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Chemical analysis and *in vitro* antioxidant and anticholinesterase activities of essential oils and extracts from different parts of *Erica manipuliflora*

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ABSTRACT: This study reports the chemical composition of essential oils and *in vitro* antioxidant and anticholinesterase activities of essential oils, hexane, ethyl acetate, methanol, and butanol extracts of aerial, flower and leaves parts of *Erica manipuliflora*. GC and GC/MS analyses were used for identification of essential oils. Totals of 47 compounds were detected in the essential oils of aerial, flower and leaves parts accounting for 99.99%, 99.88% and 99.97%, respectively. The major components of the aerial, flower and leaves parts of essential oils were Germacren D (14.76%, 15.55% and 13.58%), tau-cadinol (7.53%, 4.11% and 8.96%), caryophyllene oxide (3.92%, 5.17% and 8.55%), β-caryophyllene (7.24%, 5.97% and 7.73%), and α-terpineol (6.85%, 6.14% and 4.18%), respectively. The essential oils of aerial (34.49%) and leaves (37.01%) parts consisted of mainly sesquiterpene hydrocarbons whereas essential oil of flower part (42.58%) included monoterpenoids. The essential oils and extracts were screened for their antioxidant and anticholinesterase activities. The results of activities showed that extracts possessed the highest antioxidant activity while essential oils had the highest anticholinesterase activity. This finding supposes that *E. manipuliflora* may be considered as valuable natural source with antioxidant and anticholinesterase properties for food, cosmetic and pharmaceutical industries.

KEYWORDS: *Erica manipuliflora*; chemical composition; essential oil; extracts; different parts; antioxidant activity; anticholinesterase activity.

1. INTRODUCTION

The genus *Erica* L. is a member of the family Ericaceae and more than 800 species naturally grow in the world, mainly in the coastal areas of the Mediterranean Sea [1-4]. *Erica* species which are narrow-leaved and evergreen in winter have a wide variety of flower structures and colours. *Erica* species are called as "funda", "püren" or "süpürge çalısı" locally in Turkey. Totally 5 species are reported in Turkey; namely, *E. arborea, E. manipuliflora, E, bocquetii, E. sicula subsp. libanotica and E. spiculifolia salisb. E, bocquetii* is endemic to South Western Anatolia (between Elmalı and Fethiye region) [3, 5-7]. *Erica manipuliflora* is the most common *Erica* species in southwest part of Turkey. Since ancient times, *Erica* species have been used as a traditional medicine for the treatment of burns and wounds by the local people in Anatolia. The studies have been showed that *Erica* species possess antiulcer [8,9], antimicrobial [10,11], antibacterial, cytotoxic [12-15] and antioxidant [16-20] activities. It has also been reported that *Erica* species have (poly) phenolic, flavonoid, coumarin and triterpenoid, compounds [21-28]. According to our knowledge, there is no study in the literature on anticholinesterase activity and chemical composition of essential oil of *E. manipuliflora* growing in Turkey.

The aim of this study was to investigate the antioxidant and anticholinesterase activities of hexane, ethyl acetate, methanol, butanol extracts obtained from aerial, flower, and leaves parts of *E. manipuliflora* which is widely grown in Anatolia and used for various purposes by the local people. In addition, determination of chemical composition, antioxidant and anticholinesterase activities of essential oil obtained via hydro-distillation of aerial, flower, and leaves parts of the plant were aimed.

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2. RESULTS AND DISCUSSION

2.1. Chemical composition of essential oils

The essential oils obtained from aerial, flower and leaves parts of *E. manipuliflora* were analyzed using the GC and GC-MS techniques. The chemical compositions of the essential oils, relative percentages (%) and Kovats index of compounds are presented in Table 1. A total of 47 components were detected in each of the three parts of essential oils, and all of them were identified. The main compounds of the aerial, flower and leaves parts of essential oils were Germacren D (14.76%, 15.55% and 13.58%), tau-cadinol (7.53%, 4.11% and 8.96%), caryophyllene oxide (3.92%, 5.17% and 8.55%), β-caryophyllene (7.24%, 5.97% and 7.73%), and α-terpineol (6.85%, 6.14% and 4.18%), respectively (Table 1).
Sesquiterpene hydrocarbons were the most abundant compounds in aerial (34.49%) and leaves (37.01%) parts of essential oils while monoterpenoids were found to be most abundant compounds in flower part (42.58%) of essential oil.

In a previous report, the essential composition of *E. manipuliflora* collected from two different regions of Greece was studied and Heptacosane, caryophyllene oxide, β-caryophyllene, 1-octen-3-ol, α-terpineol and linanol were recorded as main compounds [29]. The similarities and differences were found in the chemical composition of the essential oils of *Erica species* when our results were compared to literature findings.

2.2. Antioxidant activity

The antioxidant activities of essential oils, hexane, ethyl acetate, methanol and butanol extracts obtained from aerial, flower and leaves parts of *E. manipuliflora* were tested with five different assays i.e. β-carotene-linoleic acid, DPPH radical scavenging, ABTS cation radical scavenging, cupric-reducing antioxidant capacity (CUPRAC) and metal chelating activity. The results are showed in Table 2. The antioxidant capacities compared with α-tocopherol, BHA, and EDTA. The essential oils and extracts were tested at different concentrations and IC_{50} values were determined.

The highest lipid peroxidation inhibition activity by β-carotene-linoleic acid was detected in flower part of ethyl acetate extract of *E. manipuliflora* with the IC_{50} value of 45.61±1.28 µg/mL followed by leaves part of ethyl acetate extract (IC_{50}: 48.16±1.35 µg/mL). It was determined that all studied samples had low lipid peroxidation inhibition activities than standards.

Radical scavenging activities of the samples were measured by monitoring the removal of DPPH$^{\bullet}$ radical at 515 nm and ABTS$^{\bullet+}$ radical at 734 nm. Antioxidants capture DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, cause to disappear of the colours of these radicals and decrease in the absorbance. In DPPH$^{\bullet}$ assay, the aerial, flower and leaves parts of butanol extract exhibited the highest activity among the all studied extracts and essential oils. The flower part of butanol extract (IC_{50}: 35.64±1.15 µg/mL) indicated higher DPPH$^{\bullet}$ radical scavenging activity than α-tocopherol (IC_{50}: 37.20±0.41 µg/mL). In ABTS$^{\bullet+}$ assay, the leaves part of methanol extract (IC_{50}: 20.01±0.63 µg/mL), and the aerial part of hexane extract (IC_{50}: 38.12±1.57 µg/mL) exhibited higher radical scavenging activities than α-tocopherol (IC_{50}: 38.51±0.54 µg/mL).

CUPRAC assay was used to evaluate reducing properties of the samples. The aerial ($A_{0.05}$: 15.47±0.05 µg/mL), flower ($A_{0.05}$: 12.77±0.04 µg/mL) and leaves ($A_{0.05}$: 10.22±0.06 µg/mL) parts of butanol extracts were found to be more active than BHA ($A_{0.05}$: 24.40±0.69 µg/mL) and α-tocopherol ($A_{0.05}$: 66.72±0.81 µg/mL) used as standards. Also, the aerial ($A_{0.05}$: 44.57±0.12 µg/mL), flower ($A_{0.05}$: 23.70±0.06 µg/mL) and leaves ($A_{0.05}$: 49.78±0.06 µg/mL) parts of methanol extract showed significant reducing activity (Table 2).

Another significant mechanism of measuring antioxidant activity is chelation of iron and transition metals (arsenic, copper, vanadium, chromium, cadmium, cobalt, nickel,). The highest ability to chelate ferrous ion was shown in the flower part of ethyl acetate extract with 27.45±1.25 % inhibition at 400 µg/mL concentration and followed by the leaves part of essential oil (21.33±1.88 %). In general, the all studied samples exhibited low metal chelating activity (Table 2). In a recent study, the antioxidant capacities of various extracts (chloroform, ethyl acetate, methanol, butanol and water) of *E. manipuliflora* were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging and the thiobarbituric acid (TBA) test systems [30]. These results are consistent with previous studies on the antioxidant activity of *Erica* species.

2.3. Anticholinesterase activity

Alzheimer's disease (AD) is a cognitive disorder that occurs in association with aging and is widespread in recent years.

Table 1. Chemical composition of the essential oils of aerial, flower and leaves parts of *Erica manipuliflora*.

No	Compounds[a]	Aerial part (%[b])	Flower part (%[b])	Leaves part (%[b])	RI[c]	Identification Methods[d]
1	*cis*-Linalol oxide	0.38	1.22	0.16	1078	Co-GC, MS, RI
2	Linalool	1.12	4.17	0.72	1104	Co-GC, MS, RI
3	*cis*-Limonene oxide	1.96	5.56	0.62	1124	Co-GC, MS, RI
4	β-Phenylethanol	*tr*	0.48	*tr*	1136	MS, RI
5	Verbenone	0.67	0.84	0.15	1141	MS, RI
6	*a*-Campholenal	0.12	0.16	*tr*	1145	MS, RI
7	trans-pinocarveol	5.78	5.51	1.82	1150	Co-GC, MS, RI
8	*cis*-Verbenol	0.34	0.57	0.11	1154	MS, RI
9	Borneol	2.56	2.75	0.47	1168	Co-GC, MS, RI
10	Verbenol	0.67	1.17	0.25	1172	Co-GC, MS, RI
11	*a*-Terpineol	6.85	6.14	4.18	1178	Co-GC, MS, RI
12	Myrtenol	0.98	*tr*	1.51	1183	Co-GC, MS, RI
13	*cis*-Carveol	0.43	0.53	0.20	1206	Co-GC.MS. RI
14	2-Coumarone	0.77	1.34	0.32	1224	MS. RI
15	*trans*-Geraniol	2.63	3.2	0.86	1239	Co-GC.MS. RI
16	*cis*-7-Decenal	0.40	0.48	0.85	1245	MS, RI
17	Mesitol	0.24	0.31	0.32	1249	MS, RI
18	Benzocycloheptatrien	0.57	1.05	0.14	1256	MS, RI
19	Carvacrol	4.03	4.12	3.57	1262	Co-GC, MS, RI
20	Thymol	0.02	1.43	0.55	1268	Co-GC, MS, RI
21	2-Methoxy-4-vinylphenol	0.59	0.96	0.12	1278	MS, RI
22	(E.E)-2.4-Decadienal	0.75	*tr*	0.45	1283	MS, RI
23	Eugenol	0.22	0.67	0.3	1326	Co-GC, MS, RI
24	*β*-Bourbonene	0.92	2.76	2.10	1332	MS, RI
25	*cis*-Jasmone	2.91	2.71	2.09	1342	Co-GC, MS, RI
26	*β*-Caryophyllene	7.24	5.97	7.73	1349	Co-GC, MS, RI
27	*β*-Cubebene	1.39	*tr*	1.10	1381	MS, RI
28	Germacrene D	14.76	15.55	13.58	1464	Co-GC, MS, RI
29	*β*-İonone	1.34	0.9	2.15	1467	MS, RI
30	*a*-Amorphene	1.43	0.76	1.67	1471	MS, RI
31	*δ*-Cadinene	1.69	2.96	1.24	1495	Co-GC, MS, RI
32	*β*-İonol	1.04	0.93	1.76	1485	MS, RI
33	*β*-Guaiene	3.93	1.47	4.27	1491	MS, RI
34	Viridiflorene	3.13	2.56	5.32	1494	MS, RI
35	Caryophyllene oxide	3.92	5.17	8.55	1507	Co-GC, MS, RI
36	Viridiflorol	4.52	1.38	7.01	1530	MS, RI
37	Isoaromadendrene epoxide	0.95	1.36	2.50	1538	MS, RI
38	Spathulenol	3.84	1.96	4.17	1556	Co-GC, MS. RI
39	*tau*-Cadinol	7.53	4.11	8.96	1582	MS, RI
40	*a*-Cadinol	3.38	3.30	4.59	1615	MS, RI
41	*a*-Hexylcinnamaldehyde	0.54	0.83	0.41	1728	MS, RI
42	Benzyl benzoate	0.54	0.83	0.97	1789	MS, RI
43	Salicylic acid benzyl ester	0.63	1.18	0.43	1824	MS, RI
44	Heneicosane	0.63	*tr*	0.38	1935	Co-GC, MS, RI
45	3-Deoxy.Estradiol	0.8	*tr*	0.70	1955	MS, RI
46	Tricosane	0.47	0.53	0.15	2091	Co-GC, MS, RI
47	Heptacosane	0.32	*tr*	0.47	2295	MS, RI
	Monoterpene hydrocarbons	-	-	-		
	Monoterpenoids	34.05	42.58	21.47		
	Sesquiterpene hydrocarbons	34.49	32.03	37.01		
	Sesquiterpenoids	24.68	18.11	36.19		
	Others	6.77	7.16	5.30		
	Total identified (%)	99.99	99.88	99.97		
	Total number of compounds	47	47	47		

[a] Compounds are listed in order of their elution from a DB-5 fused silica column. [b] Percentage concentration. [c] Retention index on DB–5 fused silica column. [d] Identification methods: Co-I: Co-injection: based on comparison with authentic compounds; MS: based on comparison with WILEY, ADAMS and NIST 08 MS databases; RI: based on comparison of calculated with those reported in ADAMS and NIST 08. tr: trace

Table 2. Antioxidant activities of the essential oil and extracts of aerial, flower and leaves parts of *Erica manipufilora* by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays[a].

			Antioxidant Activity				
			β-Carotene-linoleic acid assay	DPPH• assay	ABTS•+ assay	CUPRAC assay	Metal chelating assay
			IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.50}$ (µg/mL)[c]	Inhibition %[b]
Erica manipuliflora	Essential oil	APEs	33.21±1.80[b]	4.52±0.42[b]	11.28±1.79[b]	0.095±0.16[b]	NA[f]
		FEs	24.10±1.45[b]	36.94±1.23	9.90±0.61[b]	0.102±0.01[b]	NA[f]
		LEs	43.20±1.20[b]	6.17±0.18[b]	8.57±0.45[b]	0.099±0.36[b]	21.33±1.88
	Hexane	APH	165.73±1.78	>400	38.12±1.57	>400	NA[f]
		FH	>400	>400	228.48±0.48	309.09±0.06	NA[f]
		LH	337.62±1.25	387.75±1.42	255.72±0.77	>400	NA[f]
	Ethyl Acetate	APEA	124.03±1.40	179.04±1.16	60.14±2.18	117.30±0.02	20.20±1.77
		FEA	45.61±1.28	>400	57.22±1.66	300.00±0.12	27.45±1.25
		LEA	48.16±1.35	>400	45.87±1.24	315.38±0.04	16.30±1.16
	Methanol	APM	46.35±1.12[b]	188.07±0.31	62.57±2.23	44.57±0.12	8.56±1.71
		FM	43.45±1.63[b]	72.78±1.23	82.54±0.95	23.70±0.06	7.21±1.13
		LM	48.80±1.84[b]	398.61±0.46	20.01±0.63	49.78±0.06	8.61±1.69
	Butanol	APB	250.20±1.12	51.17±0.28	261.95±1.33	15.47±0.05	8.27±1.25
		FB	>400	35.64±1.15	158.72±1.06	12.77±0.04	10.98±0.25
		LB	245.90±1.38	45.62±2.07	>400	10.22±0.06	16.73±1.69
	Standards	α-tocopherol[d]	2.10±0.08	37.20±0.41	38.51±0.54	66.72±0.81	NT[e]
		BHA[d]	1.34±0.04	19.80±0.36	11.82±0.09	24.40±0.69	NT[e]
		EDTA[d]	NT[e]	NT[e]	NT[e]	NT[e]	94.70±0.60

[a] IC_{50} values represent the means ± SEM of three parallel measurements (p<0.05). [b] % inhibition of 400 µg/mL concentration of extracts. [c] $A_{0.50}$ values represent the means ± SEM of three parallel measurements (p<0.05). [d] Reference compounds. [e] NT: not tested. [f] NA: not active. AP: Aerial part; F: Flower part; L: Leaves part.

Table 3. Anticholinesterase activities of the essential oil and extracts of aerial, flower and leaves parts of *Erica manipuliflora* [a].

			Anticholinesterase Activity	
			AChE assay IC_{50} (µg/mL)	BChE assay IC_{50} (µg/mL)
Erica manipuliflora	Essential oil	APEs	144.87±1.39	>400
		FEs	79.19±0.95	205.88±1.39
		LEs	73.82±1.36	118.02±1.17
	Hexane	APH	338.05±2.79	>400
		FH	252.12±2.19	283.42±0.62
		LH	>400	>400
	Ethyl acetate	APEA	248.61±1.15	79.66±1.79
		FEA	>400	146.54±1.97
		LEA	284.32±1.81	153.89±1.28
	Methanol	APM	>400	58.01±1.28
		FM	281.81±1.25	137.01±1.35
		LM	>400	351.94±1.13
	Butanol	APB	>400	263.04±1.75
		FB	238.61±0.83	99.91±1.12
		LB	310.43±1.12	>400
	Standard	Galantamine[b]	5.04±0.15	50.80±0.93

[a] IC_{50} values represent the means ± SEM of three parallel measurements (p<0.05). [b] Reference compound. [c] NA: Not active. AP: Aerial part; F: Flower part; L: Leaves part.

Currently, using of cholinesterase inhibitors and the increase in the level of acetylcholine is the most commonly used method for treatment of AD. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes that hydrolyse acetylcholine prevents the progression of the disease.

Table 3 shows IC_{50} values of the aerial, flower and leaves parts of essential oil, and extracts of *E. manipuliflora* for AChE and BChE assays. The leaves part of essential oil (IC_{50}: 73.82±1.36 µg/mL) exhibited moderate inhibitory activity against AChE, followed by the flower part of essential oil (IC_{50}: 79.19±0.95 µg/mL). Against BChE enzyme, the aerial parts of methanol and ethyl acetate extracts showed the highest inhibitory activity with IC_{50} values of 58.01±1.28 µg/mL and 79.66±1.79 µg/mL, respectively. In the literature survey, no study was found related to anticholinesterase activity of *E. manipuliflora*.

3. CONCLUSION

In this study, the chemical composition of essential oils of the aerial, flower and leaves parts of *E. manipuliflora* which is widely grown in Western Anatolia were reported here for the first time. In addition, this study represents the first comprehensive investigation on antioxidant and anticholinesterase activities of essential oils and extracts of various parts of *E. manipuliflora*. *E. manipuliflora* which is an important source of pollen for apiculture in pine honey production periods can be considered as a potential source of natural antioxidant and cholinesterase enzyme inhibitor. Thus, further studies are necessary to isolate and identify the bioactive components from these species. We believe that it is important to convert this source to medical products by performing *in vivo* tests.

4. MATERIALS AND METHODS

4.1. Plant material

The aerial, flower and leaves parts of *Erica manipuliflora* were collected from Ula-Muğla, Turkey in 2017. The voucher specimen has been deposited at the herbarium of Natural Product Laboratory of Muğla Sıtkı Koçman University.

4.2. Extraction

The aerial, flower and leaves parts of *E. manipuliflora* were extracted separately with hexane, ethyl acetate, methanol, and butanol respectively at room temperature for 24 h and four times. Solvents were removed with a rotary evaporator. All extracts were stored at +4°C until analysis.

4.3. Isolation of the essential oil

The essential oils of aerial, flower and leaves parts of *E. manipuliflora* were obtained via hydro-distillation in a Clevenger type apparatus for 4 h. The oils were dried over anhydrous sodium sulphate and stored under +4°C until analyses.

4.4. Analysis of the essential oil

4.4.1. Gas chromatography (GC-FID)

A Flame Ionization Detector (FID) and a DB-5 fused silica capillary non-polar column (30 m×0.25 id., film thickness 0.25 µm) were used for GC analyses. The injector temperature and detector temperature were adjusted 250 °C and 270 °C, respectively. Carrier gas was He at a flow rate of 1.4 mL/min. Sample size was 1.0 µL with a split ratio of 20:1. The initial oven temperature was held at 60 °C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 10 min. The percentage composition of the essential oil was determined with GC solution computer program.

4.4.2. Gas chromatography/mass spectrometry (GC/MS)

An Ion trap MS spectrometer and a DB-5 MS fused silica non-polar capillary column (30 m×0.25 mm ID, film thickness 0.25 µm) were used for the GC/MS analyses. Carrier gas was helium at a flow rate of 1.4 mL/min. The oven temperature was held at 60°C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 10 min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. Ion source temperature was 200°C. The injection volume was 0.2 µL with a split ratio of 1:20. EI–MS measurements were taken at 70 eV ionization energy. Mass range was from m/z 28 to 650 amu. Scan time 0.5 s with 0.1 inter scan delays. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library as well as by

comparison of the fragmentation patterns of the mass spectra with those reported in the literature [31] and whenever possible, by co-injection with authentic compounds.

4.5. Antioxidant activity

The total antioxidant activity of the extracts and essential oils of aerial, flower and leaves parts of *E. manipuliflora* was tested by β-carotene-linoleic acid test system as previously reported paper [32]. Radical scavenging activities were measured by DPPH free and ABTS cation radical scavenging assays [32]. Reducing powers were evaluated by CUPRAC assays [32]. Metal chelating activity on ferrous ions was determined according to our described method in the literature [33]. The sample concentration providing 50 % inhibition activity (IC_{50} µg/mL) was calculated from the graph of antioxidant activity percentages (Inhibition %) against sample concentrations (µg/mL). The sample concentration having 0.50 absorbance ($A_{0.5}$) was calculated from the plot of CUPRAC absorbance against sample concentration.

4.6. Anticholinesterase activity

Anticholinesterase activities of three parts of *E. manipuliflora* were measured the spectrophotometric method developed by Ellman et al. [34]. Galantamine was used as reference compound. The results were given as 50 % inhibition activity (IC_{50} µg/mL).

4.7. Statistical analysis

All data on the antioxidant and anticholinesterase activities were the mean of three parallel sample measurements. The data were recorded as the average ± S.E.M (Standard error meaning). Significant differences between the means were determined by student's *t* test, and *p* values <0.05 were regarded as significant.

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