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ORIGINAL ARTICLE

Chemical Composition of Essential Oil (GC-FID and GC-MS), Phenolic Compounds Content (LC–ESI–MS), and Antioxidant Activity of *Eucalyptus marginata* Leaves from Northern Tunisia: Effect of Season

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Abstract

Eucalyptus marginata is an aromatic and medicinal plant species used in several industrial sectors, owing to the chemical properties of its essential oil. The present study aimed to evaluate the chemical composition and antioxidant activities of *Eucalyptus marginata* leaves collected in three seasons of the year (winter, spring and summer). Therefore, essential oil yields, total phenolic, flavonoid, tannin and chlorophyll contents have been evaluated. Essential oil chemical composition was carried out by combination of GC-FID and GC–MS. While, individual phenolic quantification has been done using LC–ESI–MS. This study demonstrated that spring seasons presented the highest contents of total phenolic compounds (491.01 mg GAE/g DW), chlorophyll α (134.55 mg/g DW), chlorophyll β (216.61 mg/g DW), chlorophyll α + β (350.61 mg/g DW), DPPH (1.099 mg/g DW) and ABTS (0.909 mg/g DW). The major compounds of *Eucalyptus marginata* essential oil for the three seasons were 1,8-Cineol (22.42 − 30.52%), while, the quantification of the identified phenolic compounds showed a significant difference between the three season ($p < 0.05$). Results revealed that *Eucalyptus marginata* is a nature source of compounds with antioxidant proprieties, and the difference in chemical composition leads to change in the antioxidant activity of plant, which contributes to seasonal change.

Keywords Antioxidant · Aromatic plants · *Eucalyptus marginata* · Essential oil · Seasonal variation

1 Introduction

The family *Myrtaceae* includes over 5800 species distributed worldwide. *Myrtaceae* species are considered as a valuable source of essential oils that are well exploited in aromatherapy, cosmetic and pharmaceutical industries [1]. *Eucalyptus* is a widespread genus of the *Myrtaceae* family that is represented by more than 900 species [2]. This genus was native to Australia and introduced worldwide, including Tunisia [2, 3]. Leaves of *Eucalyptus* species accumulate a very large number of secondary metabolites and essential oils that possess many types of medicinal, cosmetic, and food applications thanks to its richness of bioactive products such as phenolic compounds and essential oils [4]. Consequently, the production of secondary metabolites especially their composition on essential oils and phenolic compounds could be affected by the meteorological elements and locale climate, which included many factors such as rainfall, temperature, relative humidity and photoperiod [5, 6]. This variation was caused by seasonal and daily changes [7, 8]. In addition, many studies have shown that the variation in secondary metabolites production could be attributed to the seasonality [9, 10]. These letters mentioned that seasonal changes significantly affect the biosynthesis of antioxidant compounds such as total phenolic and flavonoid

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contents and the trend of changes are very different due to seasonal variations. Therefore, it is recommended to take into account seasonal changes, when harvesting plants in order to separate antioxidant ingredients, and important medicinal and industrial compounds, because awareness of the impact of seasonal changes, may can help producers choose the best period for harvesting plants and producing plant products richer than the desired compounds for use in the pharmaceutical and food industries.

The literature survey indicates no works dealing with the effect of season on the phenolic and essential oil compositions extracted from *Eucalyptus marginata* leaves' cultivated in Tunisia. Herein, and for the first time, we aimed to characterize *Eucalyptus marginata* essential oil and ethanolic-aqueous extract using the following parameters: GC-MS and GC-FID in order to identify the essential oil composition, identify and quantify individual phenolic compounds present in the ethanolic-aqueous leaves extract using LC-ESI-MS, colorimetric quantification of total phenol, flavonoid and condensed tannin contents as well as the evaluation of its antioxidant activities via DPPH and ABTS assays.

2 Experimental Section

2.1 Plant Material

The *Eucalyptus marginata* leaves' were collected at different seasons (winter, spring, and summer) from the arboretum of Souiniet (492 m 35°54′N 8°48′E) in winter, spring and summer of 2020. Voucher specimens (EMHWI2020, EMHSP2020 and EMHSU2020) were deposited at the herbarium of INRGREF. Souiniet arboretum is characterized by an average annual rainfall of 1553 mm (winter: 714 mm; spring: 377 mm and summer: 48 mm), the average annual temperature is 15.2 °C, with a minimum of 2.3 °C in January and a maximum of 25 °C in July. The humid bioclimate at the upper level with a temperate winter. The soil humus is a mull on sandstone and the parent rock forms the "Numidian Flysh". The soil is clay-sandy, moderately rich in organic matter. There are three months of drought during June, July and August.

2.2 Sample Preparation

Extraction of phenolic compounds: Ethanolic-aqueous plant extract of *Eucalyptus marginata* leaves' was prepared as follow: at room temperature for 24 h under stirring at 11,000 rpm (Fisher brand Seastar digital orbital shaker, UK) using ethanol-water (80/20, v/v) as solvent. Five grams of each origin dry leaves powder were suspended in 50 mL of solvent. Finally, each extracts were filtered by using filter paper (10–20 µm) and they were stored in sealed dark bottles at 4 °C until use for assay.

Extraction of Condensed Tannin Tannin compounds have been extracted according to the procedure described by Yahyaoui et al. [11].

2.3 Determination of Total Phenol, Flavonoid and Condensed Tannin Contents

The total phenolic content of the extract was determined by the Folin–Ciocalteu method [12].

The computation of total phenolic content was derived from the calibration curve ($y = 0.2649x$, $r^2 = 0.997$). The findings were articulated as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). While, the total flavonoid content of each extract was determined by the aluminium chloride colorimetric method [13]. The determination of total flavonoid content relied on a calibration curve ($y = 0.235x$, $r^2 = 0.994$), with the outcomes presented as milligrams of Rutin equivalent per gram of dry weight (mg RE/g DW). On the other hand, condensed tannins have been investigated according to the method proposed by Yahyaoui et al. [11]. For this, 50 µL of each extract were mixed with 3 mL of vanillin reagent (4%) and 1.5 mL of H_2SO_4 (4%). After 15 min, the absorbance was measured at 500 nm. The determination of condensed tannin content involved the utilization of a calibration curve established using a catechin solution ($y = 0.0602x - 0.0047$, $r^2 = 0.9987$). The findings were reported as milligrams of catechin equivalent per gram of dry weight (mg CE/g DW). All measurements were performed in triplicates.

2.4 Analysis of Individual Phenolic Compounds by Analytical LC–ESI–MS

In order to explore the composition of *Eucalyptus marginata* leaves' ethanolic-aqueous extract using LC–ESI–MS analysis which was performed on LC Agilent Technologies 1100 Infinity series (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler model 1100, a quaternary pump model 1100, and diode array detector model 1100. The analysis employed a C-18 column (250 mm × 4.0 mm, 5 µm, Bischoff Analysentechnik GmbH, Leonberg, Germany). The mobile phase consisted of two solvents: (A) 0.025% trifluoroacetic acid (TFA) in H_2O and (B) acetonitrile. The sample, prepared at a concentration of 10 mg/mL in methanol/H_2O (1:1), was filtered through a 0.45 µm Millipore filter (Millipore Corp., Bedford, Mass., USA). The elution program, at a flow rate of 1 mL/min, proceeded as

follows: 10–50% B (0–40 min), 50–100% B (40–41 min), 100% B (41–50 min), 100 – 10% B (50–55 min), and 10% B (55–59 min). A 10 µL injection volume was utilized, and peaks were monitored at 280 nm. Identification of peaks involved matching retention times and UV spectra with standards for confirmation. The contents of the identified compounds were obtained from a calibration curve made with standards [4].

2.5 Essential Oil Extraction and Analysis

Leaves of *Eucalyptus marginata* collected at different seasons were air dried and grounded using an electric grinder to get a fine powder that was kept in closed containers (vials) until analyses. In summary, each sample consisting of 50 g of powdered material underwent hydro-distillation during 4 h using a Clevenger apparatus. The resulting essential oil (EO) volumes were directly measured in the extraction burette, and the percentage of obtained EO was determined as the volume (mL) of EO per 100 g of dry plant material. Subsequently, the essential oils were dehydrated using anhydrous Na_2SO_4 and stored in a cool, dark environment prior to analysis. The identification and quantification of the essential oils were conducted through a combination of gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC–MS). The GC analysis employed a Hewlett-Packard 6890 chromatograph equipped with a flame-ionization detector (FID) and a split-splitless injector connected to an HP-INNOWAX polyethylene glycol capillary column (30 m × 0.25 mm). Meanwhile, GC–MS analysis utilized an HP model 5975B inert MSD equipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm: i.d; 0.25 µm film thickness) and coupled to a mass selective detector (MSD5975B). Helium served as the carrier gas with a flow rate of 1.0 ml/min. The injector and detector temperatures were set at 250 and 230 °C, respectively. The GC oven temperature initiated at 100 °C, held for 1 min at 260 °C, and then maintained for 10 min with a program rate of 4 $°C.min^{-1}$. Injection of a one µl sample occurred in split mode (1:100). Compound identification in the volatile oil involved the calculation of retention indices based on linear interpolation relative to retention times of C_5–C_{28} n-alkanes, and comparison with reference compounds in the laboratory database or literature data. Mass spectra were matched with reference spectra from the Wiley/NIST database, published data, and authentic compound spectra. Relative amounts of individual components were determined based on GC peak areas without FID response factor correction [14].

2.6 Antioxidant Assays

DPPH and ABTS radical scavenging assays were utilized to assess the antioxidant activity of the extracts using methods described by Chargui et al. [15] and Riguene et al. [16]. Then, concentrations providing 50% of inhibition (IC_{50}) were calculated and expressed as mg/g of dry matter. All measurements were performed in triplicate.

2.7 Statistical Analysis

One-way ANOVA was performed for all collected data. Means significant differences were performed using the Newman–Keuls's tests at $p = 0.05$. All statistical analysis performed using SAS software (means with the same letters are not significantly different).

3 Results and Discussion

3.1 Total Phenol, Flavonoid, Condensed Tannin and Chlorophyll Contents

Table 1 demonstrated the secondary metabolites of the ethanolic- aqueous extracts prepared from *Eucalyptus marginata* of the three seasons. The analysis of Table 1 showed that at spring season the *Eucalyptus marginata* leaves had the highest content on phenolic compounds (491.01 mg GAE / g DW), followed by winter (436.02 mg GAE / g DW) and summer which showed the low value in total polyphenols during this year (191.03 mg GAE / g DW). These results could be explained by the biosynthesis of phenolic compounds during spring seasons. On the other hand, Ralepele et al. [17] mentioned that the decrement in the total

Table 1 Essential oils yields, total phenolic, Flavonoid, tannin and Chlorophyll contents of *Eucalyptus marginata* leaves collected in different seasons

Season	EO Yields (%)	Phenolic content (mg GAE/g DW)	Flavonoid content (mg RE/ g DW)	Tannin content (mg CE/g DW)	Chlorophyll α content (mg/g DW)	Chlorophyll β content (mg/g DW)	Chlorophyll α + β content (mg/g DW)
Winter	0.165 ± 0.015^a	436.02 ± 0.06^a	28.09 ± 0.04^a	21.38 ± 0.00^a	36.77 ± 5.85^a	59.11 ± 9.41^a	95.86 ± 9.41^a
Spring	0.139 ± 0.016^b	491.01 ± 0.07^b	29.04 ± 0.09^a	17.31 ± 0.04^b	134.55 ± 4.07^b	216.61 ± 6.55^b	350.61 ± 9.41^b
Summer	0.132 ± 0.024^b	191.03 ± 0.07^c	35.02 ± 0.01^b	22.15 ± 0.00^a	108.96 ± 13.83^c	175.26 ± 2.25^c	284.23 ± 9.41^c

Values in the same row with different superscript were significantly different at $p < 0.05$. All the data were made in triplicates and were presented as mean ± SD. EO: Essential Oil, GAE: Gallic Acid Equivalent, RE: Rutin Equivalent, CE: Catechin Equivalent, DW: Dry Weight

phenolic concentration in winter can be explained by the gradual reduction in phenolic biosynthesis due to lower temperatures. This adaptive response suggests that plants conserve some resources to endure the cold season, ensuring a successful re-sprouting in the subsequent spring. The obtained results have been confirmed by the analysis of variance (ANOVA) which showed that there is highly significant difference between the three seasons ($p < 0.05$). These variations can be related to the changing of climatic conditions throughout the seasons, inducing the plants' adaptive responses to the diverse environmental changes in each season. According to Ravn et al. [18], Phenolic levels were highest in spring and lowest during summer and fall. On the other hand, flavonoids represent a very wide range of natural compounds belonging to the family of polyphenols, considered as almost universal plant pigments. These compounds are one of the most studied classes of polyphenols today and are generally present in several medicinal plants that have very significant antioxidant activity. The quantity of flavonoids was determined from the rutin calibration curve, and the results obtained are expressed in milligram equivalent of rutin per gram of dry weight. The analysis of Table 1 showed that summer season presented the highest content of flavonoids (35.02 mg RE/g DW), compared to spring (29.04 mg RE/g DW) and winter (28.09 mg RE/g DW), respectively. Statistical analysis revealed that there is no significant difference in the flavonoid contents between the three seasons ($p = 0.5234 > 0.05$). In addition, condensed tannins are phenolic compounds that exhibit antioxidant properties; they were determined by the method of vanillin in an acid medium. The assay results were determined from the catechin calibration curve. Results in Table 1 showed that summer and winter seasons were characterized by the highest levels (22.159 mg CE/g DW and 21.388 mg CE /g DW, respectively) in tannins compared to spring season (17.315 mg CE/g DW). These results were confirmed by the analysis of variance which shows a highly significant difference between the three seasons ($p < 0.05$). These differences in the content of secondary metabolites would probably be due to many environmental factors such as humidity, rainfall, temperature, sunshine, etc. [19]. Additionally, Koudoro et al. [20] showed that the ethanolic or hydroethanolic extracts of *Eucalyptus citriodora* were characterized by 4.52 mg GAE g^{-1} DW and 4.38 mg GAE g^{-1} DW of total phenolic compounds, 78.76 mg RE· g^{-1} DW and 81.56 mg RE g^{-1} DW of flavonoids and 62.62 mg CE g^{-1} DW and 67.09 mg CE·g^{-1}MS of condensed tannins contents. In addition, these researchers revealed that *Eucalyptus pauciflora* was composed of 45.43 mg GAE g^{-1} DW of total phenolic compounds, 12.29 mg RE g^{-1} DW of flavonoids and only 1.07 mg CE g^{-1} DW of condensed tannins contents. Another phytochemical study of oily extracts of *Eucalyptus* revealed that they were more abundant in polyphenols, flavonoids and condensed tannins with values: 1352.09 mg GAE/L of oil, 288.64 mg RE/L of oil and 992.30 mg CE/L of oil, respectively [21]. Moreover, the differences between our study and others with regard to the ideal season for the production of certain constituents can be explained as the differentiated response of each plant to its environment, as an efficient synthesis of these metabolites which are intrinsic characteristics of each species [22]. Thus, the seasonal variation of secondary metabolites can be caused by physiological requirements like plant growth, defense and reproduction; environmental differences such as water stress, light, nutrient deficiency, temperatures extremes and also to the type of solvent [19, 23]. Moreover, our research team determined the chlorophyll α, β and $\alpha + \beta$ contents in the different seasons. From Table 1, we can conclude that the chlorophyll content reached its maximum in spring seasons, while we noted its minimum values in winter seasons. The difference between the total chlorophyll content in the three studied seasons can be due to different factors such as temperature, water flow, light, weather, and other factors.

3.2 Phenolic Compound Analysis by LC–ESI–MS

LC-ESI-MS analysis was performed in order to increase the nutritional value of *Eucalyptus marginata* leaves collected in different seasons. The identification of the phenolic compounds was carried out by mass spectra, comparison with reference compounds and with literature data [4, 24, 25]. Table 2 illustrates all the identified peaks with their retention times, the pseudo-molecular ions as well as the concentration of each identified phenolic compound.

The LC-ESI-MS analysis showed that there are differences between the molecules present in the extracts of the three seasons. Twenty phenolic compounds were identified in *Eucalyptus marginata* leaves extract including nine phenolic acid and eleven flavonoids. A total of 16 phenolic compounds were identified in the winter season by comparison with reference standards. The LC-MS analysis of *Eucalyptus marginata* revealed that 16 compounds was dominant in the extracts obtained in winter (289.71 mg/kg of Extract). It was detected only in this season. This compound with $[M-H]^-$ at *m*/*z* 47 was characterized as *trans*-cinnamic acid. Also, the analysis of Table 2 demonstrated the presence of 14 phenolic compounds in the spring season. Moreover, Catechin (+) and Quercetin (quercetin-3-*O*-rhamonoside) compounds were abundant with concentrations varying between 62.04 and 62 mg/kg of Extract, respectively and their molecular formula were respectively $C_{15}H_{14}O_6$ and $C_{21}H_{20}O_{11}$. The mass spectrum of these molecular ion were at $[M-H]^-$ m/z 289 and 447. All of these reason confirmed that these two compounds were respectively Catechin (+) and Quercetin

Table 2 Phenolic composition of *Eucalyptus marginata* leaves' extracts by LC–ESI–MS

N°	Phenolic Compounds	Molecular formula	Molecular Mass	$[M\text{-}H]^-$ m/z	R_t (min)	Winter (mg/kg of Extract)	Spring (mg/kg of Extract)	Summer (mg/kg of Extract)
1	Qunic acid	$C_7H_{12}O_6$	192	191	1.750	25.18 ± 0.04^{a}	19.75 ± 0.08^{b}	20.63 ± 0.06^{c}
2	Gallic acid	$C_7H_6O_5$	170	169	2.627	17.22 ± 0.05^{a}	17.95 ± 0.09^{a}	13.75 ± 0.05^{b}
3	Catechin (+)	$C_{15}H_{14}O_6$	290	289	7.479	13.42 ± 0.08^{a}	62.04 ± 0.06^{b}	70.97 ± 0.08^{c}
4	Protocatechuic acid	$C_7H_6O_4$	154	153	8.617	ND	ND	ND
5	*p*-coumaric acid	$C_9H_8O_3$	164	163	15.833	5.25 ± 0.07^{a}	ND	29.94 ± 0.02^{b}
6	*trans*-Ferulic acid	$C_{10}H_{10}O_4$	194	193	19.150	ND	ND	ND
7	Hyperoside (quercetin-3-*O*-galactoside)	$C_{21}H_{20}O_{12}$	464	463	20.744	17.26 ± 0.00^{a}	41.46 ± 0.09^{b}	28.57 ± 0.01^{c}
8	Rutin (quercetin-3-*O*-rutinoside)	$C_{27}H_{30}O_{16}$	610	609	21.742	2.13 ± 0.06^{a}	3.37 ± 0.07^{b}	2.34 ± 0.04^{a}
9	Luteolin-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	448	447	22.323	12.60 ± 0.05^{a}	29.27 ± 0.01^{b}	18.64 ± 0.05^{c}
10	*o*-coumaric acid	$C_9H_8O_3$	164	163	22.906	3.76 ± 0.06	ND	ND
11	Quercetin (quercetin-3-*O*-rhamonoside)	$C_{21}H_{20}O_{11}$	448	447	24.147	31.06 ± 0.03^{a}	62.00 ± 0.00^{b}	40.97 ± 0.03^{c}
12	Naringin (Naringenin-7-*O*-neohesperidoside)	$C_{27}H_{32}O_{14}$	580	579	24.246	20.76 ± 0.02^{a}	39.73 ± 0.09^{b}	27.78 ± 0.07^{c}
13	Rosmarinic acid	$C_{18}H_{16}O_8$	360	359	25.00	4.26 ± 0.07	ND	ND
14	Apegenin-7-*O*-glucoside	$C_{21}H_{20}O_{10}$	432	431	25.280	2.16 ± 0.01^{a}	3.08 ± 0.04^{b}	0.46 ± 0.01^{c}
15	Salviolinic acid	$C_7H_6O_3$	138	137	26.643	ND	0.59 ± 0.00^{a}	0.59 ± 0.00^{a}
16	*trans*-cinnamic acid	$C_9H_8O_2$	148	147	28.794	289.71 ± 0.07	ND	ND
17	Quercetin	$C_{15}H_{10}O_7$	302	301	29.118	0.84 ± 0.05^{a}	0.71 ± 0.02^{b}	0.38 ± 0.00^{c}
18	Naringenin	$C_{15}H_{12}O_5$	272	271	31.478	0.81 ± 0.01^{a}	0.92 ± 0.07^{a}	0.67 ± 0.00^{b}
19	Apegenin	$C_{15}H_{10}O_5$	270	269	31.737	ND	4.36 ± 0.09	ND
20	Acacetin	$C_{16}H_{12}O_5$	284	283	38.189	0.03 ± 0.00^{a}	0.19 ± 0.03^{b}	0.03 ± 0.00^{a}

All the data were made in triplicates and were presented as mean ± SD. Means with different letters in the same line were significantly different at $p < 0.05$

(quercetin-3-*O*-rhamonoside). However, in the summer, the Catechin (+) was the most abundant polyphenolic compound with 70.97 mg/kg of Extract. The quantification of the identified phenolic compounds (Table 4) showed a significant difference between the three season ($p < 0.05$). The main phenolic compounds found in the *Eucalyptus marginata* leaves was *trans*-cinnamic acid. On the other hand, *trans*-ferulic and protocatechuic acids were absent in all extracts. According to Hasni et al. [4], ten phenolic compounds were identified in *Eucalyptus marginata* leaves: four phenolic acids mainly gallic acid (27.77 ± 0.06 µg/g DW) and protocatechuic acid (37.66 ± 0.04 µg/g DW) and six flavonoid compounds such as quercetin (150.78 ± 0.02 µg/g DW) and hyperoside (39.19 ± 0.03 µg/g DW). Based on the above, we concluded that *Eucalyptus marginata* leaves were rich sources of phenolic compounds.

3.3 Seasonal Effect on Yield and Chemical Composition of *Eucalyptus marginata* Essential Oils

The highest essential oil yield was obtained for leaves harvested in winter (0.165%), followed by those collected in spring (0.139%) and summer (0.132%) (Fig. 1; Table 1). However, no significant variation was noted in essential oil yield among seasons. This paper presents the first study of the variation in chemical composition of *E. marginata* essential oil according to the season of leaves collection. The chemical composition of EO_S was analyzed by GC-MS, which allowed the identification of 16 terpenic compounds representing 90.29–90.93% of the total essential oil (Table 3). The major compounds of *Eucalyptus marginata* essential oil for the three seasons were 1,8-Cineole (22.42 −30.52%), *p*-Cymene (8.14 −12.92%), 2-Cyclohexen-1-one, 4-Isopropyl (21.07 −21.82%), *p*-Cumic aldehyde (10.58 −11.44%) and 1-Terpinen-4-ol (4.46 −5.31%). Other compounds were identified as minor ones including Cymenene, Spathulenol and Caryophyllene oxide. Moreover, analysis of the chemical composition of *Eucalyptus marginata* essential oils revealed significant variation for all identified compounds among seasons (Table 3). However, regardless of the season of collection, 1,8-cineol, 2-Cyclohexen-1-one, 4-Isopropy and *p*-Cymene were always identified as the major components. The study of Ghazghazi et al. [14] reported similar findings on the composition of essential oil from *E. marginata*. The difference in the chemical compounds content might be attributed to the harvest period [26], the nature of the soil [27] and the climate [28–30], seasonal and geographic conditions [30–33], the extraction

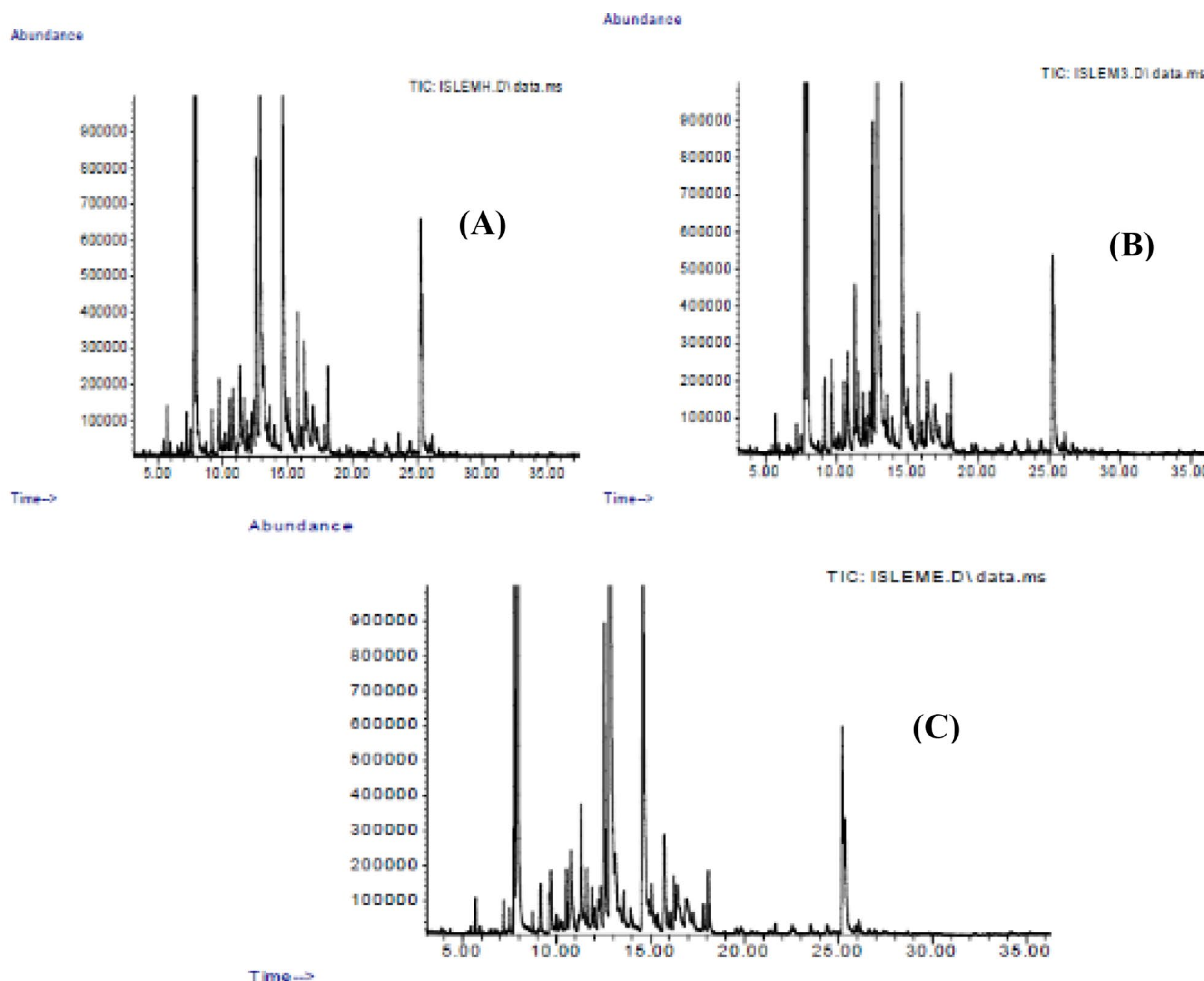

Fig. 1 TIC chromatograms showing the chemical profiles of the essential oils of *Eucalyptus marginata*

method and technique [21, 34] and the age of leaves [35] and trees [2, 21]. In the same context, Hasni et al. [36] showed that various factors could influence the monoterpene emission in *Eucalyptus* species, such as seasonal variation and diurnal emission activity cycles.

3.4 Impact of Growing Season on Antioxidant Activities

At present, there is no universal, unique and reliable method reflecting the antioxidant capacity. For this, to judge the overall antioxidant effect of an extract of a plant or food resource, it is necessary to use several potency tests. It is known that the strongest antioxidant activity is that which has the lower IC_{50} and vice versa. Figure 2 exhibited the antioxidant capacity of *Eucalyptus marginata* leaves extract recovered in summer, spring and winter.

Based on the theory of radical scavenging activity, the results indicate that the *Eucalyptus marginata* leaves collected in the spring season had the highest antioxidant activity (IC_{50} = 1.099 mg/g DW), followed by, the *Eucalyptus marginata* leaves collected in the winter season (IC_{50} = 1.315 mg/g DW) and finally the summer season IC_{50} = 1.963 mg/g DW (Fig. 2). As previously reported by Hasni et al. [36], the IC_{50} of extracts obtained by maceration and ultrasound assisted extraction were respectively 23.204 µg/ml and 21.264 µg/ml and 78.922 mg BHTE/g DW and 116.901 mg BHTE/g DW. However, based on the ABTS test, the highest activity is found in the spring season with IC_{50} = 0.909 mg/g DW, followed by winter with IC_{50} = 1.158 mg/g DW and finally summer with IC_{50} = 1.287 mg/g DW. This may be due to the mechanism of the two tests one generally relies on electron transfer only and the other involves the transfer of protons and electrons both [37]. In this study, the obtained results demonstrate that

Table 3 Chemical composition (%) of essential oils extracted from *Eucalyptus marginata* leaves collected in different seasons

Compounds	RI	Spring	Summer	Winter
α-Phellandrene	1009	Tr[b]	Tr[b]	0.52 ± 0.00[a]
p-Cymene	1029	10.38 ± 0.06[b]	8.14 ± 0.04[c]	12.92 ± 0.04[a]
1.8-Cineol	1035	26.24 ± 0.14[b]	30.52 ± 0.10[a]	22.42 ± 0.19[c]
Furanoid linalool oxide	1077	0.90 ± 0.03[a]	0.72 ± 0.02[b]	0.65 ± 0.00[b]
Cymenene	1096	1.07 ± 0.03[b]	0.94 ± 0.02[b]	1.32 ± 0.07[a]
α-Thujone	1122	0.74 ± 0.02[b]	0.82 ± 0.03[a]	0.67 ± 0.02[b]
Sabina ketone	1163	0.58 ± 0.02[a]	Tr[b]	Tr[b]
1-Terpinen-4-ol	1184	4.46 ± 0.03[c]	5.31 ± 0.05[a]	4.95 ± 0.04[b]
2-Cyclohexen-1-one, 4-Isopropyl	1194	21.82 ± 0.09[a]	21.24 ± 0.06[b]	21.07 ± 0.04[b]
Myrtenal	1202	1.55 ± 0.03[a]	1.36 ± 0.04[b]	1.65 ± 0.06[a]
p-Cumic aldehyde	1247	11.41 ± 0.06[a]	10.58 ± 0.04[b]	11.44 ± 0.04[a]
Piperitone	1261	1.00 ± 0.01[a]	0.93 ± 0.01[b]	Tr[c]
Phellandral	1282	2.32 ± 0.02[b]	2.12 ± 0.02[c]	3.10 ± 0.03[a]
p-Cymen-7-ol	1303	2.32 ± 0.05[a]	0.86 ± 0.03[c]	1.05 ± 0.03[b]
Spathulenol	1590	3.14 ± 0.02[c]	4.18 ± 0.02[b]	4.54 ± 0.03[a]
Caryophylene oxide	1595	2.96 ± 0.04[b]	2.89 ± 0.03[b]	3.95 ± 0.03[a]
Total		90.93 ± 0.31 [a]	90.66 ± 0.32 [b]	90.29 ± 0.17 [c]

Means with different letters in the same column were significantly different at $p < 0.05$. All the data were completed in triplicates and were presented as mean ± SD. RI: retention indices calculated in regard to standards mixture of hydrocarbons (C_5-C_{28}), *tr*: trace

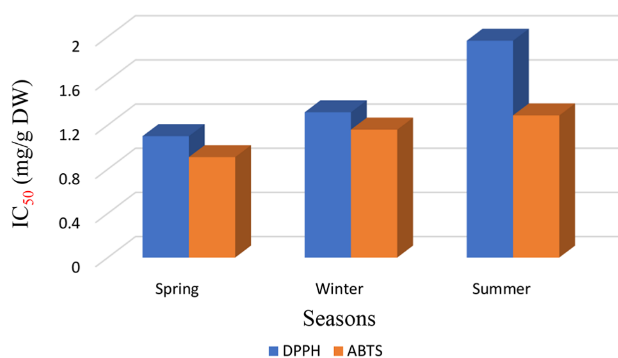

Fig. 2 Variation in the antiradical activity of *Eucalyptus marginata* leaves extract by DPPH and ABTS tests depending on the season

the ABTS test was the most sensitive in identifying the antioxidant capacity. This may be due to by the higher reactivity of the ABTS to react with compounds hydrogen atom transfer [38]. Also, it is reported that the antioxidant activity was due to the presence and abundance of phenolic compounds [39]. The antioxidant or antiradical activity can also be correlated by some conditions (temperature, pH, type of solvent and the concentration of samples [40–42].

By comparing the total polyphenol content with the antioxidant capacity (ABTS, DPPH), it has been found there is significant correlations between the antioxidant activity and total phenolic compounds. From these results, we can conclude that the *Eucalyptus marginata* leaves collected in spring have maximum values of phenolic compounds and concurrently, intense antioxidant activity, followed by the *Eucalyptus marginata* leaves collected in winter and finally summer season.

4 Conclusion

The current paper succeeded to study the chemical composition of essential oil, phenolic compounds content, and antioxidant activity of *Eucalyptus marginata* leaves collected from the Northeast of Tunisia regarding season. The Essential oil yields were mostly depending on seasons of the year (winter, spring and summer). Samples collected in spring yielded more essential oils and phenolic compounds. These variations could be due to external environmental factors or internal factors depending on the life cycle of the plant, but still remain uncertain. The study should therefore be conducted over multiple years to confirm the effect of the harvesting season.

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Declarations

Informed Consent Not applicable.

Conflict of interest The authors declare no conflict of interest.

Ethical Approval Not applicable.

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