

Full Length Research Paper

Chemical composition and antioxidant and antimicrobial activities of the essential oil from *Teucrium polium geyrii* (Labiatae)

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Accepted 9 May, 2013

The composition of the essential oil from Algerian *Teucrium polium geyrii* (Labiatae) plant was analysed by gas chromatography–mass spectrometry (GC-MS); 85 constituents (87.72% of the total oil) were identified. The main compounds were (11.18%) dl-Limonene, (10.02%) δ -cadinene and (9.15%) trans β -caryophyllene. The antimicrobial activity of the essential oil was evaluated against *Staphylococcus aureus* ATCC 27923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Candida albicans* using disc diffusion method. The oil showed an efficient fungicidal activity against *C. albicans*. The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH') and ferric reducing antioxidant power (FRAP) assays. The oil of *T. polium geyrii* plant exhibited noticeable scavenging effects in DPPH free radical scavenging and FRAP assays.

Key words: *Teucrium polium geyrii*, essential oil, labiatae, antimicrobial activity.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Abdollahi et al., 2003). Many natural compounds extracted from plants have exhibited biological activities. Among these various sorts of natural compounds, essential oils obtained from aromatic plants are receiving special attention (Neffati et al., 2009). The *Teucrium polium geyrii* is a wild-growing flowering plant belonging to the family Labiatae and is found abundantly in south western Asia, Europe and north Africa. *Teucrium* species have been used as medicinal herbs for over 2000 years as diuretic, diaphoretic, tonic, antipyretic, antispasmodic, cholagogic, and many of them are used in folk medicine (Ozenda, 1983; Abdollahi et al., 2003). Anti-inflammatory,

anti-rheumatoid, antihypertensive, antioxidant, hypoglycemic and anorexic effects are other reported activities of *T. polium* (Suleiman et al., 1998; Abdollahi et al., 2003; Mazokopakis et al., 2004; Baluchnejadmojarad et al., 2005; Boulila et al., 2008; Sharififar et al., 2009).

Several researchers have evaluated *T. polium* grown in different geographic areas phytochemically. Most of these studies, based on the gas chromatography (GC) analysis of the extracts, revealed the presence of several volatile compounds varying in the major constituent(s) and their concentration depending on the geographic origin (Velasco-Negueruel and Perez-Alonso, 1990; Perez-Alonso et al., 1993; Kamel and Sandra, 1994; Kovacevic et al., 2001; Boulila et al., 2008). Literature

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survey did not reveal previous work on the chemical composition and biological activities of the essential oil of *T. polium geyrii* from Algeria. The present work was undertaken to study the chemical composition, antioxidant and antimicrobial activities of essential oil of *T. polium geyrii*.

MATERIALS AND METHODS

Plant

The aerial parts of *T. polium geyrii* were collected in November, 2007 from Tamanrasset (central Hoggar), in south Algeria and was dried at room temperature in the shade. The identification was performed according to the flora of Sahara (Ozenda, 1983) and the botanists of the National Agronomic Institute in El-Harrach, (Algeria).

Essential oil extraction and analysis

Plant material and isolation procedure

The essential oils were extracted by hydrodistillation of dried plant material (100 g of each sample in 500 ml of distilled water) using a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4°C prior to analysis. Yield based on dry weight of the sample was calculated. The composition of the oils was investigated by GC and gas chromatography–mass spectrometry (GC/MS). The analytical GC was carried out on an HP 6890-series II gas chromatograph (Agilent Technologies) equipped with flame ionization detectors (FID) under the following conditions: the fused silica capillary column, apolar HP-5 (60 m to 0.32 mm ID, film thickness of 0.15 µm). The oven temperature was held at 45°C for 8.5 min then programmed at rate of 2°C/min to 250°C and held isothermally for 14 min. The carrier gas was nitrogen at a flow rate of 0.5 ml/min; injector temperature: 240°C, detector: 250°C; the volume injected: 01 µl by split less method. The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/MS was performed in a Hewlett-Packard 5973 MSD System. An HP-5 MS capillary column (60 m to 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 0.7 ml/min. Oven temperature was programmed (45°C for 6 min then programmed at a rate of 2°C/min to 250°C) and held isothermal for 6.5 min. Injector port: 250°C, detector: 250°C. Volume injected: 01 µl by split less method; mass spectrometer: HP5973 recording at 70 eV; scan time: 2.83 s; mass range (m/z): 27 to 550 Th. Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the essential oil constituents was based on a comparison of their retention indices relative to (C₈-C₂₄) n-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their MS data compared to the National Institute of Standards and Technology (NIST) mass spectral library and published mass spectra (Adams, 2001; Velasco-Negueruel and Perez-Alonso, 1990)

Microbial strains

The essential oil was tested against four microorganisms.

Reference strains were: *Staphylococcus aureus* ATCC 27923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 25853; clinically isolated strain was *Candida albicans*.

Antimicrobial screening

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil. Briefly, a suspension of the test microorganism (2×10^8 Cfu/ml) was spread on the solid media plates. Filter paper discs (5 mm in diameter) were individually impregnated with 10 µl of the oil, then placed on the inoculated plates, and were incubated at 37°C for 24 h for the bacteria and at 30°C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters. A broth micro dilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (National Committee for Clinical Laboratory Standards (NCCLS), 2001). The essential oils were dissolved in dimethylsulphoxide (DMSO). All tests were performed in Mueller Hinton broth (MHB) and Sabouraud for the yeasts. Each test was performed in duplicate and repeated three times. The MBC is defined as the lowest concentration of the essential oil at which inoculated bacteria was totally killed. Levofloxacin and DMSO solution served as positive and negative controls, respectively.

Antioxidant activities

Measurement of free radical-scavenging activity (DPPH[•] assay)

The free radical-scavenging activity was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]) assay described by Blois (1958). Briefly, 6×10^{-6} M solution of DPPH[•] in methanol was prepared and 3 ml of this solution was added to 100 µl of sample solution of essential oil. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The ability to scavenge the DPPH[•] radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging effect (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Ascorbic acid was used as a standard and results were expressed in ascorbic acid equivalents (AEAC)/g dried extracts.

Measurement of ferric reducing antioxidant power (FRAP assay)

The reducing power was determined by using FRAP assay described by Benzie and Strains (1996) with some modifications. Briefly, 0.2 ml of sample compounds were mixed with 1.8 ml of the freshly prepared FRAP reagent which consisted of 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM FeCl₃ and 25 ml of 0.3 M acetate buffer (pH 3.6). The absorption of the reaction mixture was measured at 595 nm. Quercetin was used as a standard and results were expressed in Quercétine equivalent capacity (QEAC).

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil was obtained by hydrodistillation from

Table 1. Main component (%) of the essential oil from the aerial parts of *T. polium geyrii* plant.

| No. | Compound | RI | (%) |
|-----|-------------------------------------|------|-------|
| 01 | β -pinene | 953 | 0.25 |
| 02 | β -myrcene | 977 | 0.11 |
| 03 | α -Phellandrene | 987 | 0.08 |
| 04 | dl-Limonene | 1014 | 11.18 |
| 05 | γ -Terpinene | 1041 | 0.15 |
| 06 | α -terpinolene | 1070 | 0.08 |
| 07 | Dehydro-p-cymene | 1073 | 0.14 |
| 08 | Isopulegol | 1085 | 0.03 |
| 09 | β -thujone | 1095 | 0.20 |
| 10 | Octen-1-ol, acetate | 1098 | 1.08 |
| 11 | Campholenal | 1105 | 0.10 |
| 12 | 4-Terpineol | 1155 | 0.08 |
| 13 | Myrtenal | 1171 | 0.21 |
| 14 | Verbenone | 1185 | 0.03 |
| 15 | Cis-carveol | 1199 | 0.17 |
| 16 | (+)-Carvone | 1220 | 0.75 |
| 17 | Linalool acetate | 1235 | 0.05 |
| 18 | Chrysanthenyl acetate | 1237 | 0.35 |
| 19 | Ionone | 1250 | 0.24 |
| 20 | L-bornyl acetate | 1259 | 0.69 |
| 21 | 4-Terpinenyl acetate | 1274 | 0.18 |
| 22 | Carvacrol | 1290 | 0.04 |
| 23 | (-)-Myrtenyl acetate | 1298 | 0.40 |
| 24 | Elixene | 1306 | 0.12 |
| 25 | α -Cubebene | 1318 | 0.91 |
| 26 | α -Terpineol acetate | 1320 | 0.60 |
| 27 | Ledene | 1328 | 0.03 |
| 28 | (+)-Cycloisosativene | 1331 | 0.07 |
| 29 | α -Copaene | 1340 | 0.46 |
| 30 | β -bourbonene | 1347 | 0.13 |
| 31 | β -Damascenone | 1350 | 0.08 |
| 32 | β -Cubebene | 1353 | 0.46 |
| 33 | β -Elemene | 1356 | 0.40 |
| 34 | Isocaryophyllene | 1366 | 0.24 |
| 35 | α -Gurjunene | 1369 | 0.15 |
| 36 | Trans β -caryophyllene | 1381 | 9.15 |
| 37 | γ -Elemene | 1385 | 0.15 |
| 38 | Germacrene B | 1393 | 3.07 |
| 39 | α -Guaiene | 1396 | 0.53 |
| 40 | α -Humulene | 1410 | 3.20 |
| 41 | Aromadendrene | 1416 | 0.36 |
| 42 | Trans β -Farnesene | 1420 | 0.16 |
| 43 | Cis-Z- α -Bisabolene epoxide | 1431 | 1.02 |
| 44 | β -Cadinene | 1434 | 1.51 |
| 45 | Germacrene D | 1440 | 0.30 |
| 46 | β -Selinene | 1445 | 0.26 |
| 47 | β -Cubebene | 1452 | 1.29 |

Table 1. Contd.

| | | | |
|------------------|---------------------------|------|-------|
| 48 | Epizonarene | 1454 | 1.22 |
| 49 | Calarene | 1457 | 0.61 |
| 50 | α -Murolene | 1465 | 0.88 |
| 51 | β -Maliene | 1472 | 2.11 |
| 52 | γ -Cadinene | 1479 | 1.42 |
| 53 | Germacrene D-4-ol | 1483 | 0.41 |
| 54 | δ -Cadinene | 1495 | 10.02 |
| 55 | α -Guaiene | 1504 | 0.19 |
| 56 | Eudesma-3,7(11)-diene | 1506 | 0.30 |
| 57 | α -Calacorene | 1509 | 1.45 |
| 58 | Cis- α -Bisabolene | 1512 | 3.43 |
| 59 | γ -Gurjunene | 1519 | 1.28 |
| 60 | 9-Methoxycalamenene | 1525 | 0.52 |
| 61 | Caryophyllenyl alcohol | 1530 | 1.08 |
| 62 | Caryophyllene oxide | 1542 | 4.75 |
| 63 | Eremophilene | 1548 | 0.23 |
| 64 | Alloaromadendrene | 1552 | 0.61 |
| 65 | Globulol | 1556 | 0.34 |
| 66 | Torreyol | 1579 | 2.18 |
| 67 | Tau -Cadinol | 1591 | 4.30 |
| 68 | Aglaiene | 1595 | 0.80 |
| 69 | β -Eudesmol | 1597 | 0.56 |
| 70 | α -Cadinol | 1602 | 2.02 |
| 71 | Isoaromadendrene epoxide | 1605 | 0.35 |
| 72 | Bisabolol oxide A | 1608 | 1.13 |
| 73 | Cadalene | 1621 | 1.31 |
| 74 | Uroazulene | 1623 | 0.68 |
| 75 | Anymol | 1632 | 1.06 |
| 76 | Valencene | 1640 | 0.27 |
| 77 | Longipinocarvone | 1653 | 0.21 |
| 78 | Spathulenol | 1663 | 0.30 |
| 79 | Cubenol | 1718 | 0.18 |
| 80 | n-Octadecane | 1745 | 0.13 |
| 81 | Isopropyl myristate | 1772 | 0.03 |
| 82 | Nonadecane | 1844 | 0.04 |
| 83 | Heneicosane | 2253 | 0.03 |
| 84 | Dotriacontane | 2462 | 0.02 |
| 85 | Tetratetracontane | 2670 | 0.03 |
| Total identified | | | 87.72 |

RI = retention indices; % = percentage calculated by GC-FID on non-polar HP-5 capillary column.

the aerial parts of *T. polium geyrii* with a yield of 0.1% (v/w) on a dry weight basis. 85 constituents, representing 87.72% of the oil, were identified. Qualitative and quantitative analytical results are shown in Table 1. dl-Limonene (11.18%) was the main compound of the essential oil, followed by δ -Cadinene (10.02%) and the trans β -caryophyllene (9.15%). Among other compounds

Table 2. Antimicrobial activity of the essential oil from the *Teucrium polium geyrii* plant.

| Parameter | Essential oil | | | Levofloxacin | | |
|--|---------------|------------------|------------------|-----------------|------------------|------------------|
| | DD | MIC ^a | MBC ^a | DD ^b | MIC ^c | MBC ^c |
| <i>Staphylococcus aureus</i> ATCC 27923 | 12 | 4.90 | 4.90 | 34 | 0.30 | 0.30 |
| <i>Escherichia coli</i> ATCC 25922 | 10 | 12.25 | 19.60 | 34 | 0.30 | 0.61 |
| <i>Pseudomonas aeruginosa</i> ATCC 25853 | 09 | 12.25 | 12.25 | 20 | 0.15 | 0.30 |
| <i>Candida albicans</i> | 14 | 2.45 | 2.45 | NT | NT | NT |

DD, diameter of zone of inhibition (mm) including disc diameter of 5 mm. NT, not tested. ^aValues given as mg/ml. ^bTested at a concentration of 5 µg/disc. ^cValues given as µg/ml.

were considerable amounts of caryophyllene oxide (4.75%), tau-cadinol (4.30%), cis- α -bisabolene (3.43%) and α -humulene (3.20%). The oil was characterized by a higher content of sesquiterpenes. However, literature review showed variation between chemical compositions depending on the geographic origin (Kovacevic et al., 2001; Boulila et al., 2008; Bakkali et al. 2008). For example, myrcene (15.3%), germacrene D (9.0%), α -pinene (6.6%) and α -cadinol (5.1%) were the major components of the Tunisian *Teucrium polium* L. essential oil (Boulila et al., 2008), the major components of *T. polium* from Iran were α -pinene (12.52%), linalool (10.63%), caryophyllene oxide (9.69%), β -pinene (7.09%) and β -caryophyllene (6.98%) (Moghtader, 2009), but the major compounds of the Northwestern Algerian *T. polium* plant were germacrene D (25.81%), bicyclogermacrene (13%), β -pinene (11.69%) and carvacrol (8.93%) (Belmekki et al., 2013). The major component of the Jordanian *T. polium* essential oil was 8-cedren-13-ol (24.8%) (Aburjai et al., 2006). Generally, these major components determine the biological properties of the essential oils (Bakkali et al., 2008).

Antimicrobial activity

The antimicrobial activity of the essential oil was evaluated in *S. aureus* ATCC 27923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 25853 and *C. albicans*, using disc diffusion and broth micro dilution methods. The disc diameters of zone of inhibition (DD), MIC and MBC of the essential oil for the microorganisms tested are shown in Table 2. The data obtained from the disc diffusion method indicated that *C. albicans* was the most sensitive microorganism tested, with the strongest inhibition zone (14 mm), followed by *S. aureus* ATCC 27923 and *E. coli* ATCC 25922 with strong inhibition zones (10 to 12 mm). *P. aeruginosa* ATCC 27853 exhibited a weak inhibition zone (09 mm) but the inhibition zones were lower than those of antibiotics, which showed wide inhibition zones at very low concentrations.

The results of the MIC determination indicated the oil

inhibited all microorganisms tested. *S. aureus* ATCC 25923 and *C. albicans* had the lowest MIC (2.45 to 4.9 mg/ml). *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 had the highest MIC (12.25 mg/ml). As shown in Table 2, the MBC and MIC results varied for the four micro-organisms tested. The lowest MBC was 2.45 mg/ml for *C. albicans* and *E. coli* ATCC 25922 had a high MBC (19.60 mg/ml) detected. The activity of the oil is expected to be related to the respective composition of plant volatile oils. D-Limonene, δ -cadinene and the β -caryophyllene have been shown to have antimicrobial properties (Bakkali et al., 2008; 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 (Belmekki et al., 2013).

Antioxidant activities

Several assays have been done for the measurement of antioxidant activity of plant extracts including DPPH[•] and FRAP assays. DPPH[•] assay provided information on the reactivity of the compounds with a stable free radical. Because of the odd electron, DPPH[•] shows a strong absorption band at 517 nm in visible spectrophotometry (deep violet color). As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Rice-Evans et al., 1996).

The *Teucrium* essential oil that was investigated in the present study exhibited significant activities towards scavenging DPPH[•] radicals, with 79.02 ± 0.00 mg ascorbic acid equivalents (AEAC)/g dried extracts. FRAP method was used to evaluate the reducing potential of *Teucrium* essential oil. In this method, the reduction of ferric-tripyridyltriazine complex to its ferrous colored form is evaluated in the presence of antioxidants (Benzie and Strains, 1996). Using this assay, the essential oil from *Teucrium* exhibited a high reducing potential with 755.44 ± 0.48 mg Quercetine equivalents (QE)/g dried extracts.

Many studies have shown a significant antioxidant effect of different extracts from *T. polium* plant (Ljubuncic et al., 2006; Ardestani and Yazdanparast, 2007; Sharififar et al., 2009). Additional studies are needed to characterize the bioactive compounds responsible for the observed activities.

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