https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27(3s) (October2024); 4452 – 4457
Research Article

Variation of The Chemical Composition of Essential Oils and Antioxidant Activity of *Teucrium Polium* L. Growing Wild in Jordan at Different Flowering Stages

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Abstract:
The composition of hydro-distilled oils of *Teucrium polium* L. at different stages (pre-flowering and flowering) was evaluated by Gas Chromatography/Mass Spectrometry (GC/MS). Furthermore, the antioxidant activity of the essential oils extracted from pre-flowering and full-flowering of *T. polium* was examined utilizing DPPH free radical scavenging and $ABTS^{\cdot+}$ cation radical scavenging. The essential oils were analysis yielded 59 and 57 components, respectively. Oxygenated sesquiterpenes accounted for (49.90%) of the total amount in the fresh aerial portion at the pre-flowering stage, followed by monoterpenes hydrocarbons (17.75%) and sesquiterpene hydrocarbons (8.00%). The majority of the fresh aerial portion at the full-flowering stage portion was composed of oxygenated sesquiterpenes (45.71%) monoterpenes hydrocarbons (25.49%) and sesquiterpene hydrocarbons (12.14%). epi-α-Cadinol accounted for (11.90%) of the fresh plant at the pre-flowering stage, followed by (E)-β-santalol (6.15%). After flowering, the concentrations of both components were dropped to (10.13%) and (5.14%), respectively. The results of the study indicate that *T. polium* has antioxidants in both the pre-flowering and full-flowering stages. Compared to the full-flowering sample, the pre-flowering essential oil showed greater antioxidant activity.

Keywords: Antioxidant, DPPH, essential oil, hydro-distilled, *Teucriumpolium*

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Received: 20/10/2024 *Accepted: 24/10/2024*

DOI: https://doi.org/10.53555/AJBR.v27i3S.3067

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Introduction:

Antioxidants defend the human body against harm and free radicals [1,2]. Antioxidant molecules found in food are known to protect against human health and also lower the risk of chronic diseases. Cancer, heart disease, and gastrointestinal disorders [3-5]. Phenolic acids can kill free radicals and reduce their harmful effects [6-10]. A member of the Lamiaceae family has demonstrated potent antioxidant qualities due to the presence of significant polyphenols [11]. *Teucerium polium* L. is the most prominent species of *Teucrium* belonging to the subfamily Lamiaceae [12, 13]. *Teucerium polium* L. is a perennial plant that is locally called "Jaada" [14]. The Teucrium genus is widespread in Southwest Asia, Europe, North Africa, and the Mediterranean region. It can be found in deserts, slopes, and low rock meadows. *Teucerium polium* L. is a little fragrant, pubescent shrub

that grows 20–40 cm tall. It has dense white flower heads and oval, 1-3 cm leaves with enrolled sides [15]. In many nations, they are used as herbal tea, appetizers, and spices. *T. polium* is also used to treat a variety of illnesses, including rheumatism, pain, the common cold, diuretics, antirheum, tonics, antifungals, carminatives, antipyretics, antibacterials, and antispasmodics and digestive issues [16, 17]. In Jordan, *T. polium* is widely used in traditional medicine to treat stomach and gastrointestinal conditions as well as for its hypoglycemic qualities [14].

The research revealed qualitative and quantitative differences in the oil composition that were linked to the various plant habitats, settings, and climates in which the plants were cultivated [18-20]. Finding the development stage that yields the optimum essential oil content and compounds, however, is crucial to figuring out the most suitable period [21-24]. In Jordan, no research has been done on the variation in germander components in pre-flowering and full-flowering of *Teucrium polium*. Thus, this study aims to assess the quality attributes of *Teucrium polium* by contrasting it with various growth stages (pre-flowering and full flowering) in Jordan. Furthermore, the essential oils extracted from the pre-flowering and full-flowering *T. polium* plants growing wild in Jordan were tested for antioxidant activity.

Materials and Methods Collection of Plants and Extraction of the Oils

The aerial flowering parts of *T. polium* were collected from the Jerash Region, north of Jordan, during the pre-flowering and full-flowering stages (April 2021 and June 2021, respectively). Fresh samples were used immediately for the extraction of their essential oils. The samples are collected at different flowering stages. Then hydro-distillation in a Clevenger-type apparatus for 3 h was used to extract their essential oils. After being collected separately, the oils were dried over anhydrous Na_2SO_4 and kept in amber glass vials at 4°C in a refrigerator until analysis. The extraction process was carried out twice.

GC-MS Spectrometry Analysis

The essential oils obtained from fresh *Teucrium polium* at both pre-flowering and full flowering stages were analysed with GC/MS. A CR-5 MS column (5% diphenyl, 95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 µm film thickness), a gas chromatography-mass spectrometer (Chromatic Crystal 9000 GC-MS, Yoshkar Ola, Russia) was used for the GC/MS analysis. The electron ionization mode in the MS detector was run at 70 eV. After being programmed to remain at 40 °C for one minute, the column temperature was increased to 280 °C at a steady rate of 3 °C per minute. The carrier gas utilized was helium (1.0 mL/min). The relative percent concentrations of the identified substances were computed using the relative peak regions. According to van Den Dool and Dec Kratz [25], the essential oils' chemical components were determined by comparing their computed Kovats retention index (KI) (about C8–C30). By comparing their mass spectra to those published by Adams [25] and the NIST library (2008), the constituents were identified.

Evaluation of Antioxidant Activity

DPPH Free Radical-Scavenging Capacity

One of the most often used techniques for assessing antioxidant activity is the DPPH free radical scavenging activity [26]. The method described in reference [27] was used in this work, and it covered a concentration range of (10–1000) µg/mL. A positive control was ascorbic acid. The mixture's absorbance was measured with a Cary Bio 100 double-beam UV-VIS spectrophotometer at a wavelength of 517 nm. The following formula was used to determine the proportion of free radical-scavenging capacity:

$$\left(\frac{Ac - A_s}{A_C}\right) x100\%$$

Where Ac is the absorbance of the blank and As is the absorbance in the presence of extract. When exposed to radical scavengers, the concentration of DPPH radicals dramatically drops. IC50 (µg/mL) was used to express the antiradical activity. Plots of the percentage of radical inhibition against the logarithm of concentration were linearly regression-analysed to determine the IC50 values.

$ABTS^{\bullet+}$ Radical-Scavenging Capacity

Using 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) and a slightly modified technique from reference [27], the essential oils from fresh *Teucrium polium* at both pre-flowering and flowering stages were tested for their ability to scavenge radicals throughout a concentration range of (10–1000) µg/mL. A positive control was ascorbic acid. The Cary Bio 100 UV-VIS spectrophotometer was used to measure the mixture's absorbance at a wavelength of 734 nm. The following formula was used to determine the $ABTS^{\bullet+}$ inhibition:

$$\left(\frac{Ac - A_s}{A_C}\right) x100\%$$

Where As is the absorbance when the extract is present, and Ac is the absorbance of the blank. Plots of the percentage of radical inhibition against the logarithm of concentration were linearly regression-analysed to determine the IC50 values.

Statistical Analysis

The essential oils' ability to scavenge DPPH and $ABTS^{\bullet+}$ radicals was examined in triplicate, and the results are shown as mean ± standard deviation. Dunnett's test and the one-way ANOVA test (analysis of variance) were used for statistical analysis. $P < 0.05$ was regarded as statistically significant.

Results and Discussion Essential Oil Composition

A total of 62 compounds were identified using GC/MS analysis of the hydro-distilled essential oils extracted from the fresh flowering portions of *T. polium*at the pre- and full flowering stages (Table 1). Oxygenated

monoterpenes made up the largest portion of the various terpenoidal classes found in both oils, making up (49.90%) of the total composition of the pre-flowering and (45.71%) of the stage of the fully grown flower.

The primary components found in this class during the pre-flowering stage were epi-α-Cadinol, (11.90%) and (E)-β-Santalol (6.15%). However, after flowering, the concentrations of both components were dropped to (10.13%) and (5.14%), respectively. Significant levels of Cabreuva oxide B were found in both phases (6.01% and 5.05%, respectively).1,7-diepi-α-Cedrenalalso found in both phases (5.87% and 4.65%, respectively).

The content of monoterpenes significantly increased from (17.75%) to (25.49%) upon flowering. The components found in this class during the pre-flowering stage were α-pinene (5.63%), Caryophyllene (3.65%), and trans-meta-Mentha-2,8-diene (3.51%). The concentration of the three compounds, however, increased upon flowering to (11.01%), (4.22%) and (4.91%), respectively. Sesquiterpene hydrocarbons were found also in both oils but were detected at higher concentrations in the oils levels extracted from the full-flower stage compared to their content during the pre-flower stages (12.14% and 8.00%, respectively).

Table 1: Volatile organic compound of *Teucrium polium* at the different stages (pre- and full flowering).

No.	KIexp	KI_{lit}		% composition	
				Pre-flowering	Full-flowering
1	918	923	Isocitronellene	1.02	0.52
2	920	926	Tricyclene	-	0.33
3	934	939	α-pinene	5.63	11.01
4	945	950	β-Citronellene	1.22	0.54
5	950	952	α-Fenchene	0.11	0.12
6	955	960	Thuja-2,4(10)-diene	-	0.03
7	980	983	trans-meta-Mentha-2,8-diene	3.51	4.91
8	981	984	trans-Isolimonene	1.14	0.62
9	1001	1002	δ-2- Carene	0.09	1.44
10	1003	1004	ρ-Mentha-1(7),8-diene	2.01	0.85
11	1002	1007	(2E,4E)-Heptadienal	0.71	0.36
12	1403	1408	Caryophyllene	3.65	4.22
13	1015	1017	α-Terpinene	0.36	0.54
14	1022	1024	ρ-Cymene	0.01	0.76
15	1024	1025	Methyl heptanoate	0.67	0.74
16	1027	1029	Limonene	0.08	0.14
17	1032	1031	1,8-Cineole	0.96	0.99
18	1036	1035	(3E)-Octen-2-one	0.75	0.87
19	1406	1409	α-Gurjunene	2.11	1.32
20	1416	1417	α-Santalene	0.64	0.69
21	1417	1420	β-Cedrene	0.14	0.45
22	1420	1424	4,8-β-epoxy-Caryophyllane	1.31	0.48
23	1433	1439	α-Guaiene	0.52	0.13
24	1441	1442	(Z)-β-Farnesene	0.69	0.20
25	1443	1446	Bakerol	1.08	0.01
26	1446	1447	epi-β-Santalene	0.74	0.47
27	1450	1454	α-Humulene	0.11	0.98
28	1455	1456	(E)-β-Farnesene	1.53	3.25
29	1456	1459	Rotundene	3.08	3.69
30	1457	1459	β-Santalene	2.87	0.96
31	1462	1461	Cyclamen aldehyde	0.63	0.82
32	1463	1464	Cabreuva oxide B	6.01	5.05
33	14661	1468	Cabreuva oxide C	3.41	3.65
34	1480	1482	α-Cyclogeranyl acetate	0.25	1.04
35	1495	1495	cis-Cadina-1,4-diene	1.32	-
36	1498	1499	Curzerene	1.00	-
37	1520	1522	β-sesquiphellandrene	4.63	0.47
38	1638	1640	epi-α-Cadinol	11.90	10.13
39	1640	1641	1,7-diepi-α-Cedrenal	5.87	4.65
40	1643	1642	Daucol	0.02	1.22
41	1644	1646	α-Muurolol	3.08	-

42	1714	1715	(2E,6Z)-Farnesol	1.97	5.42
43	1716	1715	Phenethyl 2-furoate	1.21	-
44	1718	1716	(Z)-β-Santalol	0.86	0.76
45	1720	1722	Neocnidilide	-	0.47
46	1722	1723	(2Z,6E)-Farnesol	1.02	2.10
47	1734	1739	(E)-β-Santalol	6.15	5.14
48	1750	1753	Cuparenal	0.18	0.28
49	1752	1754	Isobaeckeol	0.53	0.29
50	1760	1761	(Z)-Lanceol	1.96	0.58
51	1765	1769	Bancroftinone	0.03	0.74
52	1770	1774	n-Pentadecanol	4.36	3.15
53	1773	1778	(E)-α-Atlantone	1.01	3.14
54	1786	1787	Butyl dodecanoate	0.98	0.74
55	1788	1790	1-Octadecene	0.41	0.27
56	1794	1795	α-Eudesmol acetate	0.58	0.63
57	1799	1800	n-Octadecane	0.63	1.78
58	1801	1803	Benzoin	0.14	2.45
59	1810	1815	Torquatone	0.85	0.96
60	1821	1822	(2Z,6E)-Farnesyl acetate	0.36	1.02
61	1830	1837	Occidol	1.85	2.05
62	1865	1866	Encecalin	0.02	-

Classification	**% Composition**	
	Pre-flowering	**Full-flowering**
Monoterpenes hydrocarbons (MH)	17.75	25.49
Oxygenated monoterpenes (OM)	2.18	1.53
Oxygenated sesquiterpenes (OS)	49.90	45.71
Sesquiterpene hydrocarbons (SH)	8.00	12.14

3.2 Antioxidant Activity

According to the data in **Table 2** the antioxidant activities demonstrated by two methods, ABTS $^{\bullet+}$ and DPPH, were measured by using different concentrations of essential oil solution in methanol ($1*10^4$-$1*10^6$) µg/ml. The absorbance for the DPPH procedure was measured at 517 nm against blank samples using a UV-VIS spectrophotometer. On the other hand, the absorbance for the ABTS $^{\bullet+}$experiment was measured at 713 nm against blank samples using a UV-VIS spectrophotometer.

The results are expressed as percentage inhibition of absorbance. It showed the essential oils from pre-flowering and full-flowering *Teucrium polium* L possessed strong antioxidant and radical scavenging activities determined by DPPH and ABTS $^{\bullet+}$ compared to ascorbic acid. Thus, based on the IC_{50} values of the essential oil in pre-flower and full-flower *Teucrium polium* L, the antioxidant is preserved **Table 3**. shows that the percent of DPPH or ABTS$^{\bullet+}$ inhibition of the oil was proportional to increased concentration according to the selected concentrations.

Table 2. Antioxidant activity of essential oils from *Teucrium polium* at the different stages (pre- and full flowering).

	DPPH			**ABTS $^{\bullet+}$**		
Concentration (µg/ml)	**Pre-Flower**	**Full Flower**	**Ascorbic acid**	**Pre-Flower**	**Full Flower**	**Ascorbic acid**
1.00 × 10	18.12 ± 0.19	15.01 ± 0.47	25.11 ± 0.11	25.96 ± 0.17	22.63 ± 0.65	32.96 ± 0.11
2.00 ×10	33.42 ± 0.87	22.65 ± 0.96	35.09 ± 0.36	39.58 ± 0.69	29.75 ± 0.12	41.35 ± 0.63
1.00×10^3	42.56 ± 0.12	32.32 ± 0.35	43.36 ± 0.96	47.77 ± 0.73	38.14 ± 0.36	49.74 ± 0.45
3.50×10^2	64.87 ± 0.25	58.95 ± 014	65.13 ± 0.57	65.36 ± 0.97	48.69 ± 0.77	71.01 ± 0.63
5.00×10^2	73.55 ± 0.23	69.11 ± 0.52	74.37 ± 0.39	76.45 ± 0.01	66.36 ± 0.75	81.63 ± 0.20
1.00×10^3	88.44 ± 0.97	78.96 ± 0.63	90.77 ± 0.97	90.54 ± 0.03	85.63 ± 0.41	95.47 ± 0.01

IC_{50} values of essential oils from pre-flowering and full-flowering *Teucrium polium* are shown in **Table 3**. A lower IC_{50} value indicates higher antioxidant activity. The DPPH IC_{50} value of the essential oil from the pre-flowering stage of *T. polium* is (0.09×10^2 µg/mL). The DPPH IC_{50} value of the essential oil from the full-flowering stage of *T. polium* is (2.20×10^2 µg/mL). On the other hand, the ABTS IC_{50} value of the essential oil from the pre-flowering stage of *T. polium* is (1.01×10^2 µg/mL). The DPPH IC_{50} value of the essential oil from the full-flowering stage of *T. polium* is (2.12×10^2 µg/mL). According to the values of $IC_{50.}$ The values of IC_{50} of the essential oil from the pre-flowering stage of *T. polium*is lower. As a result, the essential oil from the pre-flowering stage of *T. polium*is better as an antioxidant than that from the essential oil from the pre-flowering stage.

Table 3. IC_{50} values of essential oils from *Teucrium polium* at the different stages (pre- and full flowering).

	DPPH IC_{50} value (µg/ml)	**ABTS $^{\bullet+}$ IC_{50} value (µg/ml)**
pre-flowering	$(0.09 \pm 0.05) \times 10^2$	$(1.01 \pm 0.03) \times 10^2$
full-flowering	$(2.20 \pm 0.82) \times 10^2$	$(2.12 \pm 0.11) \times 10^2$
Ascorbic acid	$(0.07 \pm 0.02) \times 10^2$	$(0.50 \pm 0.03) \times 10^2$

Conclusion

This study is the first to take into consideration how the chemical makeup of the essential oil derived from *T. polium* varies depending on the stage of full-flowering. The composition of the oils was evaluated by GC/MS. From fresh pre-flowering and full-flowering stages of *T. polium*oils, four primary classes were identified: oxygenated sesquiterpenes, monoterpenes hydrocarbons, sesquiterpene hydrocarbons, and oxygenated monoterpenes.

The results of this investigation provide credence to the suggestion that *T. polium* should be collected before flowers open rather than after. The antioxidant activity of the essential oil components was assessed by their ability to scavenge DPPH and $ABTS^{\bullet+}$ radicals. According to the study's findings, *T. polium* naturally contains antioxidants in both the pre-flowering and full-flowering stages. The pre-flowering essential oil exhibited higher antioxidant activity than the full-flowering sample.

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