ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 6, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

CHEMICAL COMPOSITION, ANTIBACTERIAL ACTIVITY, AND ANATOMICAL STUDY OF *TEUCRIUM POLIUM* L.

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Received: 19 February 2019, Revised and Accepted: 15 April 2019

ABSTRACT

Objective: The aim of this work is the simplification of the exploitation of medicinal plants that are widely spread in Algeria such as *Teucrium polium* and search for new biological molecules; first, we performed an anatomical study of the plant to identify their characteristics, then a chemical analysis of its essential oils to identify the active substances contained in this plant; finally, we tested the antibacterial properties of this essential oil.

Methods: The extraction of essential oils was carried out by hydrodistillation method using a Clevenger-type apparatus. The chromatographic analysis of essential oil of this plant was performed with a Hewlett Packard gas chromatograph (GC) controlled pore glass (CPG)/(Flame ionization detectors) 7890, coupled to a GC: CPG/mass spectrometry (MS) 7890/5975C. The antimicrobial activity was determined by the disc diffusion method. The cross sections were manually prepared and the coloring process was done using double coloration method.

Results: The results show that the extraction yield was 0.7. The extraction produced yellowish essential oils with a very strong odor. Twenty-seven compounds were identified by GC and GC/MS, the major compounds were β-pinène (30.61%) followed by carvacrol (13.09%) and α-pinène (10.40%). Essential oils of this plant exhibited an antibacterial effect on *Escherichia coli* and *Staphylococcus aureu,* and *Pseudomonas aeruginosa* was resistant. The anatomical study of this plant shows three different types of glandular trichomes on leaves and stems.

Conclusion: This study allowed identifying the anatomical characteristics of *T. polium* as well as the chemical composition of their essential oil. It has also been shown that this essential oil has an important and significant antibacterial activity.

Keywords: *Teucrium polium* L., chemical composition, antibacterial activity, anatomical study.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i6.32686

INTRODUCTION

Teucrium genus is spread in Southwest Asia, Europe, North Africa, and the Mediterranean region. It is located in low rock meadows, hills, and deserts [1].

Teucrium polium L. is a grassy plant, branched in the base, with linear leaves green and gray. The cup is green-gray, the coriander is white. The inflorescences in the compact heads are located at the top of the legs. This plant is widely spread in Algeria [2].

In Algerian folk medicine, this plant is used to treat gastrointestinal diseases and treat wounds. The aerial parts of the plant (stems, leaves, and flowers) are used as tea, which is very useful for gastric ulcers and intestinal diseases.

The aim of this study is to know the anatomical structure of *T. polium* L. and its chemical composition as well as its antibacterial activity.

METHODS

Plant material

Aerial parts (leaves, stems, and flowers) of *T. polium* were collected during flowering period at the end of May 2018 from North East of Algeria (Sétif) at an altitude of 800 m. Samples were identified at Laboratory of Natural Resource Valorisation, University of Setif 1.

Extraction and chemical composition of the essential oil

The aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil analysis was carried out using gas chromatograph (GC)-FID and GC/mass spectrometry (MS).

The essential oils were analyzed on a Hewlett Packard GC CPG/FID 7890, coupled to a gas chromatograph: CPG/MS 7890/5975C, equipped with a colon apolar: DB5 MS: The column used was a 40x m 0,18x mm, 0,18 µm film thickness, programming from 50°C for 5 min to 5°C/min until 300°C. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30), injector and detector temperature is 280°C with split 1/100. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the m/z range of 33,450.

The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library [3,4] and those described by Adams [5], as well as on comparison of their retention indices either with those of authentic compounds or with literature values [5].

Antibacterial activity

Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and four Gram-negative bacteria (*Pseudomonas aeruginosa ATCC27853, Escherichia coli ATCC 25922, Salmonella enterica* ssp. Arizona CIP 81, and *Shigella sonnei*) were used in this study. The non-reference strains used were isolated from human specimens at the Laboratory of Bacteriology of the University Hospital Center of Sétif.

The antibacterial activity of essential oil was evaluated using the agar diffusion test [6]. Mueller-Hinton agar was poured in Petri dishes, solidified, and surface dried before inoculation by bacterial inoculums (using selected strains for this study). Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution, and diluted essential oil (1:1, 1:2, 1:5, and 1:10 w: w of DMSO). DMSO was used as negative control. The antibiotic gentamicin was used as positive control.

Then, Pétri dishes were incubated at 37°C during 18–24 h aerobically. After incubation, inhibition zone diameters were measured and documented.

Preparation of sections for anatomical study

Young sections of the plant containing stems and leaves were selected to make cross sections by hand with sharp blade and then coloring them using double coloration method [7]. Light microscope was used to check up transverse sections.

RESULTS AND DISCUSSION

Extraction and composition analysis

The hydrodistillation of the essential oil of *T. polium* gave a viscous liquid with a yellowish color. The yield of the essential oil was 0.11%, whereas the analysis of the essential oils of *T. polium* by GC and GC/MS identified 27 components corresponding to 99.72% of the total oil (Table 1 and Fig. 1), the gas chromatogram of the oil on an HP-5 MS capillary column is shown in Fig. 2. The major compounds were β-pinene (30.61%), carvacrol (13.09%), α-pinene (10.40%), and 28.15 germacrene D (9.30%).

These values were different compared to those obtained by Lograda *et al*. [8], probably the reason for the difference is due to changing climatic conditions or collection conditions; however, results of Moghtader [9] and Othman *et al*. [10] were similar.

Results of the present study were different with those obtained by Mahmoudi and Nosratpour [11], Asgharipour and Shabankare [12], and Kurtoglu and Tgn [13]. In the essential oil from Western Algeria, the major compounds were germacrene D (25.81%) and bicyclogermacrene (13.00%) according to Belmekki *et al*. [14]. Guetat *et al*. [15] obtained 114 compounds where y-murolene (8.72%) was the major compound. It reported that essential oil of other species as *T. capitatum* L. has different chemical composition [16].

Table 1: Chemical composition for *Teucrium polium* L.

Pc.	Composés	KI	%
1	5.85 α-Pinène	931	10.40
2	6.26 Camphene	946	0.27
3	Sabinene	969	0.54
4	7.04 β-Pinene	977	30.61
5	7.43 Myrcene	984	1.65
6	8.53 p-Cymene	1021	1.82
7	8.69 Limonene	1026	3. 0.04
8	8.96 (Z)-β-Ocimene	1094	1.35
9	1 (7),5,8-o-Menthatriene	1125	0.87
10	13.02 Nopinone	1140	6.20
11	14.07 Pinocarvone	1165	1.60
12	14.78 Cymen-8-ol-meta	1189	0.60
13	15.56 Myrtenal	1201	5.76
14	19.71 Thymol	1281	1.20
15	20.14 Carvacrol	1302	13.09
16	21.77 δ Elemene	1337	0.47
17	25.05 β-bourbonnene	1392	0.77
18	25.19 β-Funebrene	1427	0.35
19	25.36 (E-) Caryophyllene	1429	0.52
20	28.15 Germacrene D	1493	9.30
21	28.66 Valencene	1499	0.32
22	28.83 Bicyclogermacrene	1505	2.27
23	29.09 α-Z-Bisabolene	1509	0.30
24	29.35 γ-Cadinene	1521	0.35
25	29.72 δ-Cadinene	1524	1.35
26	30.11 cis - Calamenene	1529	0.30
27	35.59 (9,10-Dehydro-isolongifolene)	1589	4.41
Σ			99.72

Antibacterial activity of *T. polium* essential oil

The results were expressed by measuring the diameter of inhibition of the different concentrations of *T. polium* essential oil in DMSO (v/v) against either Gram-positive and Gram-negative pathogenic bacteria in mm after 24 h of incubation at 37°C (Table 2).

The essential oil of *T. polium* showed weak antibacterial activity against *P. aeruginosa* ATCC27853, whereas this activity was significant

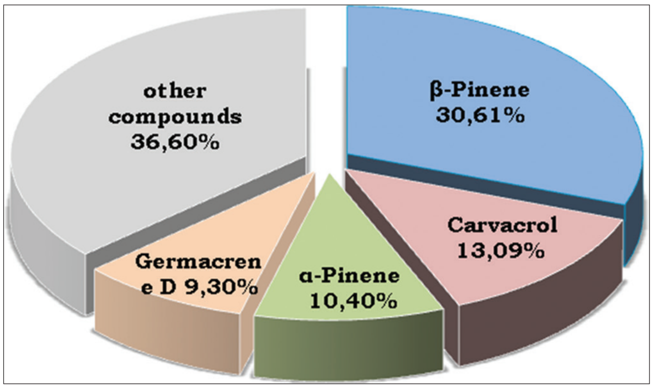

Fig. 1: Distribution of the major chemical compounds of the essential oil of *Teucrium polium*

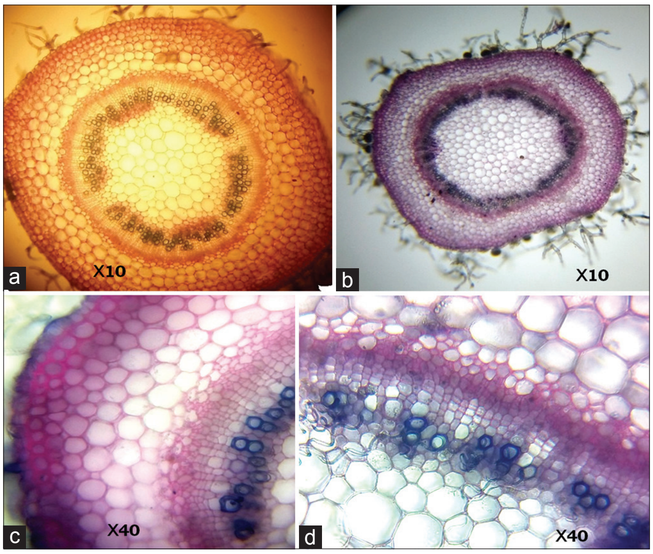

Fig. 2: Cross section in stem of *Teucrium polium*. (a) Cross section in stem showing différent tissue, (b) Cross section in stem showing covering and glandular trichomes, (c) Cross section in stem showing cortex, (d) Cross section in stem showing vascular stacks

Table 2: Antibacterial activity of *Teucrium polium* essential oils measured as diameter of inhibition

Bacterial strains	Concentration (w/w)					
	Gram	10%	20%	50%	100%	Control (GM)
Pseudomonas aeruginosa ATCC27853	Negative	-	09	09	10	24
Staphylococcus aureus ATCC 25923	Positive	12	18	23	38	25
Escherichia coli ATCC 25922	Negative	13	12	14	33	22
Shigella sonnei	Negative	11	11	16	20	13
Salmonella enterica ssp. Arizonae CIP 81	Negative	-	-	-	-	17

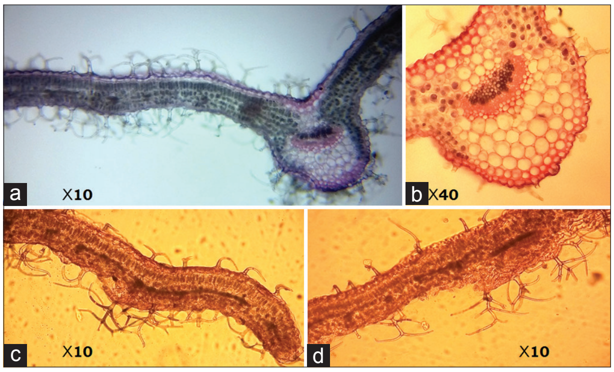

Fig. 3: Cross section in leaf of *Teucrium polium* (a) Cross section in leaf showing différent tissue, (b) Cross section in leaf showing larger vascular bundle, (c, d) protector and glandular trichomes

against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *S. sonnei*, while *S. enterica* ssp. Arizona CIP 81 was resistant.

Results of Belmekki *et al*. [14], Darabpour *et al*. [17], and Othman *et al*. [10] were weaker compared to the results of the present study, while our results were in good agreement with the findings of Lograda *et al*. [8], Thoppil et *al*. [18] for *E. coli* and different for *S. aureus.* In general, tested Gram-negative bacteria appear more resistant than Gram-positive ones [19,20]. In another study, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were resistant to essential oil of *T. polium* [21]. Previous studies have shown that *T. polium* extracts are active against microorganisms such as bacteria and fungi [22].

Anatomical study

Observations by light microscope showed the epidermis layer of stems and leaves contained two types of hairs glandular and covering trichomes (Fig. 2a, b; Fig. 3a, c, d; Fig. 4a, b, c).

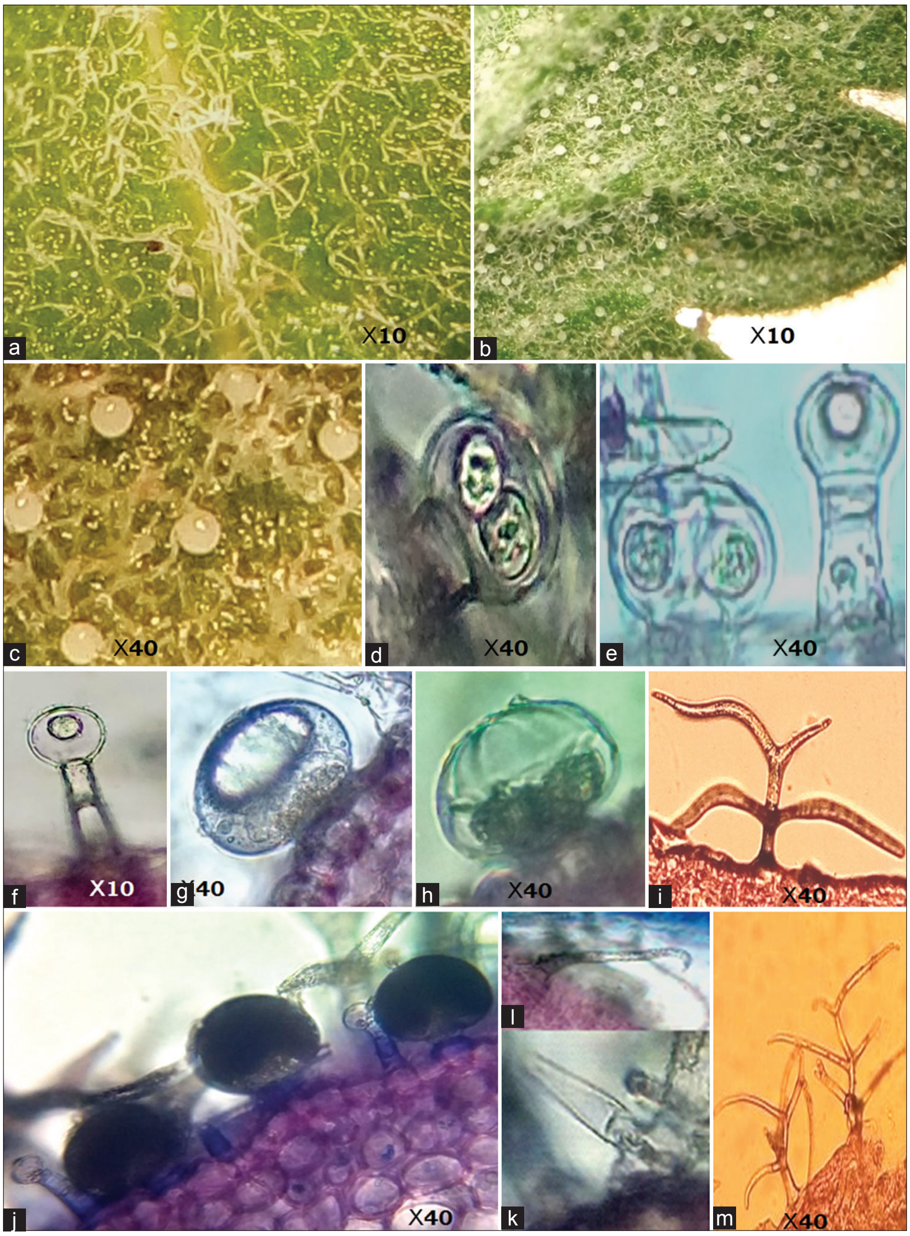

Fig. 4: Glandular and covering trichomes on young leaves and stems of *Teucrium polium.* (a) Upper surface of leaf; (b) underside of leaf; (c) glandular and protector trichomes of underside of leaf; (d, e, f) glandular trichomes unicellular and bicellular; (g, h) glandular trichomes (multicellular); (i) branched protector trichomes; (j) protector and glandular trichomes, (k) unbranched trichomes (special hair of *T. polium*); (l) unbranched trichomes; (m) branched trichomes

The anatomical study which was performed on young fresh stems showed the presence of pith, vascular stacks, cortex, and epidermis (Fig. 2d), whereas 7 apitates as tissue (supporting tissue) are existed in the cortex under the epidermis in a thin layer and irregularly a way (Fig. 2c).

The microscopic observation of *T. Polium* cross sections shows that they consist of a long, narrow blade with secondary veins. In the middle of the blade, there is a main vein that is more prominent to the underside of the leaf giving it a shape close to the letter V. The leaves are composed of the following tissues: Adaxial epidermis, palisade mesophyll, spongy mesophyll, and abaxial epidermis (Fig. 3a, b).

Three forms of glandular trichomes were observed, the first was unicellular (very small) with thin and along stalked with globule head (long 7 apitates glandular hair) (Fig. 4e, f, j); the second was big with short and large stalked with spheral head bicellular (Fig. 4d, e) or multicellular (peltate glandular hair) (Fig. 4g, h, j).

It was also observed three forms of protector trichomes as branched trichomes (Figs. 3c, d and 4i, m) and unbranched trichomes with peak sharp and the third ones were unbranched trichomes with a broad summit (Fig. 4k, l).

The protector and glandular trichomes were spread on the lower side of the leaves more than the upper side (Fig. 4a, b).

In the previous studies, the anatomical characteristics of *T. polium* were not very different [23,24]. Covering trichomes of *T. polium* from Iran were similar to those found in the present study [25]; but results of Dehshiri and Azadbakht, 2012 [26] were different. The present results correspond to those found by El Beyrouthy *et al*., 2008 [27] too.

According to the previous studies, different species of the same genus have different anatomical characterizations [28,29].

CONCLUSION

The variations in the chemical compositions and biological properties of different species across countries might be attributed to the varied agro-climatic (climatical, seasonal, and geographical) conditions of the regions, isolation regimes, and adaptive.

This study allowed us to know the biological properties of the species *T. polium* L. from Northeastern Algeria:

The major compounds were monoterpenes such as α-pinene, β-pinene, and carvacrol.

The essential oil of *T. polium* had significative antibacterial activity, especially against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

Two types of trichomes were detected, protector trichomes and secretor trichomes.

Three forms of protector trichomes: Branched trichomes, unbranched trichomes with peak sharp, and the third ones were unbranched trichomes with a broad summit.

Three forms of glandular trichomes were detected in the present anatomical study: Unicellular (very small) with thin and along stalked with globule head, the second was big with short and large stalked with spheral head bicellular, and the third was big with short and large stalked with spheral head multicellular.

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