Acta Botanica Hungarica 65(1–2), pp. 73–85, 2023
DOI: 10.1556/034.65.2023.1-2.4

CHEMICAL PROFILE AND BIOACTIVITY OF THE ESSENTIAL OIL OF *TEUCRIUM TAKOUMITENSE*: AN ENDEMIC LAMIACEAE FROM SOUTHEAST MOROCCO

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(Received: 5 October 2022; Accepted: 30 January 2023)

The *Teucrium* genus is an important source of essential oil-bearing plants. The essential oils of this genus are endowed with important phytochemical diversity, exert widespread biological properties, and have several uses in traditional medicine. *Teucrium takoumitense* is an endemic aromatic and medicinal plant, grown in a very limited area in southeastern Morocco. To the best of our knowledge, there is no published report on the phytochemical or biological studies of *Teucrium takoumitense* essential oil (EO) harvested from the Errachidia region in Southeast Morocco. GC/MS analysis, total phenolic compounds, antioxidant, anti-inflammatory, and antibacterial tests on the EO of this plant were carried out in this study. The results of GC/MS analysis showed that β-ocimene (10.12%), δ-bisabolene (8.35%), linalool (8.16%), β-eudesmol (8.05%), and δ-cadinene (7.89%) are the major compounds in the EO. Important antioxidant activity (IC_{50} DPPH = 2.4 mg/mL, IC_{50} ABTS = 1.58 mg/mL, IC_{50} FRAP = 0.71 mg/mL, and TAC value = 230.72 AAE mg/g EO) and potent anti-inflammatory effect evaluated by phenol induced inflammation in rat ears were induced by the volatile oil. Moreover, the volatile oil induced antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* In conclusion, *Teucrium takoumitense* EO was found to be rich in volatile compounds and exert antioxidant, anti-inflammatory, and antibacterial properties. More studies are recommended to be evaluated on the extracts of this plant and conservation interventions need to be carried out to maintain its sustainability.

Key words: antibacterial effect, anti-inflammatory, antioxidant, chemical composition, essential oil, *Teucrium takoumitense*

INTRODUCTION

Essential oils (EOs) are important bioactive plant secondary metabolites. These compounds are a mixture of volatile and aromatic molecules with im-

portant chemical diversity and large spectra of biological properties. These volatile compounds have been intensively used for centuries in several cultures for their therapeutic, antimicrobial, antiseptic, antioxidant properties, etc. (Popa *et al.* 2021). Furthermore, the use of these natural antioxidants as alternatives to chemical drugs suspected to be potentially harmful to the environment and human health would be safer and more effective.

Plants are a natural source of volatile phytochemicals. Numerous species produce important amounts of these bioactive compounds. Moreover, Lamiaceae is a large botanical family that includes numerous EO-bearing plants.

Teucrium which belongs to the important Lamiaceae family is a cosmopolitan genus of perennial plants that are rich in EOs characterised by various chemical profiles rich in monoterpenoid and sesquiterpenoid compounds with several biological properties (De Martino *et al.* 2020). Moreover, the species of this genus have a large implementation in the ethnomedicine of many countries. For example, they are used in the Moroccan folk medicine to treat several human diseases, such as digestive disorders, inflammation, hypertension, fever, diabetes, rheumatism, and parasitic diseases (El Atki *et al.* 2020). In traditional Palestinian medicine, *Teucrium polium* is used for its antispasmodic and antidiarrheal effects and to treat intestinal and cardiac disorders (Jaradat *et al.* 2016). Additionally, in the folk medicine of several European countries, *Teucrium chamaedrys* is widely used to treat kidney, liver, and heart diseases, diarrhoea, malaria, pulmonary disorders, coughs, asthma, abscesses, conjunctivitis, etc. (Uritu *et al.* 2018, Zdraveva *et al.* 2018). However, several species of *Teucrium* genus are still unstudied and need screenings for their phytochemical and biological properties.

Teucrium is the largest genus of the Lamiaceae family in Morocco. Additionally, several researchers have studied this genus. Sixty taxa, grouped into 52 species and arranged in 8 sections, are recognised for the genus *Teucrium* in Morocco (Fennane *et al.* 2007). Moreover, *Teucrium takoumitense* Förther et Podlech is a Moroccan endemic species that grows in a very limited area in Tazougart village in Errachidia region. As far as we know, there is no biological or chemical study reported on this *Teucrium* species. Therefore, this work aimed to determine the chemical composition of the EO of this plant and to evaluate its antioxidant, anti-inflammatory, and antibacterial properties.

MATERIAL AND METHODS

Chemicals and apparatus – All chemicals and reagents were of analytical grade. Methanol was obtained from HiPerSolv Chromanorm company. Folin-Ciocalteu reagent was purchased from Oxford Company. Sodium carbonate, ascorbic acid, and gallic acid were obtained from Fluka. Other products (cate-

chin, quercetin, DPPH, ABTS, potassium persulfate, ferric chloride, potassium ferricyanide, sodium phosphate, ammonium molybdate, phenol, and trichloroacetic acid) were purchased from Sigma-Aldrich Chemical Co. Spectrophotometric measurements were performed on visible spectrophotometer VIS-723G (Beijing Beifen-Ruili Analytical Instrument). Measurements of rat ear edema were performed using a digital calliper (POWERFIX Profi+, Germany).

Plant material – The areal parts of *T. takoumitense* were collected in Tazougart village in Errachidia region during the flowering period (May 2022). The plant was identified in the National Institute of Agronomic Research of Errachidia. The plant material was air-dried at room temperature (25 °C) for seven days, then it was stored in the dark at room temperature (25 °C) until extraction.

Extraction – Two hundred grams of areal parts of *T. takoumitense* were subjected to 3 h of hydrodistillation using a Clevenger-type apparatus as recommended by the European Pharmacopoeia (1997). The obtained volatile oil was separated and stored at + 4°C until tested and analysed.

Chemical composition – The profile of volatile oil was characterised by gas chromatography (GC) (Agilent 7890A Series) coupled with mass spectrometry (MS) equipped with a multimode type injector and a 123-BD11 column of dimension 15 m × 320 μm × 0.1 μm. Four μL of the soluble extract were injected into the column by split 1/4 mode using helium as the carrier gas at 2 mL/min. The temperatures of the ion source and quadrupoles were 230 and 150 °C, respectively. The oven temperature program was started at 30 °C and finished at 360 °C. Identification was performed using NIST 2017 MS Library.

Estimation of total phenolic compounds – The determination of total phenolic content was performed using the Folin-Ciocalteu method (Poh-Hwa *et al.* 2011). Briefly, 200 μL of each extract was mixed with 1,000 μL of Folin-Ciocalteu reagent at 10%. The reaction was neutralised with 800 μL of sodium carbonate (Na_2CO_3) at 7.5%. The mixture was further incubated in the dark for 30 min. Then, the absorbance was determined against a blank at 765 nm with a spectrophotometer. Gallic acid (7–125 μg/mL) was used to generate the calibration curve, and the results were expressed as mg of gallic acid equivalents per gram of essential oil (GAE mg/g EO).

Antioxidant activity: DPPH free radical scavenging capacity – 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was performed as described in our previous study (Elbouny *et al.* 2022). When DPPH radical accepts hydrogen from a corresponding donor (antioxidant), its solutions lose the characteristic purple colour (λ_{max} 515–517 nm). Briefly, 1 mL of methanolic solution of DPPH at 0.0023% (60 μM) was mixed with 25 μL of the volatile oil at different concentrations. The reaction mixture was vigorously shaken and incubated at room temperature for 20 min. Then, the absorbance was read at 517 nm against a methanolic blank. The control was prepared by adding

methanol instead of EO. Quercetin (0.38–6.09 μg/mL) was used as an antioxidant standard. The percentage of inhibition was calculated using the formula below:

% Inhibition of DPPH = ((Absorbance of Blank – Absorbance of Test) / (Absorbance of Blank)) × 100

Results were expressed as IC_{50} (μg/mL) value which was determined using the linear regression equation from the inhibition values.

ABTS free radical scavenging capacity – 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was performed adhering to the method of Pukalskas *et al.* (2002). Antioxidant can reduce $ABTS^{\cdot+}$ to ABTS and decolorise it. The purpose is to determine the $ABTS^{+}$ reduction potential of the EO. The $ABTS^{+}$ radical was generated through the oxidation of ABTS with Potassium Persulfate. In brief, the ABTS solution (7 mM) had reacted with Potassium Persulfate (70 mM) solution (mixed in equal volume) for generation of ABTS cations. The mixture was allowed to stand in the dark at room temperature for 16 h. Before being used in the assay, the ABTS radical cation was diluted with Methanol for an initial absorbance of about 0.700 at 734 nm. Then, different concentrations of *T. takoumitense* EO (100 μL) were added to 2 mL of ABTS solution. The absorbance was read at 734 nm and the percentage inhibition was calculated as described earlier for the DPPH test. Ascorbic acid (0.5–5 μg/mL) was used as a standard antioxidant. Results were expressed as IC_{50} (μg/mL) value.

Ferric-reducing antioxidant power test (FRAP) – The ferric reducing activity was measured spectrophotometrically with the Oyaizu method (Oyaizu 1986). In this method, the principle is to measure the ferric ion reducing antioxidant parameter. Various concentrations of EO (200 μL) were mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$). After 20 min incubation at 50 °C, 2.5 mL of 10% (w/v) trichloroacetic acid was added to the mixture. About 2.5 ml of each concentration was taken and 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride ($FeCl_3$) were added. The intensity of the blue-green colour was measured at 700 nm. Catechin (0.65 - 21.39 μg/mL) was used as a positive control. Results were expressed as IC_{50} (μg/mL) value.

Total antioxidant capacity test (TAC) – The total antioxidant activity was evaluated by the formation of phosphomolybdenum complex according to the method established by Prieto *et al.* (1999). This test is based on the ability of the EO to donate electrons to the molybdenum (VI), forming the molybdenum (V). Briefly, 200 μL of *T. takoumitense* EO was added to 2 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The absorbance was measured at 695 nm after boiling at 95 °C for 90 minutes. Ascorbic acid (15–250 μg/mL) was used as a standard to generate the

calibration curve. The total antioxidant capacity was expressed as milligrams of ascorbic acid equivalent per gram of EO (mg AAE/g EO).

Anti-inflammatory activity – The anti-inflammatory effect of the volatile oil was carried out using phenol induced ear edema in rats (Rodrigues *et al.* 2016). Edema was induced in Wister albino rats of both sexes weighing 100–120 g (n = 6) by topical application of phenol. The handling and use of animals were performed in accordance with the guidelines of the local committee (AREC-FSTE-12/2020).

Ten microliters of phenol solution (15% v/v in acetone) were applied to the inner and outer surface of the right ear of rats. Immediately, animals received topical treatment with 20 μL of essential oil (1 mg/ear), indomethacin (1 mg/ear) as a reference drug, or acetone for the negative control group. The thickness of the ear was measured 2 h after phenol applications using a digital calliper. The percentage of edema was calculated using the following formula:

$$\% \text{ of edema} = ((1 - (T(t0) / T(t2)) \times 100$$

where, T(t0) is the thickness of ear before applying the phenol and T(t2) is the thickness of ear after 2 h of applying the phenol.

Antibacterial activity: Bacterial strains – *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 (two gram-negative bacteria), and *Staphylococcus aureus* ATCC 25922 (gram-positive bacterium) were used in the antimicrobial test. Bacterial strains were revivified in Luria-Bertani plates for 24 h at 37 °C before use. The turbidity of the bacterial inoculum was adjusted using a spectrophotometer at 600 nm (10^6 UFC/mL). Microorganisms were obtained from the culture collection of the National Institute of Hygiene (Rabat).

Disc diffusion method – The assay was carried out as described by Chraibi *et al.* (2021). After the inoculation of bacterial inoculum on Petri dishes containing Muller Hinton agar culture medium. Filter paper discs (6 mm) disposed in the centre of plates were impregnated with 6 μl of volatile oil. Then, the Petri dishes were placed at 4 °C for 120 min to provide a better diffusion of volatile compounds in the medium and then incubated at 37 °C for 48–72 h. Gentamicin (10 μg/disc) and chloramphenicol (30 μg/disc) were used as antibacterial standard drugs. Assays were done in triplicate. The bacterial effect was estimated by measuring the inhibition zone (IZ) diameter (millimetres: mm) using a digital calliper.

Determination of minimum inhibitory concentration (MIC) – The MICs were determined using the micro-dilution method in a 96-well microplate according to Chraibi *et al.* (2021) with a slight modification. Briefly, the resazurin solution was prepared in sterile water and the bacterial culture was made by diluting an overnight culture of a test bacterium in physiologic water (0.1%) at a concentration of 10^6 CFU/mL. Thereafter, 50 μL of culture was added to each indicated well followed by 10 μL of resazurin solution and then 40 μL of each

diluted EO at different concentrations (0.0625–2% (v/v)) was added. After 6 h of incubation at 37 °C, results were recorded.

Determination of minimal bactericide concentration (MBC) – The MBCs were determined by inoculating a volume of 2 μL from negative wells and incubated at 37 °C for 24 h. The bactericidal potential of the EO was determined by calculating the MBC/MIC ratio. An EO is considered bactericidal when its MBC/MIC ratio is equal or less than 4, whereas the EO is bacteriostatic when its MBC/MIC ratio underpasses 4. From the MIC and MBC values, we calculated the tolerance level of the analysed EO (Bammou *et al.* 2020).

Statistical analysis – Statistical analysis was performed using "GraphPad Prism 8" software. Comparisons were made using one-way ANOVA test followed by post hoc analysis for data with three groups and data with two groups were analysed using unpaired t-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Chemical composition – The EO isolated by hydrodistillation from the areal part of *T. takoumitense* was obtained with a yield of 0.48%. The result of the GC/MS analysis of EO is shown in Table 1. Thirty-five compounds were identified representing 91.95% of the total composition. The volatile oil was rich in sesquiterpene hydrocarbons (34.28%) and oxygenated sesquiterpenes (31.42%). β-ocimene (10.12%) was the major compound followed by δ-bisabolene (8.35%), linalool (8.16%), β-eudesmol (8.05%), δ-cadinene (7.89%), eudesma-3.5-dien-1β-ol (7.67%) and linalyl acetate (6.26%) as the main components of the oil.

Antioxidant activity – The results of antioxidant tests are shown in Table 2. The Folin-Ciocalteu test showed that *T. takoumitense* essential oil has 23.34±0.72 GAE mg/g EO of phenolic compounds. Moreover, the essential oil revealed various antioxidant effects. It was able to scavenge the ABTS and DPPH radicals with half-maximal inhibitory concentrations of 1582.20±2.01 μg/mL and 2405.38±10.40 μg/mL, respectively. Moreover, the volatile oil exhibited ferrous (EC50 = 709.26±7.83 μg/mL) and molybdenum (230.72±0.52 AAE mg/g EO) reducing effects. However, the antioxidant potential of antioxidant standards was much higher than that of essential oil ($p < 0.0001$).

Anti-inflammatory activity – The results of the anti-inflammatory test are represented in Figure 1. Phenol application induced significant inflammation (% of edema = 77.59%). However, the different treatments reduced the inflammation. *T. takoumitense* at 0.5 mg/ear significantly ($P < 0.0001$) reduced ear edema (% of edema = 34.52%). Moreover, at 1 mg/ear *T. takoumitense* significantly ($P < 0.0001$) reduced ear edema with an edema percentage of 22.51%.

Table 1
The results of GC/MS analysis

No	Components	Ret	Abun	No	Components	Ret	Abun
1	β-pinene	974	0.52	24	germacrene d-4-ol	1578	4.67
2	β-myrcene	990	0.96	25	caryophyllene oxide	1584	0.46
3	d-limonene	1020	0.59	26	humulene-1.2-epoxide	1610	0.38
4	**linalool**	**1098**	**8.16**	27	muurola-4.10(14)-dien-1β-ol	1615	1.9
5	3-carene	1148	0.24	28	**β-eudesmol**	**1653**	**8.05**
6	α-terpineol	1189	2.66	29	cadalene	1674	2.07
7	**β-ocimene**	**1225**	**10.12**	30	α-bisabolol	1685	2.65
8	geraniol	1235	2.18	31	**eudesma-3.5-dien-1β-ol**	**1706**	**7.67**
9	**linalyl acetate**	**1261**	**6.26**	32	murolan-3.9(11)-diene-10-peroxy	1730	0.23
10	*cis*-β-elemene	1382	0.03	33	oplopanone	1738	0.6
11	α-cubebene	1351	0.05	34	dehydrofukinone	1813	0.22
12	α-copaene	1378	1.01	35	phytol	2125	0.15
13	neryl isobutyrate	1469	1.33		**total**	**91.95**	
14	caryophyllene	1419	0.43		monoterpene hydrocarbons	14.28	
15	geranyl acetate	1378	5.03		oxygenated monoterpenes	17.14	
16	humulene	1454	0.58		sesquiterpene hydrocarbons	34.28	
17	*allo*-aromadendrene	1461	0.16		oxygenated sesquiterpenes	31.42	
18	valencene	1491	0.99		others	2.85	
19	cubebanol	1500	1.13				
20	α-muurolene	1502	1.73				
21	**δ-bisabolene**	**1515**	**8.35**				
22	**δ-cadinene**	**1524**	**7.89**				
23	α-calacorene	1548	2.5				

Table 2
The results of antioxidant activity

Essential oil and standards	DPPH	ABTS	FRAP	TAC	TPC
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	EC_{50} (μg/mL)	AAE mg/g EO	GAE mg/g EO
T. takoumitense EO	2405.38± 10.40	1582.20± 2.01	709.26±7.83	230.72±0.52	23.34±0.72
Quercetin	5.49± 0.02****	–	–	–	–
Ascorbic acid	–	2.52±0.02****	–	–	–
Catechin	–	–	13.90±0.03****	–	–

Values are represented as mean of 3 replicates (± SD); **** = $p < 0.0001$; – = not applicable

Table 3
The results of antimicrobial activity

	T. takoumitense	Chlorophenicol	Gentamicin
Bacterial strains	IZ (mm)	IZ (mm)	IZ (mm)
Staphylococcus aureus	15.5±0.5[a]	12±0.15[b]	n.a.
Pseudomonas aeruginosa	15.25±0.7[a]	15±1.22[a]	10±0.6[b]
Escherichia coli	12.21±0.18[a]	11±0.77[a]	14±0.1[b]

n.a. = not active; Values in the same row with the same letter group are not significantly different ($P < 0.05$)

Table 4
The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) by the EO of *T. takoumitense*

Bacterial strains	MIC	MBC	MBC/MIC	Effect
Staphylococcus aureus	0.25	0.25	1	bactericidal
Pseudomonas aeruginosa	0.25	0.5	2	bactericidal
Escherichia coli	0.125	0.5	4	bactericidal

Indomethacin at 1 mg/ear exerted important anti-inflammatory activity (% of edema = 14.71%).

Antibacterial activity – The results of the antibacterial activity of *T. takoumitense* EO are shown in Table 3. The volatile oil of *T. takoumitense* exerted an important antibacterial effect against tested strains with inhibition zone diameters varying from 12.21 to 15.5 mm. The EO showed higher activity against *S. aureus* (IZ = 15.5±0.5 mm) which was more important than that of chlorophenicol (IZ = 12±0.15 mm) and gentamicin (no activity) $p < 0.05$. In general, the inhibitory effect of the essential oil was comparable or greater than that of antibiotics.

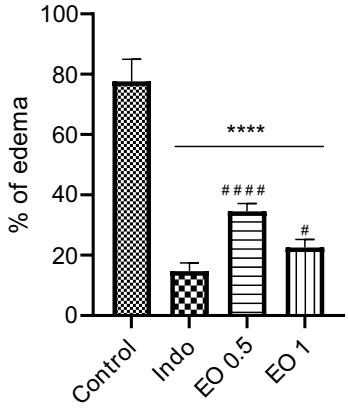

Fig. 1. The results of anti-inflammatory activity. Indo = indomethacin at 1 mg/ear. EO 0.5 = essential oil at 0.5 mg/ear. EO 1 = essential oil at 1 mg/ear. **** = $p < 0.0001$, when compared to control group. # # # # = $p < 0.0001$ and # = $p < 0.05$, when compared to indomethacin

The EO of *T. takoumitense* presented an important antimicrobial potential (Table 4). On the one side, with MIC values of 0.125 (% v/v) for *E. coli*, which proved that this species is the most sensitive. Whereas *S. aureus* and *P. aeruginosa* recorded a MIC of 0.25 (% v/v). On the other side, the MBC values ranged from 0.25 to 0.5 (% v/v). Moreover, the MBC/ MIC ratio was around 4 and 1 which confirms the bactericidal effect of the tested EO.

DISCUSSION

Teucrium species are sources of a wide variety of volatile compounds. In this present study, the composition of *T. takoumitense* EO was revealed. The oil was dominated by oxygenated and hydrocarbon sesquiterpenes (65.70%) whereas oxygenated and hydrocarbon monoterpenes have a low percentage in the essential oil (31.42%). Likewise, several *Teucrium* species were reported to contain high amounts of sesquiterpene hydrocarbons and oxygenated sesquiterpenes including *T. ramosissimum* (80.62%) from Tunisia (Ghazouani *et al.* 2017) and *T. arduini* (63.4) from Croatia (Dunkić *et al.* 2011). Moreover, *T. takoumitense* essential oil has several dominant compounds, including β-ocimene (10.12%) δ-bisabolene (8.35%), linalool (8.16%), β-eudesmol (8.05%), δ-cadinene (7.89%), eudesma-3.5-dien-1β-ol (7.67%) and linalyl acetate (6.26%). Compared to our findings, there are several chemotypes of *Teucrium* plants in the literature. Ciocarlan *et al.* (2022) reported that the major compounds of Moldovan *Teucrium* species are germacrene D (*T. polium*), *α*-himachalene (*T. hircanicum*), *β*-caryophyllene (*T. botrys*, *T. orientale*, *T. chamaedrys*), and *β*-bisabolene (*T. flavum*) (Ciocarlan *et al.* 2022). Moreover, Catinella *et al.* (2021) reported that *β*-pinene (*T. capitatum*), longifolenaldehyde (*T. montanum*), and *β*-bisabolene (*T. flavum)* are the major components of Italian *Teucrium* plants. However, there are no *Teucrium* species reported to have β-ocimene as the major compound in its essential oil, which makes *T. takoumitense* a β-ocimene chemotype *Teucrium*.

The search for antioxidants in plant extracts is a field with growing interest. Numerous natural volatile compounds exert antioxidant effects, and they are more efficient and safer than several synthetic antioxidants (Amorati *et al.* 2013). Therefore, the evaluation of the antioxidant potential of plant EOs is important. *T. takoumitense* volatile oil was found to have low phenolic compounds (23.34 μg GAE/g EO) according to the Folin-Ciocalteu estimation test, which is consistent with the results of the chemical composition in which no phenolic compound was detected. Moreover, our findings revealed that *T. takoumitense* volatile oil exerted various antioxidant effects, which are DPPH and ABTS radicals scavenging potential and ferrous and molybdenum reducing effects. Furthermore, the antioxidant capacity of this species can be considered as a moderate antioxidant effect (DPPH IC_{50} = 2,405.38 μg/mL; ABTS IC_{50} = 1,582.20; FRAP IC_{50} = 709.26; TAC value = 230.72 mg AAE/g EO) compared to the antioxidant effect of other *Teucrium* plants reported in previous studies. On one hand, some *Teucrium* species displayed very low antioxidant activity, such as *T. polium* from Iran (DPPH IC_{50} = 9200 μg/mL) (Mahmoudi and Nosratpour 2013), Moroccan *T. polium* subsp. *aureum* (DPPH IC_{50} = 3,700 μg/mL; FRAP IC_{50} = 2,310 mg/mL) (El Atki *et al.* 2020), and *T. polium* subsp.

capitatum from Algeria (DPPH IC_{50} = 5,550.33 μg/mL; TAC value = 508.91 μg EAA/mg) (Chabane *et al.* 2021). On the other hand, other species were reported to exert higher antioxidant potential, such as *T. pseudochamaepitys* (DPPH IC_{50} = 770 μg/mL) (Hammami *et al.* 2015), *T. luteum* subsp. *flavovirens* (DPPH IC_{50} = 8.39 μg/mL) (Majdoub *et al.* 2022) from Tunisia, and Iranian *T. orientale* (DPPH IC_{50} = 121.6 μg/mL) (Amiri 2010).

T. takoumitense essential oil demonstrated significant anti-inflammatory properties in rat ear edema. Likewise, several studies reported that the EOs from *Teucrium* species exert several anti-inflammatory effects. *T. scordium* volatile oil from Italy was tested on lipopolysaccharide-stimulated macrophages and produced anti-inflammatory action by reducing nitric oxide production without affecting cell viability (Piras *et al.* 2021). The same anti-inflammatory action was exhibited by the volatile oils of *T. flavum*, *T. montbretii*, *T. polium*, and *T. brevifolium* from Greece (Menichini *et al.* 2009). Moreover, the essential oil of Palestinian *T. pruinosum*, was proven to exert efficient anti-inflammatory effects by inhibiting the activity of cyclooxygenase-1 and cyclooxygenase-2 enzymes. Its activity was comparable to that of the non-steroidal anti-inflammatory drug etodolac (Jaradat *et al.* 2018). These anti-inflammatory actions are generally due to the bioactive volatile compounds of these plants. Thus, these previous findings, along with our results, prove that the volatile oils from *Teucrium* species are a source of efficient anti-inflammatory compounds. Furthermore, more research on the anti-inflammatory activities of *T. takoumitense* essential oil and its major components is highly recommended to reveal the mechanisms of action and to identify the compounds responsible for this biological effect.

Plant volatile oils are an important source of antibacterial agents. EOs volatile compounds exert their antibacterial effects by disrupting bacterial membranes, which is the most common mechanism of antimicrobial action (Álvarez-Martínez *et al.* 2021). In the same manner, *T. takoumitense* essential oil exerted important antibacterial effects. Moreover, the antimicrobial properties of the volatile oils obtained from other *Teucrium* species have been largely explored. El Atki *et al.* (2020) investigated the antibacterial activity of *T. polium* volatile oil against six bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Citrobacter koseri*, and *Staphylococcus aureus*) and reported that the oil of this species was active against all strains (El Atki *et al.* 2020). Moreover, Küçük *et al.* (2006) tested the EOs of *T. chamaedrys* and *T. orientale* against several microbial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Yersinia pseudotuberculosis*, *Serratia marcescens*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *C. tropicalis)* and found that *Teucrium* oils exerted antibacterial effects against bacterial strains. However, no antifungal effect was observed against *Candida albicans* and *C. tropicalis* (Küçük *et al.* 2006). In general, *Teucrium* species, including *T.*

takoumitense along with other Lamiaceae plants, exert potent antibacterial effects, and they are an important source of antibacterial volatile agents.

CONCLUSION

The findings of the present study indicate that *T. takoumitense* EO has a special chemical profile and possesses important anti-inflammatory, antioxidant, and antibacterial effects. This study provides a scientific base for future investigations on the volatile and non-volatile extracts of *T. takoumitense.*

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Acknowledgement – This work was supported by National Centre of Scientific and Technical research (CNRST), Morocco.

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