PAPER

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *ALOYSIA TRIPHYLLA* L. ESSENTIAL OILS AND METHANOLIC EXTRACT

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ABSTRACT

The essential oil variability in aerial parts of *Aloysia triphylla,* collected from four different Tunisian regions was assessed. In addition, total polyphenols, flavonoids, and condensed tannins as well as antioxidant, antibacterial, and antifungal activities of methanolic extract and essential oils were determined. Chromatographic analysis of *Aloysia triphylla* essential oils showed the predominance of monoterpene aldehydes represented mainly by neral and geranial. RP-HPLC analysis of *Aloysia triphylla* methanolic extract revealed the predominance of *p*-coumaric acid among the 15 phenolic acids identified. Antiradical activity was region-dependent and the methanolic extract of Kairouan sample had the strongest activity. Concerning the reducing power, the methanolic extract of Kairouan, Siliana, Kairouan, and Gabes samples were less active than the positive control. Siliana sample showed the least ability to prevent the bleaching of β-carotene, whereas Kairouan sample exhibited the strongest activity. Obtained results of antimicrobial and antifungal activities showed that *Aloysia triphylla* essential oil was endowed with important antibacterial and antifungal properties. Overall, based on its methanolic extract and essential oil features, *Aloysia triphylla* may be considered as a valuable source of new multipurpose products for industrial, cosmetic, and pharmaceutical utilization.

Keywords: *Aloysia triphylla* L., regions, phenolic, essential oils, antioxidant activity, antimicrobial activity

1. INTRODUCTION

The lemon verbena, *Aloysia triphylla* (l'Herit), Britt's = *Lippia citriodora* (Lam.), is a perennial shrub belonging to the Verbenaceae family (SHARMA *et al.*, 2016). It grows naturally in South America and was introduced into North Africa (Tunisia) and in Southern Europe in the late seventeenth century (CARNAT *et al.*, 1999; BENSABAH *et al.*, 2014). Leaves of *Aloysia triphylla* are used for culinary purposes as food seasoning and beverage flavoring (DOMINGUEZ-AVILA *et al.*, 2016). Furthermore, due to their aromatic, antispasmodic and digestive properties, leaves of *Aloysia triphylla* are commonly used as infusion or herbal tea (BENSABAH *et al.*, 2014). Traditional applications of *Aloysia triphylla* include its use as herbal remedy for colds, fever, spasms asthma, flatulence, colic, diarrhea, indigestion, insomnia, anxiety, analgesic and sedative (VALENTAO *et al.*, 1999). These therapeutic benefits were attributed to phenolic compounds (mainly flavonoids, phenolic acids, and phenylpropanoids) (PASCUAL *et al.*, 2001; QUIRANTES-PINÉ *et al.*, 2009). Antioxidants acting as radical scavengers are able to protect the human body as well as processed foods from oxidative damage. Presently, much attention has been focused on the antioxidant effect of plant natural compounds due to their wide application in food. Medicinal plants, being a promising source of phenolics, flavonoids, anthocyanins and carotenoids, are usually used to add flavor and improve the shelf life of dishes and processed food products. Regarding these beneficial effects, low cost and properties of plant phenolics, the interest is to increase research on natural antioxidants, in order to improve on their use in the food industry and as preventive medicine (EL BABILI *et al.*, 2013). Antioxidant properties of extracts of *Aloysia triphylla* leaves, as well as chemical composition of their essential oil obtained from different localities were investigated by several authors (PASCUAL *et al.*, 2001; CATALAN and LAMPASONA, 2002; CRABAS *et al.*, 2003; KIM and LEE, 2004; SANTOS GOMEZ *et al.*, 2005; CHOUPANI *et al.*, 2014). The essential oil has been shown to exhibit antimicrobial and anti-Candida activities (DUARTE *et al.*, 2005; TEIXEIRA *et al.*, 2007). According to ALI *et al.* (2011), antimicrobial activity of *Aloysia triphylla* essential oil may be attributed to the presence of high concentration of long-chain alcohols and aldehydes especially citral, citronellol, menthol, and β-caryophyllene. Otherwise, antifungal activity is related to eugenol, camphene, and β-caryophyllene contents (MAGWA *et al.*, 2006). There is hardly any available datum concerning *Aloysia triphylla* cultivated in Tunisia. In our present study, we have studied essential oil and methanolic extracts composition of *Aloysia trihpylla* cultivated in Tunisia and gathered from four distinct regions (Kairouan, Korba, Siliana, and Gabes). Moreover, we have evaluated their antioxidant, antimicrobial, and antifungal activities. This report also stressed on the underlying variability of *Aloysia triphylla* essential oil and methanolic extracts and their biological activities as affected by the collection site.

2. MATERIAL AND METHODS

2.1. Plant material

The aerial parts of four accessions of Tunisian *Aloysia triphylla* were gathered from four different regions in Tunisia, namely Kairouan (center), Korba (northest), Siliana (northwest), and Gabes (southeast) (Table 1). The aerial part samples were air- dried and conserved in a desiccator at room temperature (~25°C) in darkness for further extraction.

Table 1. Geographical coordinates and bioclimatic classification of the collecting zone of aerial parts of *Aloysia triphylla*.

	Longitude	Latitude	Elevation (m)	Bioclimatic zone
Kairouan	10°05'30,93''E	35°40'33,29''N	62	arid superior
Korba	10°51'43,49''E	36°34'50,18''N	12	semi-arid superior
Siliana	9°21'52,32''E	36°05'19,39''N	425	semi-arid superior
Gabes	10°05'51,08''E	33°53'17,08''N	7	arid inferior

2.2. Essential oil extraction

Dried aerial parts (100 g) were subjected to hydro distillation in a Clevenger type apparatus for 3h according to the European Pharmacopoeia (2017).

2.3. Essential oil analysis

Analysis of volatile compounds by GC was carried out on a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar polyethylene glycol (PEG) HP Innowax capillary column (30 m × 0.25 mm, 0.25 mm film thickness; Hewlett-Packard, CA, 127 USA) was used. The flow of the carrier gas (N_2) was 1.6 mL/min. The split ratio was 60:1. The analysis was performed using the following temperature program: oven temperature kept isothermally at 35°C for 10 min, increased from 35°C to 205°C at the rate of 3°C/min and kept isothermally at 205°C for 10 min. Injector and detector temperatures were held at 250°C and 300°C, respectively. The individual peaks were identified by comparing their relative retention indices to n-alkanes (C6-C22) with those of literature (ADAMS, 2004) and/or with those authentic compounds available in our laboratory. Volatile aroma compounds analysis by GC/MS was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer (Palo Alto, CA, USA) with electron impact ionization (70 eV is the ionization energy). A HP-5 MS capillary column (30 m × 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; Hewlett-Packard, CA, USA) was used. The column temperature was programmed to rise from 50°C to 240°C at a rate of 5°C/min. The carrier gas was helium with a flow rate of 1.2 mL/min; split ratio was 60:1. Scan time and mass range were 1 s and 40-300 m/z, respectively. Identification of aroma compounds was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra.

2.4. Polyphenols extraction

The air-dried aerial parts of *Aloysia triphylla* were finely ground with a blade-carbide gringing (IKA-WERK Type: A: 10). Triplicate sub-samples of 1 g of each ground organ were separately extracted by stirring with 10 mL of pure methanol for 30 min. The extracts were then kept for 24 h at 4°C, filtered through a Whatman No. 4 filter paper, evaporated under vacuum to dryness and stored at 4°C until analyzed (MAU *et al.* 2004).

2.5. Total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteu reagent, following the Singleton's method, which was slightly modified by DEWANTO *et al.* (2002). An aliquot

(0.125 mL) of a suitable diluted methanolic organ extract was added to 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 760 nm. Total phenolic contents of aerial parts (three replicates per treatment) were expressed as mg gallic acid equivalents per gram (mg GAE/g of DW) through the calibration curve with gallic acid. The calibration curve range was 50-400 mg/mL (R^2 = 0.99). All experiments were performed in triplicates.

2.6. Total condensed tannins content

The total tannin content was measured using the modified vanillin assay described by SUN *et al.* (1998). A total of 3mL of 4% methanol vanillin solution and 1.5mL of concentrated H_2SO_4 were added to 50 μL of suitably diluted sample. The mixture was kept for 15 min, and the absorbance was measured at 500 nm against the blank (methanol). The amount of total condensed tannins was expressed as milligrams of (+)-catechin equivalent per gram of dry weight (mg of CE/g of DW) through the calibration curve with catechin. Triplicate measurements were taken for all samples.

2.7. Total flavonoid content

Total flavonoid content was measured according to DEWANTO *et al.* (2002). The 250 µL appropriately diluted methanolic aerial extract was mixed with 75 µL $NaNO_2$ (5%). After 6 min, 150 µL of 10% $AlCl_3$ and 500 µL of NaOH (1 M) were added to the mixture. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid contents of aerial parts (three replicates per treatment) were expressed as mg catechin equivalents per gram (mg CE/g of DW) through the calibration curve with catechin. The calibration curve range was 50-500 mg/mL.

2.8. Isolation and identification of phenolic compounds

Dried samples from aerial parts of *Aloysia triphylla* were treated and used to hydrolize phenolic compounds following the method of PROESTOS *et al.* (2006). Thereafter, the analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an ultraviolet (UV)-vis multi wavelength detector. The separation was carried out on a 250 × 4.6-mm, and 4 μm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15% A/85% B 0-12 min, 40% A/60% B 12-14 min, 60% A/40% B 14-18 min, 80% A/20% B 18-20 min, 90% A/10% B 20-24 min, and 100% A 24-28 min. Phenolic compounds were identified based on their retention times and spectral characteristics of their peaks against those of the standards, as well as by spiking the sample with standards. Analyses were performed in triplicate.

2.9. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

The scavenging capacity of the obtained extracts was measured by bleaching of the purple-colored solution of the DPPH radical according to the method of HANATO *et al.* (1988). A total of 1 mL of different concentrations of extracts prepared in methanol was

added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity was expressed as IC_{50} (μg/mL), which is the concentration required to cause 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect \%} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. BHT was used as a positive control.

2.10. Reducing power assay

The method of OYAIZU (1986) was used to assess the reducing power of different extracts. A total of 1 mL of different concentrations of extracts in methanol was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ and incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged at 650g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue-green color was measured at 700 nm. Results were expressed as EC_{50} (mg/mL), which was the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against the extract concentration. Ascorbic acid was used as a positive control.

2.11. β-Carotene bleaching test

The β-carotene bleaching method is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (KULISIC *et al*., 2004). A modification of the method described by KOLEVA *et al.* (2002) was employed. β-Carotene (2 mg) was dissolved in 20 mL of chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 mL of oxygenated ultra-pure water was added, thereafter, the emulsion was vigorously shaken. Reference compound (BHT), sample extracts were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of different concentrations of extract (1, 10, 100 and 200 μg/mL). The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 120 min, when the absorbance was measured again. BHT was used as the positive control. For the negative control, the extract was substituted by an equal volume of methanol. The antioxidant activity (%) of the *Aloysia triphylla* aerial parts extracts was evaluated in terms of bleaching of the β-carotene using the following formula:

$$\text{\% Inhibition} = \frac{At - Ct}{C0 - Ct}$$

where At and Ct are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min. C_0 is the absorbance values for the control measured at zero time during the incubation. The results are expressed as IC_{50} values (μg/mL), which is the concentration required to cause a 50% β-carotene bleaching inhibition. Tests were carried out in triplicate.

2.12. Screening of antibacterial and antifungal activities

Antibacterial activity was analyzed by the disc diffusion method against four human pathogenic bacteria including *Escherichia coli (ATCC 35218)*, *Pseudomonas aeruginosa (ATCC 4141), Enterococcus faecalis (ATCC 2212), and Bacillus subtilis (CIP 5265*) according to the method of RIOS and RECIO (2005).
The same agar-disc diffusion method was used for screening the antifungal activity of *Aloysia triphylla* aerial parts extracts. Four yeast strains (*Candida albicans* (ATCC 2091), *Candida kefyr*, *Candida parapsilosis, and Candida glabrata* (ATCC 90030)) were first grown on Sabouraud chloramphenicol agar plate at 30°C for 18-24 h. Several colonies of similar morphology of the clinical yeast were transferred into API suspension medium and adjusted to 2 McFarland turbidity standards with a Densimat. The inocula of the respective yeast were streaked on to Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterilized 6 mm paper disc was loaded with 20 μL (10 mg/mL) of aerial parts extract. The treated Petri dishes were placed at 4°C for 1-2h and then incubated at 37°C for 18-24h. The inhibition of fungal growth was also evaluated by measuring the diameter of the transparent inhibition zone around each disc. The susceptibility of the standard was determined using a disc paper containing Nystatin.

2.13. Data analysis

All analyses were performed in triplicate and the results expressed as mean values±standard deviations (SD). The data were subjected to statistical analysis using statistical program package STATISTICA (STATSOFT, 1998). The one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed and the differences between individual mean values were significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Essential oil yields

Essential oil yields were determined by hydrodistillation of 100 g of *Aloysia triphylla* dry aerial parts gathered from different localities. From these results, the optimal yield was observed in the sample of Korba (0.56%) followed by the samples of Kairouan, Siliana and Gabes. Statistical examination revealed significant differences in the essential oil yield of *Aloysia triphylla* originated from Korba, and those from Siliana, Kairouan, and Gabes. These values are closer to those reported by DI LEO LIRA *et al.* (2013) on essential oil yields of *Aloysia triphylla* from Argentina. When compared with other studies reported by MOEIN *et al.* (2014) and EL-HAWARY *et al.*(2011) on *Aloysia citriodora* Palau leaves and on *Lippia citriodora* Kunth fresh leaves, essential oil yields are higher than those of Tunisia region, bearing essential oil contents of 1.3% and 0.9%, respectively. According to TAGHI EBADI *et al.* (2015), EL-HAWARI *et al.* (2015), and BENSABAH *et al.* (2014), variation in the essential oil yield of *Aloysia triphylla* is attributed to cultivation climates, edaphic variables, water quality irrigation, ripening stages, and drying methods.

3.2. Variability in chemical composition of essential oil

Essential oils analyzed are divided into seven classes based on their chemical functional groups (Table 2).

Table 2. Essential oils composition (peak area % w/w±SD) and analysis of variance analysis results (*p*-values) of essential oil *Aloysia triphylla* aerial parts.

Compound*	RRI	Collection region				*P*
		Kairouan	Korba	Siliana	Gabes	
α-Pinene	1031	0.42 ^{c}±0.03	0.55 ^{a}±0.04	0.35 ^{d}±0.02	0.44 ^{b}±0.03	0.002**
Sabinene	1132	0.22 ^{a}±0.01	0.13 ^{d}±0.01	0.20 ^{b}±0.01	0.19 ^{c}±0.02	0.268^{NS}
Myrcene	1176	0.38 ^{d}±0.04	0.41 ^{c}±0.03	0.45 ^{b}±0.05	0.52 ^{a}±0.06	0.001**
Limonene	1202	**7.27** ^{c}±0.54	**6.34** ^{d}±0.43	**7.52** ^{b}±0.74	**7.80** ^{a}±0.81	0.000***
(Z)-β-Ocimene	1245	1.59 ^{d}±0.13	2.00 ^{c}±0.21	2.20 ^{b}±0.19	2.41 ^{a}±0.31	0.309^{NS}
γ-Terpinene	1265	0.21 ^{c}±0.05	0.22 ^{b}±0.03	0.23 ^{a}±0.08	0.19 ^{d}±0.02	0.150^{NS}
cis -Limonene oxide	1450	0.42 ^{b}±0.04	0.44 ^{a}± 0.03	0.39 ^{c}±0.03	0.35 ^{d}±0.03	0.501^{NS}
trans- Sabinene hydrate	1474	0.26 ^{a}±0.02	0.15 ^{d}±0.01	0.19 ^{c}±0.02	0.24 ^{b}±0.01	0.918^{NS}
cis- Sabinene hydrate	1556	0.23 ^{a}±0.02	0.22 ^{b}±0.02	0.20 ^{c}±0.01	0.22 ^{b}±0.02	0.034*
Linalol	1552	0.50 ^{d}±0.04	0.55 ^{c}±0.05	0.58 ^{b}±0.06	0.60 ^{a}±0.05	0.444^{NS}
Terpinene-4-ol	1611	0.12 ^{c}±0.01	0.13 ^{b}±0.01	0.11 ^{d}±0.01	0.15 ^{a}±0.01	0.483^{NS}
α-Cadinol	1620	0.49 ^{d}±0.05	0.54 ^{c}±0.05	0.58 ^{a}±0.06	0.57 ^{b}±0.07	0.009**
trans-Chrysanthenol	1684	0.89 ^{a}±0.07	0.77 ^{b}±0.06	0.69 ^{b}±0.07	0.68 ^{bc}±0.06	0.042*
α-Terpineol	1705	0.74 ^{c}±0.06	0.70 ^{d}±0.05	0.78 ^{a}±0.07	0.75 ^{b}±0.08	0.480^{NS}
cis-Chrysanthenol	1762	0.58 ^{a}±0.06	0.51 ^{b}±0.05	0.40 ^{c}±0.03	0.38 ^{d}±0.03	0.004**
Nerol	1798	0.25 ^{a}±0.02	0.24 ^{b}±0.02	0.20 ^{d}±0.01	0.23 ^{c}±0.02	0.774^{NS}
Geraniol	**1856**	**5.57** ^{d}±0.54	**6.02** ^{b}±0.59	**5.87** ^{c}±0.61	**6.42** ^{a}±0.54	**0.024***
Geranyl acetate	1765	1.79 ^{a}±0.14	1.80 ^{a}±0.12	1.72 ^{b}±0.15	1.70 ^{c}±0.17	0.296^{NS}
Chrysanthenone	1507	0.55 ^{d}±0.05	0.66 ^{a}±0.06	0.65 ^{b}±0.06	0.61 ^{c}±0.06	0.192^{NS}
Neral	**1240**	**17.22** ^{b}±1.80	**14.81** ^{d}±1.25	**15.60** ^{c}±1.44	**18.73** ^{a}±1.75	**0.000*** **
Geranial	**1742**	**25.15** ^{c}±2.15	**26.85** ^{b}±2.17	**27.41** ^{a}±2.45	**24.85** ^{d}±2.51	**0.000*** **
1-8-Cineole	1213	1.62 ^{d}±0.14	1.70 ^{c}±0.15	1.77 ^{b}±0.18	1.78 ^{a}±0.16	0.051^{NS}
α-Cubebene	1350	0.29 ^{b}±0.03	0.33 ^{a}±0.02	0.25 ^{c}±0.03	0.28 ^{bc}±0.01	0.075^{NS}
β-Cubebene	1466	0.11 ^{c}±0.01	0.10 ^{cd}±0.01	0.12 ^{b}±0.01	0.15 ^{a}±0.01	0.223^{NS}
δ-Elemene	1479	0.45 ^{d}±0.03	0.48 ^{c}±0.05	0.50 ^{b}±0.06	0.55 ^{a}±0.06	0.318^{NS}
α-Copaene	1497	0.16 ^{c}±0.01	0.18 ^{b}±0.01	0.19 ^{ab}±0.02	0.20 ^{a}±0.02	0.069^{NS}
β-Bourbonene	1535	0.86 ^{b}±0.07	0.88 ^{a}±0.08	0.85 ^{c}±0.07	0.85 ^{c}±0.08	0.110^{NS}
cis-α-Bergamotene	1560	0.46 ^{a}±0.05	0.38 ^{b}±0.03	0.35 ^{c}±0.03	0.34 ^{c}±0.02	0.003**
β-Copaene	1580	0.30 ^{b}±0.03	0.32 ^{a}±0.03	0.28 ^{c}±0.02	0.25 ^{d}±0.02	0.000***
α-Cedrene	1583	0.52 ^{b}±0.05	0.50 ^{d}±0.04	0.53 ^{a}±0.03	0.51 ^{c}±0.06	0.910^{NS}
β-Gurjunene	1597	0.36 ^{a}±0.03	0.26 ^{b}±0.02	0.21 ^{c}±0.02	0.19 ^{d}±0.01	0.036*
α-Caryophyllene	1610	1.64 ^{d}±0.15	2.21 ^{c}±0.20	2.23 ^{b}±0.21	2.24 ^{a}±0.25	0.244^{NS}
β-Caryophyllene	1612	1.06 ^{a}±0.12	1.00 ^{b}±0.11	0.98 ^{cd}±0.07	0.99 ^{c}±0.08	0.000***
Allo-Aromadendrene	1630	0.48 ^{a}±0.04	0.44 ^{b}±0.03	0.41 ^{c}±0.05	0.48 ^{a}±0.05	0.000***
Aromadendrene	1661	1.03 ^{d}±0.11	1.22 ^{b}±0.10	1.12 ^{c}±0.12	1.25 ^{a}±0.10	0.459^{NS}
α-Amorphene	1679	1.23 ^{d}±0.12	1.40 ^{b}±0.11	1.33 ^{c}±0.10	1.52 ^{a}±0.14	0.279^{NS}
β-Acoradiene	1688	0.41 ^{b}±0.03	0.40 ^{c}±0.05	0.44 ^{a}±0.04	0.38 ^{d}±0.03	0.255^{NS}
α-Zingiberene	1720	0.94 ^{a}±0.08	0.85 ^{b}±0.07	0.84 ^{c}±0.08	0.83 ^{d}±0.09	0.000***
Germacrene-D	1725	0.44 ^{d}±0.05	0.45 ^{c}±0.03	0.47 ^{b}±0.05	0.49 ^{a}±0.05	0.439^{NS}
Bicyclogermacrene	1755	4.54 ^{a}±0.42	4.21 ^{c}±0.41	4.25 ^{b}±0.39	4.12 ^{d}±0.40	0.000***
ar-Curcumene	**1760**	**5.24** ^{a}±0.45	**4.31** ^{d}±0.32	**5.22** ^{b}±0.61	**5.13** ^{c}±0.44	**0.861^{NS}**
α-Cadinene	1773	0.42 ^{c}±0.04	0.40 ^{d}±0.03	0.47 ^{b}±0.05	0.48 ^{a}±0.04	0.344^{NS}
δ-Cadinene	1776	0.63 ^{d}±0.05	0.68 ^{c}±0.06	0.72 ^{b}±0.07	0.77 ^{a}±0.08	0.621^{NS}

Caryophyllene Oxide	2008	0.91 ^{b}±0.08	0.85 ^{d}±0.06	0.92 ^{a}±0.07	0.90 ^{c}±0.08	0.485^{NS}
(E)-Nerolidol	2050	1.41 ^{d}±0.12	1.54 ^{b}±0.14	1.56 ^{a}±0.16	1.47 ^{c}±0.13	0.995^{NS}
Spathulenol	2150	1.06 ^{c}±0.10	1.20 ^{a}±0.11	1.08 ^{b}±0.09	1.09 ^{b}±0.09	0.030*
T-Cadinol	2185	0.53 ^{d}±0.04	0.62 ^{a}±0.05	0.55 ^{c}±0.05	0.59 ^{b}±0.06	0.023*
Isospathulenol	2230	2.60 ^{c}±0.24	2.65 ^{b}±0.25	2.64 ^{bc}±0.26	2.66 ^{a}±0.29	0.995^{NS}
Chemical classes						
Monoterpene hydrocarbons		10.77 ^{c}±1.34	10.46 ^{d}±2.01	11.73 ^{b}±1.32	12.36 ^{a}±2.45	0.000***
Monoterpene alcohols		9.14 ^{d}±0.76	9.46 ^{b}±0.95	9.21 ^{c}±0.83	9.78 ^{a}±0.89	0.000***
Monoterpene esters		1.79 ^{a}±0.14	1.80 ^{a}±0.12	1.72 ^{b}±0.15	1.70 ^{c}±0.17	0.296^{NS}
Monoterpene Ketones		0.55 ^{a}±0.05	0.66 ^{a}±0.06	0.65 ^{a}±0.06	0.61 ^{a}±0.06	0.192^{NS}
Monoterpene aldehydes		42.37 ^{d}±4.37	41.66 ^{c}±3.98	43.01 ^{b}±4.38	43.58 ^{a}±5.12	0.000***
Monoterpene ethers		1.62 ^{d}±0.14	1.70 ^{c}±0.15	1.77 ^{b}±0.18	1.78 ^{a}±0.10	0.051^{NS}
Sesquiterpenes		28.08 ^{d}±2.56	27.86 ^{a}±3.11	28.51 ^{c}±2.73	28.71 ^{b}±3.54	0.000***
Total identified		94.29 ^{c}±7.52	93.60 ^{d}±8.64	96.60 ^{b}±8.72	98.52 ^{a}±7.24	0.000***

RRI: relative retention index; ∗Compounds in order of elution on HP-innowax; values of volatile essential oil percentages are the average of three determinations (n = 3). These values with different letters (a-d) are significantly different at P <0.05. NS: not significant. **P < 0.01. ***P < 0.001. *P*: probability.

A total of 48 compounds were identified representing 94.29%, 93.6%, 96.6%, and 98.52% of total volatiles in the samples of Kairouan, Korba, Siliana, and Gabes, respectively. These different identified compounds vary significantly (P < 0.05) from one region to another and are highly (P <0.001) affected by the regional factor (Table 2). The major contribution was attributed to the monoterpene aldehydes fraction which represents 42.3%, 41.66%, 43.01%, and 43.58% of all compounds detected in Kairouan, Korba, Siliana, and Gabes samples, respectively. Indeed, this latter fraction is dominated by geranial (24.85% in Gabes and 27.41% in Siliana) and neral (14.81% in Korba and 18.73% in Gabes). These results are in agreement with previous studies reported by MOEIN *et al.* (2014) and TAGHI EBADI *et al.* (2015). Our studies have reported that limonene was the third major compound in the essential oil of *Aloysia triphylla* samples with a content ranging between 6.34% and 7.8% for Korba and Gabes samples, respectively.

This result is similar to that reported by MOEIN *et al.* (2014), on essential oil of *Aloysia citriodora* Palau cultivated in gardens where neral, geranial, and limonene rates reached 13.46%, 16.7%, and 12.41%, respectively. Some authors reported citral to be present at a higher percentage than limonene in *L. citriodora* (KIM and LEE, 2004), while others reported the opposite (ÖZEK *et al.*, 1996). SANTOS-GOMES *et al.* (2005) reported that the percentage of citral exceeded that of limonene. The *ar*-curcumene is the main component of sesquiterpene fraction with a rate ranging between 4.31% and 5.24% for the samples of Korba and Kairouan, respectively. From a statistical point of view, this compound seemed not to be affected by any regional factor. Geraniol represented the main constituent in the monoterpene alcohols fraction with a percentage of 5.57%, 6.02%, 5.87%, and 6.42% in the sample of Kairouan, Korba, Siliana, and Gabes, respectively. With regard to the 1-8 cineole recognized for its antimicrobial and antifungal properties, this component showed lower amounts in all localities studied varying between 1.62% and 1.78% for Kairouan and Gabes samples, respectively. TAGHI EBADI *et al.* (2015) reported higher levels of 1-8 cineole in *Lippia citriodora* Kunth essential oil than those reported in our study. The 1-8 Cineole content amounted to 4.5% and 7.3% in freeze dried and oven dried leaves, respectively. It must be pointed out that a variety of geographical and ecological factors can lead to qualitative and quantitative differences in the essential oil produced. At the

same time, essential oil composition can be affected by a number of other factors such as the development stage of the plant, its physiology, the age of leaves, and the growing conditions (SANTOS GOMES *et al.*, 2005) as well as, the conditions and isolation method (CRABAS *et al.*, 2003; KIM and LEE, 2004; SANTOS GOMES *et al.*, 2005).

3.3. Total polyphenols, flavonoids, and condensed tannins

The aerial parts of *Aloysia triphylla* were gathered from different localities, ranging from the center (Kairouan), north (Korba and Siliana), and south (Gabes) in Tunisia characterized by diverse geographic and climatic conditions as mentioned in Table 1. Depending on its geographical origin, total polyphenols, flavonoids, and condensed tannins contents of *Aloysia triphylla* extracts are illustrated in Fig. 1.

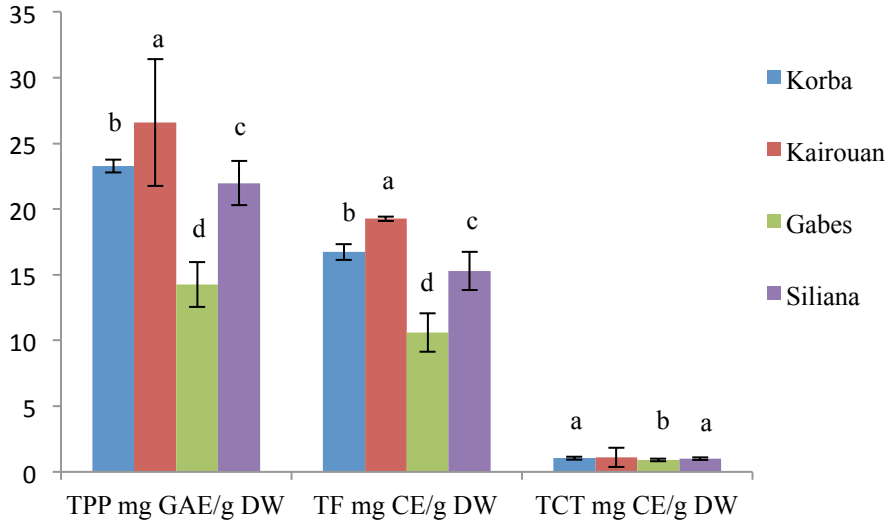

Figure 1. Total polyphenols (TPP), total flavonoids (TF), and total condensed tannin (TCT) contents of different regions of *Aloysia triphylla* aerial parts. GAE: gallic acid equivalents; CE: catechin equivalents. The letters (a-d) indicate significant differences ($p < 0.05$).

The results showed that the plant is a valuable source of phenolics with content ranging from 26.58 mg GAE/g for Kairouan to 14.25 mg GAE/g for Gabes, respectively. These results are lower than those cited by CHEURFA and ALLEM (2016) on hydro-alcoholic and aqueous extracts of Algerian *Aloysia triphylla* leaves. Moreover, ZHANG and WANG (2001) reported a total phenolic content of 1.55 mg GAE/ g of fresh weight on *Aloysia triphylla* herb extracted with phosphate buffer. Statistical analysis revealed that the total polyphenol contents varied significantly ($p < 0.05$) between the four studied localities. Likewise, the importance of the solvent type used in the extraction has been mentioned by CHOUPANI *et al.* (2014) and BETTAIEB REBEY *et al.* (2012). According to VASCO *et al.* (2008), differences in total phenol content may also be attributed to varieties, ripening stage, and post-harvest conditions (MSAADA *et al.*, 2007). Besides, the highest total flavonoid content was observed in Kairouan (19.26±0.74 mg EC/ g) followed by Korba (16.72±0.59 mg EC/g), Siliana (15.3±1.46 mg EC/g), and Gabes 10.59±1.1 mg EC/g), respectively. These results are much higher than those reported by VINHA *et al.* (2012) on the aqueous extract of *Aloysia triphylla*, showing a total flavonoid content of 43.38 mg C/100 g). It is well known that an important function of flavonoids and phenolic acids is

their action in plant defense mechanisms (DIXON and PAIVA, 1995). Indeed, flavonoids have several biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release, potent nitric oxide radical scavenging activity and exhibiting antibacterial, antiviral, anti-inflammatory and antiallergenic effects (COOK and SAMMAN, 1996).
Among the different localities studied, no significant differences ($p > 0.05$) were found in the total condensed tannins contents of *Aloysia triphylla* methanolic extracts. Kairouan showed the highest total condensed tannins (1.10±0.17 mg CE/g of DW), followed in a descending order by Korba (1.04±0.11 mg CE/g of DW), Siliana (1.01±0.10 g CE/g of DW), and Gabes (0.89±0.09 g CE/g of DW). Interestingly, no condensed tannins were observed in aqueous extracts (infusion and decoction) of *Aloysia citriodora* aerial parts (PORTMANN *et al.*, 2012). This finding was attributed to these authors in the absence of proanthocyanidins. Meanwhile, EL BABILI *et al.* (2013) reported a condensed tannins content of 1.97 g CE/kg dry in aqueous extract of verbena (*Verbena officinalis* L.) belonging to the same botanical family (Verbenacea).

3.4. Individual phenolic compounds

The phenolic compounds in methanol extracts of the aerial parts of *Aloysia triphylla* were identified by a RP-HPLC system. This system is a high resolution chromatographic technique widely used for simultaneous separation and quantification of phenolic substances. The results related to phenolic compounds are summarized in Table 3. It is fair to say that methanol extracts of *Aloysia triphylla* are rich in phenolics. In total, 15 compounds were identified. Statistical analysis revealed that the identified compounds were significantly affected ($P < 0.001$) by the regional factor. The *p*-coumaric acid was the predominant phenolic compound. The highest content of *p*-coumaric acid was observed in Kairouan (54.90%), followed by Korba, Siliana, and Gabes. The second major compound was catechol with a content ranging from 6.00% to 12.23% for Kairouan and Korba, respectively. Likewise, RP-HPLC analysis was used for the identification and quantification of phenolic compounds in leaves of *Aloysia triphylla* after extraction with a mixture of 62.5% aqueous methanol. Authors reported the presence of only four compounds: caffeic acid (0.84%), ferulic acid (0.82%), hydroxytyrosol (0.4%), and apigenin (0.24%). The presence of caffeic and ferulic acids in methanol extracts of *Aloysia triphylla* is worth noting with concentrations ranging between 0.28% and 2.39%, and 0.36% and 3.28% for Korba and Gabes, and Gabes and Siliana, respectively. Furthermore, apigenin and hydroxytyrosol were not identified in our present study. The *O*-hydroxybenzoic acid, hydroxycaffeic acid, and 3-nitro-phthalic acid were also identified by GC-MS after sialylation according to PROESTOS *et al.* (2006). Three phenolic compounds; two phenolic acids, dihydrocaffeic acid and 4-hydroxycinnamic acid and a flavonoid glycoside, luteolin-7-*O*-glucoside, were isolated and identified from the ethyl acetate fraction of the fresh aerial parts of *Lippia citriodora* Kunth cultivated in Egypt (EL HAWARY *et al.*, 2012). Luteolin 7-*O* glucoside was also identified in methanol extracts of the aerial parts of *Aloysia triphylla*, which originated from Korba (2.46%), Siliana (0.16%), Gabes (2.10%), and Kairouan (0.35%). This flavonoid glycoside is one of the main flavonoid constituents in many herbs and is known to possess low oxygen radical absorbance capacity (ORAC) values (ZHANG and WANG, 2001).

3.5. Antioxidant activities of methanol extracts

The results for antioxidant activities from the different accessions are displayed in Table 4. ANOVA analysis (Table 4) showed that the methanol extracts are highly influenced by the

regional effect (antiradical activity was region-dependent). Methanolic extract of Kairouan sample shows the highest antioxidant activity (IC_{50} = 5.78±0.08 μg/mL), which was stronger than that of the positive control: BHT (11.5±1.23 μg/mL). The reduced activity was observed in the sample of Gabes (30.67 μg/mL). CHEURFA and ALLEM (2016) have reported antiradical of aqueous extract of Algerian *Aloysia triphylla* leaves activity with an IC_{50} of 27.4 mg/mL. This value was significantly higher ($P < 0.05$) than that of hydro-alcoholic extract witnessing an IC_{50} of 23.52 mg/mL. On the other hand, the positive control BHT exhibited a significantly lower IC_{50} value ($P < 0.05$) when compared to the two studied extracts (6.96 mg/mL). These antiradical activities are lower than those reported in our present study. Other studies conducted on culinary decoction of verbena (*Verbena officinalis* L.), belonging to the same botanical family of *Aloysia triphylla*, showed an IC_{50} of 15.76 mg/mL (EL BABILI *et al.*, 2013). It is worth mentioning now that there is a linear correlation between total polyphenols, flavonoids, and condensed tannins, and the scavenging activity against DPPH for the methanolic extracts of the aerial parts of *Aloysia triphylla*. In fact, the methanolic extract of Kairouan sample, rich in polyphenols, flavonoids, and condensed tannins, was a more effective scavenger of DPPH radicals than the poor ones observed in Korba, Siliana, and Gabes samples. Consistency can be seen in our results and those obtained by CHEURFA and ALLEM (2016) who analyzed the total polyphenols, flavonoids, and scavenging activity of DPPH in aqueous and hydro-alcoholic extracts of *Aloysia triphylla* leaves. Meanwhile, EL-BABILI *et al.* (2013) failed to show any positive correlation between phenol contents and anti-oxidant activities according to the ABTS/DPPH assays on culinary decoction of *Verbena officinalis* L.

Besides, Table 4 showed that the Fe^{3+} reducing power of *Aloysia triphylla* methanolic extracts differs greatly depending on accession provenance. Gabes sample showed the higher reducing capacity (EC_{50} = 482.00 μg/mL) followed by Siliana (EC_{50} = 371.00 μg/mL), Korba (EC_{50} = 322.66 μg/mL), and Kairouan (EC_{50} = 209.33 μg/mL). Furthermore, in comparison with the positive control: ascorbic acid (EC_{50} = 37.33 μg/mL), Kairouan, Korba, Siliana, and Gabes samples methanolic extracts exhibited 6, 9, 10, and 13 fold lower activities, respectively. These results indicate that the different methanolic extracts are able to act as electron donor and, therefore, react with free radicals, converting them to a more stable products and, thereby, terminating radical chain reactions. On the other hand, statistical analysis revealed a higher region effect ($P < 0.001$) on reducing power (Table 4).

The antioxidant activity of *Aloysia triphylla* methanolic extract was also evaluated by the β-carotene-linoleate bleaching method (Table 4). This method was based on the loss of the yellow color of β-carotene due to its reaction with radicals formed after linoleic acid oxidation in emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (KULISIC *et al.*, 2004). Siliana sample showed the lowest ability to prevent the bleaching of β-carotene (IC_{50} = 4066.67 μg/mL), whereas Kairouan sample exhibited the strongest activity (IC_{50} = 500 μg/mL).

On the other hand, all the methanolic extracts had lower antioxidant activities than BHT with IC_{50} of 75.00 μg/mL (Table 4). In addition, the β-carotene-linoleate bleaching values were highly ($P < 0.001$) affected by the accession provenance.

It is worth mentioning that differences in antioxidant activities according to the DPPH/β-carotene bleaching inhibition/reducing power might reflect differences in the ability of anti-oxidant compounds to act against the different radicals present or formed during each specific reaction.

3.6. Antibacterial activity

The test results of the antibacterial effect are summarized in Table 5. The results showed that the diameter of the inhibition zone (IZ) is highly affected by the region's factor for

Escherichia coli (ATCC 35218) and *Bacillus subtilis (CIP 5265)* strains ($P < 0.001$). On the other hand, essential oils of *Aloysia Triphylla* did not exhibit an antibacterial activity against *Pseudomonas aeruginosa (ATCC 4141) and Enterococcus faecalis (ATCC 2212)* strains. The highest antibacterial activity was observed against *Bacillus subtilis (CIP 5265)* witnessing an inhibition zone equal to 85±7.67 mm for the *Aloysia triphylla* essential oil of Kairouan, followed by that of Siliana (IZ = 32±3 mm), Korba (IZ = 26±4.08 mm), and Gabes (IZ = 25.33±9.62 mm). The lower antibacterial activity of *Aloysia triphylla* essential oils against *Escherichia coli*, when compared to that against *Bacillus subtilis,* was attributed to the cell-wall of the Gram-negative bacteria covered by an outer membrane (Lipopolysaccharide, phospholipid and some proteins) (CHAO *et al.,* 2000). This latter prevents uptake of oils or protect peptidoglycan layer from oils. Hence, Lipopolysaccharide (LPS) membrane of Gram-negative bacteria presents a permeability barrier to hydrophobic substances that can enter and inhibit the Gram-positive bacteria. In Gram-positive bacteria, the peptidoglycan layer is on the outside and more in contact with the oils.
Our results are in compliance with those reported by ALI *et al.* (2011) who reported a very strong antibacterial activity of *Aloysia triphylla* essential oil against *Bacillus subtilis (CAICC)* (IZ ≥ 16 mm) and a negative one against *Escherichia coli (ATCC 25922)* (IZ = 0 mm).
The interesting antibacterial activity against *Bacillus subtilis* was attributed to the presence of a high concentration of long-chain alcohols especially geranial and neral, particularly active against Gram-positive bacteria (DELAQUIS *et al.,* 2002). In addition, ALI *et al.* (2011) reported that the antibacterial nature of *Aloysia triphylla* essential oil was apparently related to the presence of β-caryophyllene, showing in vitro activity against *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* (SACCHETTI *et al.* 2004).

3.7. Antifungal activity

Aloysia triphylla essential oils had a significant antifungal activity against the four strains of *Candida* species studied as shown in Table 5. The highest antifungal activity was recorded for the *Aloysia triphylla* essential oil of Kairouan, showing inhibition diameter zones of 85±8.27 mm, 85±7.89 mm, 85±6.59 mm, and 85±8.57 mm against *Candida albicans (ATCC 2091)*, *Candida kefyr*, *Candida parapsilosis*, and *Candida glabrata (ATCC 90030),* respectively. These antifungal activities were higher than that observed for Nystatin (IZ = 25±2.68 mm) and used as a reference substance. Higher antifungal activities were also observed for the *Aloysia triphylla* essential oil of Siliana with inhibition diameter zones of 85±8.04 mm for *Candida albicans (ATCC 2091)*, 85±8.45 mm for *Candida parapsilosis,* and 85±8.81 mm for *Candida glabrata (ATCC 90030)*, respectively. Moreover, *Aloysia triphylla* essential oil of Gabes, showed an antifungal activity higher than that of Korba against *Candida albicans* (IZ = 85±2.48 mm vs 46.33±2.61 mm) and lower than those observed for the three other strains of *Candida* studied. Our results are higher than those observed by ALI *et al.* (2011) on antifungal activity of *Aloysia triphylla* essential oil against *Candida albicans* CAICC 51, witnessing an inhibition diameter zone between 10 and 15 mm. Antifungal activity presented by *Aloysia tripylla* essential oils could be associated with the presence of citral. In fact, literature points to the fact that citral acts as a fungicidal agent because it is capable of forming a charge transfer complex with an electron donor of fungal cells, resulting in fungal death (KURITA *et al.,* 1981).

Table 3. Phenolic composition (peak area %, w/w) and analysis of variance results (*p*-value) of methanol extracts of *Aloysia tripyllla* aerial parts.

Compound	Collection region				*P*
	Kairouan	Korba	Siliana	Gabes	
Resorcinol	1.19 ^{d}±0.07	1.96 ^{a}±0.07	1.72 ^{b}±0.02	1.25 ^{c}±0.01	0.000***
Catechol	6.00 ^{d}±0.39	12.23 ^{a}±0.34	11.14 ^{b}±0.08	8.23 ^{c}±0.11	0.000***
Epigallocatechin	1.30 ^{b}±0.06	0.09 ^{cd}±0.03	0.10 ^{c}±0.01	1.34 ^{a}±0.02	0.000***
Caffeic acid	1.50 ^{b}±0.10	0.28 ^{d}±0.07	1.11 ^{c}±0.09	2.39 ^{a}±0.03	0.000***
Syringic acid	1.92 ^{b}±0.28	2.10 ^{a}±0.13	1.49 ^{c}±0.13	0.96 ^{d}±0.36	0.000***
p-Coumaric acid	54.90 ^{c}±1.33	38.91 ^{a}±0.53	38.81 ^{a}±2.50	38.23 ^{b}±3.34	0.000***
Sinapic acid	3.97 ^{a}±0.36	1.82 ^{c}±0.18	0.86 ^{d}±0.10	3.73 ^{b}±0.32	0.000***
Ferulic acid	0.38 ^{c}±0.03	1.24 ^{b}±0.04	3.28 ^{a}±0.26	0.36 ^{d}±0.03	0.000***
Luteolin 7-*O*- glucoside	0.35 ^{c}±0.02	2.46 ^{a}±0.10	0.16 ^{d}±0.03	2.10 ^{b}±0.07	0.000***
Coumarin	1.45 ^{c}±0.06	0.22 ^{d}±0.02	1.63 ^{b}±0.05	1.92 ^{a}±0.21	0.000***
Rutin	0.52 ^{b}±0.03	0.25 ^{c}±0.01	1.03 ^{a}±0.10	1.04 ^{a}±0.36	0.000***
Rosmarinic acid	1.20 ^{b}±0.09	0.22 ^{d}±0.01	1.33 ^{a}±0.03	0.49 ^{c}±0.16	0.000***
Resveratrol	0.20 ^{d}±0.01	0.61 ^{b}±0.02	0.44 ^{c}±0.11	0.76 ^{a}±0.06	0.000***
Ellagic acid	0.28 ^{d}±0.13	1.26 ^{a}±0.11	0.31 ^{c}±0.02	0.70 ^{b}±0.06	0.000***
Quercetin	0.26 ^{d}±0.06	0.45 ^{b}±0.05	1.39 ^{a}±0.03	0.38 ^{c}±0.40	0.000***

The values of the levels and percentages of phenolic compounds represent the average of three replicates ($n = 3$). Letters (a-d) indicate significant differences at $P < 0.05$. *** Significant effect at $P < 0.001$. *P*: probability.

Table 4. DPPH scavenging activity (IC_{50} μg/mL), reducing power (EC_{50} μg/mL), and β-carotene bleaching (IC_{50} μg/mL) and analysis of variance results (*p*-value) of methanol extracts of *Aloysia tripyllla* aerial parts.

	Collection region					BHT	Ascorbic acid
	Kairouan	Korba	Siliana	Gabes	*P* value		
DPPH scavenging activity (IC_{50} μg/mL)	5.78 ^{d}±0.08	6.07 ^{c}±0.13	13.23 ^{b}±0.28	30.67 ^{a}±1.31	0.000***	11.5±1.23	
Reducing power (EC_{50} μg/mL)	209.33 ^{d}±1.30	322.66 ^{c}±2.84	371.00 ^{b}±1.13	482.00 ^{a}±2.26	0.000***		37.33±3.41
β-carotene bleaching (IC_{50} μg/mL)	500.00 ^{c}±11.32	3966.67 ^{a}±65.33	4066.67 ^{a}±65.33	1566.67 ^{b}±130.66	0.000***	75±5.12	

Values are means of triplicates±SD. Values in the same row with different superscripts (a–d) are significantly different at $P < 0.05$. *** $P < 0.001$.

Table 5. Antibacterial and antifungal (IZ mm) activities and analysis of variance results (*p*-value) of essential oils of *Aloysia tripyllla* aerial parts.

	Collection region				*P*	Tetracycline 10 μg/mL	Nystatin 10 μg/mL
	Kairouan	Korba	Siliana	Gabes			
bacteria							
Escherichia coli (ATCC 35218)	12 ^{a}±1.96	11 ^{c}±1.13	8.67 ^{d}±0.65	11.33 ^{b}±0.65	0.000***	23±2.55	
Pseudomonas aeruginosa (ATCC 4141)	na	na	na	na	-	22±2.71	
Enterococcus Faecalis (ATCC 2212)	na	na	na	na	-	24±2.64	
Bacillus Subtilis (CIP 5265)	85 ^{a}±7.67	26 ^{c}±4.08	31.67 ^{b}±3.27	25.33 ^{d}±9.62	0.000***	25±2.11	
Fungi							
Candida albicans (ATCC 2091)	85 ^{a}±8.27	46.33 ^{a}±2.61	85 ^{a}±8.04	85 ^{b}±2.48	0.000***		25±2.68
Candida kefyr	85 ^{a}±7.89	21.67 ^{c}±4.71	28.33 ^{b}±1.73	19.33 ^{d}±1.31	0.000***		24±2.18
Candida parapsilosis	85 ^{a}±6.59	28.67 ^{b}±9.15	85 ^{a}±8.45	25 ^{c}±2.56	0.000***		25±3.05
Candida glabrata (ATCC 90030)	85 ^{a}±8.57	18 ^{b}±4.08	85 ^{a}±8.81	14 ^{c}±1.96	0.000***		23±2.33

Results are the mean of three replications. The diameter of disc was 6 mm. Values with different superscripts (a-d) are significantly different at $P < 0.05$. na: not active. NS: not significant. *P*: probability. ** $P < 0.01$. *** $P < 0.001$.

4. CONCLUSIONS

This study has revealed that *Aloysia triphylla* methanolic extracts and essential oils features are markedly influenced by the regional factor. Korba sample have high potential for selecting variety rich in essential oil, while Kairouan sample was suitable for being a valuable source of antioxidants, including condensed tannins, flavonoids, phenolic compounds, and polyphenols.
The high content of these bioactive compounds both highlights their nutritional and medicinal values and provides a high protection against oxidation phenomenon. Essential oil of *Aloysia triphylla,* which originated from Kairouan stood out for presenting the best selective antibacterial and antifungal performances. Additional researches on the bioactivities of *Aloysia triphylla* might pave the way for its broad application in industrial, pharmaceutic, and cosmetic fields.

REFERENCES

Adams R.P. 2004. Identification of essential oil components by gas chromatography/mass spectrometry. Allured, IL: Carol Stream.

Ali H.F.M., El-Beltagi H.S. and Nasr N.F. 2011. Evaluation of antioxidant and antimicrobial activity of *Aloysia Triphylla*. Elec. J. Envir. Agric. Food Chem. 10:2689-2699.

Bensabah F, Lamiri A, and Naja J. 2014. Effect of purified wastewater from the city of Settat (Morocco) on the quality of *Lippia citriodora* essential oil and infusion. J. Saudi Soc. Agric. Sci. 14:101-108.

Bettaieb Rebey, I, Bourgou S, Ben Slimen Debez I, Jabri Karoui I, Hamrouni Sellami I, Msaada K, Limam F, and Marzouk B. 2012. Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of Cumin (*Cuminum cyminum* L.) Seeds. Food Bioprocess Technol. 5:2827–2836.

Carnat A., Carnat A.P., Fraise D. and Lamaison, J.L. 1999. The Aromatic and polyphenolic composition of *Lemon verbena* Tea. *Fitoterapia* 7:44-49.

Catalan C.A.N., de and Lampasona M.E.P. 2002. The chemistry of the Genus Lippia (Verbenaceae). *In*: Kintzios, S.E. (Ed.), Oregano: The genera Origanum and Lippia, first ed. Taylor and Francis Inc. 127-149. pp.

Chao S.C., Young D.G. and Oberg C.J. 2000. Screening of inhibitory activity of essential oils on selected bacteria, fungi and viruses. J. Essent. Oil Res. 12:639-649.

Cheurfa M. and Allem R. 2016. Evaluation of antioxidant activity of different extracts of *Aloysia triphylla* leaves (L'Herit.) from Algeria *in vitro*. Phytother. 14:181-187.

Choupani M., Arabshahi Delouee S. and Alami M. 2014. Antioxidant properties of various solvent extracts of Lemon verbena (*Lippia citriodora*) leaves. Inter. J. Adv. Biol. Biomed. Res. 2:1340-1346.

Cook N.C. and Samman S. 1996. Flavonoids- chemistry, metabolism, cardio protective effects and dietary sources. J. Nutr. Biochem. **7**:66-76.

Crabas N., Marongiu B., Piras A., Pivetta T. and Porcedda S. 2003. Extraction, separation and isolation of volatiles and dyes from *Calendula officinalis* L. and *Aloysia triphylla* (L'Her.) britton by supercritical CO_2. J. Essent. Oil Res. 15:350-355.

Delaquis P.J., Stanich K., Girard B. and Mazza G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Inter. J. Food Microbiol. 74:101-109.

Dewanto V., Wu X., Adom K. and Liu R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50:3010-3014.

Di Leo Lira P., Van Baren C.M., López S., Molina A., Heit C., Viturro C. and De Lampasona M.P. 2013. Northwestern Argentina: A center of genetic diversity of lemon verbena (*Aloysia citriodora* PALÁU, Verbenaceae). Chem. Biodiv. 10:251-261.

Dixon R.A. and Paiva N.L. 1995. Stress-induced phenylpropanoid metabolism. The Plant Cell 7:1085-1097.

Domínguez-Avila J.A., González-Aguilar G.A., Alvarez-Parrilla E. and De la Rosa L.A. 2016. Modulation of PPAR expression and activity in response to polyphenolic compounds in high fat diets. Inter. J. Mol. Sci. 17 (7):1002.

Duarte M.C.T., Figueira G.M., Sartoratto A., Rehder V.L.G. and Delarmelina C. 2005. Anti-candida activity of Brazilian medicinal plants. J. Ethnopharmacol. 97:305-311.

El Babili F., Babili M.E.L., Souchard J.P., Chatelain C. 2013. Culinary decoctions: spectrophotometric determination of various polyphenols coupled with their antioxidant activities. Pharm. Crops, 4, 15-20.

El Hawary S., Yousif M., Abdel Motaal A. and Abd-El Hameed L. 2011. Composition and bioactivities of the essential oil from leaves of *Lippia Citriodora* Kunth cultivated in Egypt. J. Biol. Active Prod. Nat. 1:112-119.

El-Hawary S.S., Yousif M.F., Abdel Motaal A. and Abd-Hameed LM. 2012. Bioactivities, phenolic compounds and *in-vitro* propagation of *Lippia citriodora* Kunth cultivated in Egypt. Bull. Fac. Pharm. Cairo Univ. 50:1-6.

European Pharmacopoeia (Ph. Eur.) 9th ed. 2017. https://www.edqm.eu/en/european-pharmacopoeia-9th-edition.

Hanato T., Kagawa H., Yasuhara T. and Okuda T. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. Chem. Pharm. Bul.l 36:1090-1097.

Kim N.S. and Lee D.S. 2004. Headspace solid-phase microextraction for characterization of fragrances of lemon verbena (*Aloysia triphylla*) by gas chromatography mass spectrometry. J. Sep. Sci. 27:96-100.

Koleva I.I., Teris A.B., Jozef P.H., Linssen A.G., Lyuba N.E. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13, 8–17.

Kulisic T., Radonic A., Katalinic V. and Milos M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 85:633-640.

Kurita N., Miyaji M., Kurane R. and Takahara Y. 1981. Antifungal activity of components of essential oils. Agric. Biol. Chem. 45:945-952.

Magwa M.L., Gundidza M., Gweru N. and Humphrey G. 2006. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J. Ethnopharmacol. 103:85-89.

Mau J.L., Chang C.N., Huang S.J. and Chen C.C. 2004. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitoymyces albuminosus mycelia*. Food Chem. 87:111-118.

Moein M.M., Zarshenas M. and Etemadfard H. 2014. Essential oil composition and total flavonoid content of *Aloysia Citriodora* Palau under different cultivation systems. Inter. J. Plant Anim. Envir. Sci. 4:353-358.

Msaada K., Hosni K., Ben Taarit M., Chahed T., Kchouk M.E. and Marzouk B. 2007. Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. Food Chem. 102:1131-1134.

Oyaizu M. 1986. Studies on products of browning reaction: antioxidative activity of products of browning reaction. Jap. J. Nutr. 44:307-315.

Özek T., Kirimer N., Baser K.H.C. and Tümen G. 1996. Composition of the essential oil of *Aloysia triphylla* (L'Herit.) Britton Grown in Turkey. J. Essent. Oil Res. 8:581-583.

Pascual M.E., Slowing K., Carretero E., Šanchez Mata D. and Villar A. 2001. *Lippia*: traditional uses, chemistry and pharmacology: a review. J. Ethnopharmacol. 76:201-214.

Portmann E., López Nigro M.M., Reides C.G., Llesuy S., Ricco R.A. and Wagner M.L. 2012. Aqueous extracts of *Lippia turbinata* and *Aloysia citriodora* (Verbenaceae): assessment of antioxidant. Inter. J. Toxicol. 31:192-202.

Proestos C., Boziaris I.S., Nychas G.J.E. and Komaitis M. 2006. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 95:664-671.

Quirantes-Piné R., Funes L., Micol V., Segura-Carretero A. and Fernández-Gutiérrez A. 2009. High-performance liquid chromatography with diode array detection coupled to electrospray time-of-flight and ion-trap tandem mass spectrometry to identify phenolic compounds from a *Lemon verbena* extract. J. Chromatogr. A 1216:5391-5397.

Ríos J.L. and Recio M.C. 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100:80-84.

Sacchetti G., Medici A., Maietti S., Radice M., Muzzoli M. and Manfredini S. 2004. Composition of functional properties of the essential oil of amazonian basil, *Ocimum micranthum* Willd. Labiatae in comparison with commercial essential oils. J. Agric. Food Chem. 52:3486-3491.

Santos-Gomes P.C., Fernandes-Ferreira M. and Vicente A.M.S. 2005. Composition of the essential oils from flowers and leaves of vervain (*Aloysia triphylla* (L'Herit.) Britton) grown in Portugal. J. Essent. Oil Res. 17:73-78.

Sharma A., Flores-Vallejo R.D.C. Cardoso-Taketa A. and Villarreal M.L. 2016. Antibacterial Activities of Medicinal Plants Used in Mexican Traditional Medicine. J. Ethnopharmacol. 208:264-329.

Statsoft. 1998. *STATISTICA for Windows (Computer Program Electronic Manual)*; Statsoft Inc.: Tulsa, OK.

Sun B., Ricardo-da-Silva J.M. and Spranger I. 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46:4267-4274.

Taghi Ebadi M., Azizi M., Sefidkom F. and Ahmadi N. 2015. Influence of different drying methods on drying period, essential oil content and composition of *Lippia citriodora* Kunth. J. App. Res. Med. Arom. Plants. 2:182-187.

Teixeira Duarte M.C., Leme E.E., Delarmelina C., Soares AA., Figueira G.M. and Sartoratto A. 2007. Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. J. Ethnopharmacol. 111:197-201.

Valentão P., Andrade P.B., Areias F., Ferreres F. and Seabra R.M. 1999. Analysis of vervain flavonoids by HPLC/diode array detector method. Its application to quality control. J. Agric. Food Chem. 47:4579-4582.

Vasco C., Ruales J. and Kamal-Eldin A. 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chem. 111:816-823.

Vinha A.F., Soares M.O., Castro A., Santos A., Oliveira M.B.P.P. and Machado M. 2012. Phytochemical characterization and radical scavenging activity of aqueous extracts of medicinal plants from Portugal. Eur. J. Med. Plants 2:335-347.

Zhang W. and Wang S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49:5165-5170.

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