. Qayyum, A.M. Hashmi, N. Munir, F.S .Khan, M. Ria,S. Ahmad, Antihyperglycemic activity of hydroalcoholic extracts of selective medicinal plants *Curcuma longa*, *Lavandula stoechas*, *Aeglemarmelos*, and *Glycyrrhizaglabra* and their polyherbal preparation in alloxan-induced diabetic mice. Dose-Response: An Int J1-6, (2019).

[7] K. Canlı, A. Yetgin, A.Benek, M.E. Bozyel, E. Murat Altuner. *In Vitro* antimicrobial activity screening of ethanol extractof *Lavandula stoechas* and investigation of ilts biochemical composition. *Adv Pharmacol Pharm Sci*, *2019*, 1-6, (2019).

[8] M. Soheili, M. Salami. *Lavandula angustifolia* biological characteristics: An in vitro study. J Cell Physiol 234(9), 16424-16430, (2019).

[9] R. Baptista, A.M. Madureira, R. Jorge, R. Adão, A. Duarte, N. Duarte, M.M. Lopes, G. Teixeira. Antioxidant and antimycoticactivities of two native *Lavandula* species from Portugal. Evid-Based Complement Alternat Med 2015, 1-10, (2015).

[10] Y. Ceylan, K. Usta, A. Usta, E. Maltas, S. Yildiz, Evaluation of antioxidant activity, phytochemicals and ESR analysis of *Lavandula stoecha*. Acta Pyhsica Polonica A 128, B483- B487, (2015).

[11] T.M. Chaouche, F. Haddouchi, O. Boudjemai, I. Ghellai, Antioxidant and hemolytic activity of *Ziziphus jujuba* Mill and *Rhamnus alaternus* L (Rhamnaceae) extracts from Algeria. Bulletin de la Société Royale des Sciences de Liège 89, 1-14, (2020).

[12] O. Mechraoui, S. Ladjel, M.S. Nedjimi,M.L. Belfar, Y. Moussaoui, Determination of polyphenols content, antioxidant and antibacterial activity of *Nigella sativa* L. seed St. Cerc St CICBIA 19(4), 411-421, (2018).

[13] R.K. Salar, S.S. Purewal, Phenolic content, antioxidant potential and DNA damage protection of pearl millet (*Pennisetum glaucum*) cultivars of North Indian region. Food Measure 11, 126-133, (2017).

[14] I.C.F.R. Ferreira, P. Baptista, M. Vilas-Boas, L. Barros, Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem100 (4), 1511-1516, (2007).

[15] J. Zhao Dong, J. Lu, J. Chen, Y. Li, L. Shan, Y. Lin, W. Fan, G. Gu, Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeumvulgare* L.). J Agric Food Chem 54, 7277-7286, (2006).

[16] J. de Lima Marques, L.M. Volcão, G.D. Funck, I.S. Kroning,W.P. da Silva, Â.M. Fiorentini,G.A. Ribeiro, Antimicrobial activity of essential oils of *Origanum vulgar*e L. and *Origanum majorana* L. against *Staphylococcus aureus* isolated from poultry meat. Ind Crops Prod 77, 444-450, (2015).
[17] C. Messaoud, H. Chograni, M. Boussaid, Chemical composition and antioxidant activities of essential oils and methanol extracts of three wild *Lavandula* L. species. Nat Prod Res 26, 1976-1984, (2012).
[18] J.C. Espin, M.T. Garcia-Conesa, F.A. Tomas-Barberan, Nutraceuricals: facts and fiction. Phytochem 68, 2986-3008, (2007).
[19] I. Rodriguez-Garcia, J.L. Guil-Guerrero, Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. Food chem 108, 1023-1026, (2008).
[20] I.K. Karabagias, V.K. Karabagias, K.A. Riganakos, Physico-chemical parameters, phenolic profile, in vitro antioxidant activity andvolatile compounds of Ladastacho (*Lavandula stoechas*) from the region of Saidona. Antioxidants 8(4), 80, (2019).
[21] D. Bayrak, G. Okmen, A. Arslan, The Biological Activities of *Lavandula stoechas* L. against Food Pathogens. Int J Sec Metabolite 4, 270-279, (2017).
[22] I. Spiridon, S. Colceru, N. Anghel, C.A. Teaca, R. Bodirlau, A. Armatu, Antioxidant capacity and total phenolic contents of oregano (*Origanum vulgare*), lavender (*Lavandula angustifolia*) and lemon balm (*Melissa officinalis*) from Romania. Nat Prod Res 25, 1657-1661, (2011).
[23] N. Karray-Bouraoui, F. Harbaoui, M.Rabhi, I. Jallali, R. Ksouri, H. Attia,N. Msilini, M. Lachaâl, Different antioxidant responses to salt stress in two different provenances of *Carthamus tinctorius* L. Acta Physiol Plant 33, 1435–1444, (2011).
[24] A.M.F. de Oliveira, L.S. Pinheiro, C.K.S. Pereira, W.N. Matias, R.A. Gomes, O.S. Chaves, M.F.V. de Souza, R.N. de Almeida, T.S. de Assis, Total phenolic content and antioxidant activity of some Malvaceae Family species. Antioxidants 1, 33-43, (2012).
[25] G. Piluzza, S. Bullitta, Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharm Biol49, 240-247, (2011).
[26] L. Cherrat, L. Espina,M. Bakkali, R. Pagan, A. Laglaou. Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processe. Innov Food Sci Emerg Technol. 22, 221-229, (2014).
[27] A.C. Gören, G. Topçu,G. Bilsel,M. Bilsel, Z. Aydoğmuş, J.M. Pezzuto. The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. stoechas. Zeitschrifttür Naturforschung C. 57, 797-800, (2002).
[28] M. Oskay, D. Oskay,F. Kalyoncu. Activity of some plant extracts against multi-drug resistant human pathogens. Iran J Pharm Res. 8(4), 293-300, (2009).
[29] A. Khosravi,M. Malecan. Effects of *Lavandula-stoechas* extracts on *Staphylococcus aureus* and other Gram negative bacteria. J Qazvin Univ Med Sci. 7(5), 3-9, (2004).
[30] B. Shan, Z.Y. Caia, J.D. Brooks, H. Corke The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol.117, 112-119, (2007).
[31] N. Balasundram, K. Sundram, S. Samman. Phenolic compounds in plants and agriindustrial by products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99, 191-203, (2007).