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Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria

Nacéra Belmekki[1], Nassima Bendimerad[1]*, Chahrazed Bekhechi[1] and Xavier Fernandez[2]

[1]Université de Tlemcen, Laboratoire des Produits Naturels, Département de Biologie, nouveau pôle rocade II Tlemcen 13000, Algérie.
[2]Université de Nice Sophia-Antipolis, Laboratoire de Chimie des Molécules Bioactives et des Arômes, UMR CNRS 6001, Institut de Chimie de Nice, Parc Valrose 06108 Nice cedex 2, France.

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The chemical composition of a yellow essential oil isolated from aerial parts by steam-distillation of Algerian *Teucrium polium* L. was analyzed by gas chromatography/mass spectrometry (GC-MS). The oil was obtained with a yield of 0.21%. 27 components were identified. The major compounds were germacrene D (25.81%), bicyclogermacrene (13%), ß-pinene (11.69%) and carvacrol (8.93%). Furthermore, the essential oil was tested against five bacteria (three Gram-positive and two Gram-negative) and three fungi at different concentrations. Results showed that the oil exhibited moderate inhibitory effects on *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus*, with a minimum inhibitory concentrations of 3 to 5 µl/ml.

Key words: *Teucrium polium* L., Lamiaceae, essential oil, germacrene D, bicyclogermacrene, β-pinene, carvacrol, antimicrobial activity.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Blumenthal, 2000).

With the increase of bacterial resistance to antibiotics, there is considerable interest to investigate the anti-microbial effects of different extracts against a range of bacteria, to develop other classes of antimicrobial useful for the infection control or for the preservation of food. Therefore the use of essential oils is less damaging to the human health (Isman, 2000; Misra and Pavlovstathis, 1997) because they are generally few toxic and they do not have side effects.

On the other hand, foodborne diseases are still a major problem in the world, food spoilage caused by a variety of microorganisms has often been recognized as inconvenient and one of the most important concerns for the food industry. The contamination of raw and processed foods with microflora can take place at various stages from production to sale and distribution (Deak et al., 1996). Thus, the food industry at present uses chemical preservatives to prevent the growth of food spoiling microbes (Alzoreky and Nakahara, 2003).

Teucrium species have been used as medicinal plants for more than 2000 years and some of them are still used in folk medicine (Hassan et al., 1979).

The genus *Teucrium* (Lamiaceae family) is represented by more than 340 species of which 20 are found in Algeria (Quézel and Santa, 1962); one of them is *Teucrium polium* L. or Jaadah as it is known in west Algeria, also many Mediterranean *Teucrium* species were characterized (Cozzani et al., 2005; Muselli et al. 2009; Djabou et al., 2010, 2011, 2012a, b). *Teucrium polium* is a dwarf, pubescent, aromatic shrub possessing oval leaves with enrolled margins and dense heads of white

*Corresponding author. E-mail: ns.bendimerad@mail.univ-tlemcen.dz. Fax: 00 213-(0) 40911082.

flowers (Al-Eisawi, 1982). It is mainly Mediterranean and west Irano-Turanian and can be found in countries such as Iraq, Saudi Arabia and Egypt (Feinbrun-Dothan, 1978).This plant is well known for its antinociceptive (Abdollahi et al., 2003), antioxidant (Couladis et al., 2003), hypolipidemic (Rasekh et al., 2001), anti-inflammatory, anti-rheumatoid (Tariq et al., 1989), and hypoglycemic (Gharaibeh et al., 1988) properties.

The essential oil of *T. polium* possess an antiphytoviral activity (Bezić et al., 2011). *T. polium* can caused significant reduction in blood glucose concentration that could be due to enhancement of peripheral metabolism of glucose rather than an increase in insulin release. (Gharaibeh et al., 1988).

The methanolic extract of *T. polium* has been studied for its antioxidant activity. Results showed that this plant is a source of polyphenols and flavonoids, confirm their antioxidants activities and underline their potential either as natural preservatives or in pharmaceutical (Belmekki and Bendimerad, 2012).

Thus, the aim of the present work is to characterize the essential oil produced by this plant growing wild in Northwestern Algeria. Then, the antimicrobial effect of the essential oil isolated from aerial has been investigated.

MATERIALS AND METHODS

Aerial part of *T. polium* were collected at full flowering at Beni-snous in the province of Tlemcen (Northwestern Algeria) in April, 2007. Botanical identification of the plant was conducted by Mr Hassani Fayçal, "Laboratoire d'Ecologie et Gestion des Ecosystèmes", Abou Bekr Belkaid, University, Tlemcen (Algeria). A voucher specimen of the plant was deposited in the herbarium of this laboratory.

Isolation of the essential oil

The essential oil was isolated by steam-distillation from the dried aerial parts of *T. polium* during 3 h. The sample oil was dried over anhydrous sodium sulphate and stored at low temperature before analysis.

Gas chromatography/mass spectrometry (GC-MS)

GC-MS analyses were carried out using a using a Hewlett-Packard 5890/5971A system fitted with a HP1 column (50 m × 0.20 mm fused silica capillary column; film thickness, 0.5 µm). GC oven initial temperature was 60°C and was programmed to 220°C at a rate of 2°C/min and 220°C during 120 min under the following operational conditions: vector gas, He; injector and detector temperatures, 250°C; injected volume: 0.2 µl, splitless. Retention indices were determined with C_7 to C_{28} alkane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35 to 400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (Wiley, MassFinder 2.1 Library, Nist98) libraries and MS literature data (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998; Joulain et al., 2001) and confirmed by comparison of retention indices with published index data (ESO, 2000).

Antimicrobial activity

Microbial strains

The essential oil of *T. polium* was tested against a panel of microorganisms. Five bacteria (reference strains) including three Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus feacalis* ATCC 29212, *Bacillus cereus* ATCC 11778) and two Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and three fungi (*Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*) were used. The bacterial strains were supplied by Institute Pasteur, Algiers. The fungal strains were obtained from the Microbiology laboratory in Biology, Science Faculty of the Tlemcen University Algeria.

Antibacterial screening

Disc diffusion method

The disc diffusion method was employed for the determination of antibacterial activities of the essential oil in question (Lesueur et al., 2007). Paper discs (6 mm diameter; Sanofi Diagnostic Pasteur) were impregnated with 15 µl of the oil and 5µl of dimethyl sulfoxide and transferred onto the Mueller-Hinton agar present in Petri dishes, which had been surface spread with 0.1 ml of bacterial suspension adjusted to 10^7 CFU/ml for *S. aureus* and 10^6 CFU/ml for the others strains (Joffin and Leyral, 2001; Pessini et al., 2003; Careaga et al., 2003). The DMSO solvent was used as the negative control. Standard antibiotics amoxicillin-clavulanic acid (30 µg/disk), tetracyclin (30 µg/disk), trimethoprim-sulphamethoxazol (25 µg/disk) and cephalexin (30 µg/disk) were used as positive controls. After incubation at 37 ± 1°C during 24 h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimal inhibition concentration (MIC) values were determined for essential oil *T. polium* tested against bacterial strains. A 100 µl of the inoculum, initially adjusted to the density cited above, was spread onto 20 ml Mueller–Hinton agar supplemented with the oil at concentrations ranging from 2-6 µl/ml in Petri dishes, with each one its equivalent in DMSO. These serially cultures were then incubated at 37 ± 1°C for 24 h. The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. As control, DMSO was used. Tests were carried out in triplicate.

Antifungal screening

The tests were carried out by insemination, with mycelia fragments of 6 mm in diameter (3 at 5 days hold), in Petri dishes containing PDA (Potato dextrose agar) (Fandohan et al., 2004). The oil was tested at the different concentrations of 0.25; 0.5; 1.25; 2.5; 5 and 10 µl/ml. These concentrations were obtained by mixing 0.5, 10, 25, 50, 100 and 200 µl of essential oil with 20 ml of melted sterile PDA respectively.

A disc (6 mm diameter) of the fungal species was cut from 1 week old cultures on PDA plates and then the mycelia surface of the disc was placed upside down on the center of a dish. Then, the plates were incubated in the dark at 25 ± 2°C. The extension diameter (mm) of hyphae from centers to the sides of the dishes was measured after 3 to 5 days.

In addition, PDA plates treated with amphotericin B (200 µg/ml) were used as positive control. The Petri dishes containing 20 ml of PDA with no oil was inoculated to serve as the negative control. Fungal growth was evaluated by measuring the average colony

diameters after 3 to 5 days (Salamci et al., 2007). The percentage of growth inhibition was calculated using the following equation:

% Inhibition = (C –T)/ C x 100.

Where C is the average of four replicates of hyphal extension (mm) of controls and T is the average of four replicates of hyphal extension (mm) of plates treated with essential oil solutions (Salamci et al., 2007).

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential yellow oil isolated by steam distillation from aerial parts of *T. polium* was obtained in a yield of 0.21 % (w/w) based on the dry weight of the sample. Aburjai et al. (2006) and Kabouche et al. (2007) obtained a yield of 0.8 % (w/w), 1.7 % (w/w) respectively.

The components of the essential oil, the percentage and the retention indices are listed in Table 1, in relation to their elution order on the HP-1 column. Chromatographic profiles of the essential oil revealed 27 identified constituents, which represented 91.14 % of total GC/MS area. The *T. polium* essential oil chemical composition was dominated by hydrocarbon compounds which contained a high percentage of sesquiterpenes hydrocarbons (46.81%) with germacrene D (25.81%) and bicyclogermacrene (13%) as major components. ß-pinene (11.69%) is the main constituent of the monoterpenes hydrocarbons class, which represented 19.26%, while carvacrol (8.93%) and spathulenol (6.53%) were the major components of the oxygenated classes. Similar results of higher amounts hydrocarbon sesquiterpenes were obtained by Bezić et al. (2011) and Djabou et al. (2012b).

The results obtained were most similar for the oils of *T. polium* from Iran (Eikani et al., 1999), and Yugoslavia (Kovacevic et al., 2001) which were mainly represented with germacrene D (23.6 to 13.2% and 11.9%, respectively), while the major components of the Turkish *T. polium* species were β-pinene (18%), β-caryophyllene (18%) and α-pinene (12%) (Cakir et al., 1998).

The major component of the Jordanian *T. polium* essential oil was 8-cedren-13-ol (24.8%) (Aburjai et al. 2006**)** and the α-cadinol (46.8%), the 3β-hydroxy-α-muurolene (22.5%) (Kabouche et al., 2007) for the eastern Algerian plant.

Antibacterial activity of the essential oil

The *in vitro* bacteriostatic activity of the essential oil of *T. polium* and the inhibition zones formed by standard antibiotic discs are showed in Table 2.

Results showed that oil inhibited the growth of bacterial strains produced a zone diameter of inhibition from 06 to 16 mm, depended on susceptibility of the tested bacteria. However, the inhibition zones were lower than those of antibiotics, which showed wide inhibition zones at very low concentrations. As it can be seen in Table 2, *S. aureus* and *E. coli* were the most sensitive micro-organisms with the highest inhibition zone (16 mm) and lowest MIC value (3 µl/ml). On the other hand, it is seen from Table 2 that *P. aeruginosa* was resistant at this essential oil.

Antifungal activity of the essential oil

Table 3 shows the *in vitro* fungistatic activity of the essential oil of *T. polium*, the commercial antifungal amphotericin B and the percentage of growth inhibition.

The results of antifungal activity assays showed that the oils moderately reduced the growth of *A. flavus* (25.9% inhibition at 10 µl/ml of essential oil) (Table 3). As well only 10.53% of inhibition were observed against *F. oxysporum* at 10 µl/ml of essential oil. Conversely the oil was significantly not active against *R. stolonifer* and that even with 10 µl/ml of essential oil. Inhibitory effects of the oil on the growth of fungal strains were lower compared to amphotericin B.

The antimicrobial activity may be attributed to the moderate presence of oxygenated compounds carvacrol, thymol terpinen-4-ol, α-terpineol, which have broad spectrum antifungal activity (Guillen and Manzanos, 1998; Edris et al., 2003; Kordali et al., 2005a, b ; Shunying et al., 2005 ; Kordali et al., 2007). This activity is related also to their high content of the germacrene D which posses an antimicrobial activity (Ngassapa et al., 2003). The essential oils containing terpenes are also reported to possess antimicrobial activity (Dorman and Deans, 2000).

In addition, the components in lower amount may also contribute to antimicrobial activity of the essential oils, involving probably some type of synergism with other active compounds (Marino et al., 2001).

Conclusion

The results of this work show that the essential oil of *T. polium* possesses antimicrobial properties, which can be used as natural antimicrobial agents for human and infectious diseases and in food preservation.

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Table 1. Chemical composition of *T. polium* essential oil.

Compound	RI[a]	Area%
α-pinene	931	3.96
Camphene	951	0.15
β-pinene	975	11.69
Myrcene	988	0.88
o-cymene	1023	0.66
Limonene	1028	1.77
γ-terpinene	1057	0.15
Linalool	1099	0.14
Pinocarveol	1140	1.65
Unknown 1	1162	0.47
Borneol	1171	0,39
terpinen-4-ol	1179	0,12
α-terpineol	1195	1,86
Unknown 2	1207	0.34
Unknown 3	1243	0.39
Thymol	1290	1.29
Carvacrol	1298	8.93
Eucarvone	1337	0.52
Unknown4	1344	0.15
α-copaene	1372	0.15
β-Bourbonene	1380	0.65
β-caryophyllene	1416	0.38
Unknown 5	1435	0.34
α-humulene	1456	0.23
γ-muurolene	1472	0.80
Germacrene D	1478	25.81
β-cubebene	1488	0.76
Bicyclogermacrene	1492	13.00
α-muurolene	1495	0.72
δ-cadinene	1515	4.31
Unknown 6	1519	0.68
Unknown 7	1534	0.32
Spathulenol	1574	6.53
Unknown 8	1640	1.50
Citronnelle	1653	3.64
Unknown 9	1670	0.64
Unknown 10	1691	4.03
Total identified (%)		91.14
Non identified compounds (%)		8.86
Monoterpene hydrocarbons (%)		19.26
Oxygenated monoterpenes (%)		14.9
Sesquiterpenes hydrocarbons (%)		46.81
Oxygenated sesquiterpenes (%)		10.17

Compounds are listed in elution order from an HP1 column using homologous series of *n*-alkanes.

Table 2. Antibacterial activities of essential oil of *T. polium* and some standards antibiotics.

Bacterial strains	Inhibition Zone (mm)	MICs	Standards antibiotics				Negative control DMSO
			SXT	CL	TE	AMC	
			25	30	30	30	
Bacillus cereus ATCC 11778	15	5	06	06	20	06	06
Enterococcus faecalis ATCC 29212	15	5	20	30	22	16	06
Escherichia coli ATCC 25922	16	4	17	40	42	06	06
Pseudomonas aeruginosa ATCC 27853	09	_	06	06	06	06	06
Staphylococcus aureus ATCC 25923	16	3	16	23	15	14	06

MIC: minimal inhibitory concentration (µl/ml). **-** : not active. SXT: sulfamethoxazole-trimethoprime, CL: cefalexin, TE: tetracyclin, AMC: amoxicillin-clavulanic acid.

Table 3. Antifungal activities of essential oil of *T. polium* and of amphotericin B against the mycelial growth of fungi.

Fungal Strains	Essential oil													
	CN	CP	0.25		0.5		1.25		2		5		10	
	G	I	G	I	G	I	G	I	G	I	G	I	G	I
Aspergillus flavus	54	88	54	0	54	0	50	7.4	42	22.2	40	25.9	40	25.9
Fusarium oxysporum	76	88.1	76	0	76	0	76	0	71	6.58	68	10.53	68	10.53
Rhizopus stolonifer	90	68	90	0	90	0	90	0	90	0	90	0	90	0

CN: negative control, CP: positive control (Amphotericin B), 0.25 to 10.0: concentrations (µl/ml), G: growth [Colony diameter (mm)], I: inhibition [The percentage of growth inhibition (%)].

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