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ORIGINAL PAPER

# Antimicrobial Activity of Essential Oils Isolated from *Phlomis crinita* Cav. ssp. *mauritanica* Munby

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**Abstract** The essential oil extracted from the leaves and flowers of *Phlomis crinita* Cav. ssp. *mauritanica* Munby were obtained by steam distillation and analyzed by gas chromatography coupled with mass spectrometry. The major constituents of flower oil were $\beta$-caryophyllene (58.1%) and germacrene D (35.1%). This oil inhibited the growth of *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhimurium* with minimal inhibition concentrations (MIC) varying between 39 and 625 μg/ml. The essential oil obtained from the leaves was mainly composed of *trans*-caryophyllene (40.8%) and germacrene D (39.1%) and exhibited an antimicrobial profile against the same strains mentioned above with MIC between 156 μg/ml and 2.5 mg/ml.

**Keywords** *Phlomis crinita* Cav. ssp. *mauritanica* Munby · Lamiaceae · Essential oil composition · Antibacterial activity · $\beta$-caryophyllene · Germacrene D

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## Introduction

The genus *Phlomis* L. (family Lamiaceae) comprises 12 species, which are naturalized in Europe, Asia and North Africa [1]. Some species of *Phlomis* are used in folk medicine as a stimulant, tonic, and for wound healing [2]. Many studies have shown various activities, such as anti-inflammatory, immunosuppressive [3], free radical scavenging [3], antimicrobial [2, 4], antiulcerogenic [5] and antimutagenic activities [3]. The *Phlomis* genus or their essential oils are used in the food and drug industries [6]. Essential oils are used as flavoring for foods and as a fragrance in the perfume and cosmetic industry. They have been proposed as natural preservative agents for cosmetic preparations because of their antimicrobial activities [7]. In view of increasing use of essential oils in the food, cosmetic and pharmaceutical industries, it is important to examine the oils from indigenous plants for antimicrobial activities. *P. crinita* Cav. ssp. *mauritanica* Munby (*P. mauritanica*) is a shrub with flowers having an intensely golden yellow corolla and is used in Tunisian folk medicine as a wound healing drug [8]. Although, other *Phlomis* species have been studied, this *Phlomis* species has not been extensively studied. Consequently, the aim of this study was to analyze the chemical composition and antibacterial activities of the essential oils extracted from *P. mauritanica*.

## Materials and Methods

The leaves and flowers of *P. crinita* Cav. ssp. *mauritanica* Munby were collected in the center of Tunisia (Monastir, the locality of Jammel), in June 2003. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy in Monastir, Tunisia.

### Isolation Procedure

The essential oils were isolated from 400 g of fresh flowers and leaves by hydrodistillation in a Clavenger-type apparatus for 2 h. The distillates were then kept in bottles covered with aluminium foil at 4 °C to prevent oxidation by light.

### Gas Chromatography–Mass Spectrometry

The oils were analyzed by GC and GC/MS techniques. For GC analysis, a Hewlett–Packard-5890-II (Global Medical Instrumentation, Inc, Ramsey, MN, USA) gas chromatograph, equipped with a flame-ionization detector (FID), coupled to an electronic integrator was used. Quantitative data were obtained by electronic integration of the FID-area data, without response factor correction. The gas chromatograph was fitted with a fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 ml/min; split ratio of 50:1; injection volume 10 μl; injection temperature, 240 °C; GC oven temperature, held for 1 min at 50 °C, then programmed to 280 °C at a rate of 9 °C/min; the final temperature of 280 °C was held for 5 min. GC/MS analyses were performed on a Hewlett–Packard-5890 gas chromatograph (controlled with the HP Chemstation software), equipped with a 5972 mass-selective detector. The mass spectrometer operated in EI mode at 70 eV, using a scanning speed of 1.5 s over the range 40–300 amu, and an ion source temperature of 180 °C, was used to determine the mass of the unknown compounds.

### Components Identification

The components of the essential oil were identified by comparison of their EI mass spectra to those of *Wiley-275* K.L GC-MS computer Library, or with authentic compounds, and confirmed by comparison of their Kováts retention index with those of authentic compounds or with the corresponding data published in the literature.

### Antimicrobial Assay

The antimicrobial activity of *P. mauritanica* extracts was tested on the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, and the Gram-negative bacteria *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, and *Salmonella typhimurium* NRRLB 4420, using the micro-dilution method [9]. An essential oil emulsion in 1‰ agar [10] (Sigma-Aldrich, CO, St Louis, USA) was obtained by sonication of the mixture with a Vibracell 72434 (Bioblock, Illkirch, France) sonicator for 10 s. Overnight-grown of the microbial suspension was standardized to ca. $10^5$ cells/ml. In each well of a 96-well microtiter-plate, a 100-μl sample of the microbial suspension was added to 100 μl of the tested oil dilution (added to final concentrations of 10 and 2.5 mg/ml, 625, 156, 39 and 9 μg/ml). Each essential oil concentration was tested in triplicate. The last row, containing only agar and microbial suspension, served as a growth control. The plate was incubated at 37 °C for 24 h, and the minimal inhibition concentration (MIC) was determined, with the help of a microplate reader, as the lowest concentration of the compound whose UV/VIS absorbance at 570 nm was comparable with that of negative–control wells. The MIC value was defined as the lowest concentration of the test sample resulting in complete inhibition of visible growth in the broth medium (Pronadisa, Madrid, Spain), in a triplicate assay. The standard antibiotic ampicillin was used as positive control to check the sensitivity of the test organism as described by Ben-Sghaier et al. [11].

## Results and Discussion

The compositions of the essential oils of *P. mauritanica* are presented in Table 1. Both essential oils extracted from flowers and leaves were light yellow, with a distinct sharp odor, with the total yield of 0.1 and 0.07%, respectively (V/W). Eight components were identified from the oil of the leaves, representing 84.7% of the total oil. The major constituents were *trans*-caryophyllene (40.8%) and germacrene D (39.1%). Fourteen components were identified in the essential oil of flowers making up 99% of total composition. $\beta$-Caryophyllene (58.2%) and germacrene D (35.1%) were identified as the main components. Thus, the composition of two essential oils was different (Table 1). The oils were qualitative and quantitative different with dominance of sesquiterpenes that represent 98.7 and 81.4%, respectively in flower and leaf essential oils; however, monoterpenes represent only 0.3% of the essential oil of the flowers.

Compositional variations can be observed in oils from different organs of the same species [1], with the location of the collection (sun, shadow, geographic location) [4], and different species in the same genus [1–4, 12–14]. Despite the difference in essential oils compositions

**Table 1** The chemical constituents of the essential oils of *P. mauritanica* leaves and flowers

| Component | KI apolar | Peak area (%) | |
|---|---|---|---|
| | | Leaves | Flowers |
| Monoterpene hydrocarbons | | – | 0.29 |
| α-Pinene | 938 | – | 0.29 |
| 1-Limoneme | 1031 | – | t |
| Sesquiterpenes | | 81.36 | 98.73 |
| δ-3-Carene | 1015 | 0.10 | – |
| *trans*-β-Ocimene | 1047 | – | 0.07 |
| α-Yalangene | 1369 | t | – |
| α-Copaene | 1387 | 0.36 | 0.18 |
| *trans*-caryophyllene | 1420 | 40.82 | 0.08 |
| β-Caryophyllene | 1434 | – | 58.15 |
| Aromadendrene | 1439 | – | 0.13 |
| Farnesene | 1445 | t | – |
| Allo-aromadendrene | 1467 | – | 1.61 |
| α-Humulene | 1469 | – | 0.67 |
| β-Selinene | 1484 | t | – |
| Germacrene-D | 1494 | 39.09 | 35.11 |
| β-Cadinene | 1536 | 0.99 | 1.95 |
| α-Calocorene | 1538 | – | t |
| Caryophyllene oxide | 1576 | – | 0.78 |
| Alkanes | | – | t |
| 6, 10, 14-Trimethyl 2-penta-decanone | 1480 | – | t |
| Total | | 81.36 | 99.02 |

– Absent, *t* trace

between species in the same genus, it is notable that every genus is distinguished by a characteristic odor due to a dominance of some compounds. Essential oils of *Phlomis* species are dominant in germacrene D and β-caryophyllene. For example, germacrene D is one of the main components of *P. chorassanica* Bunge (51.5%) [3], *P. herba venti* L. (33.9%) [1], *P. olivieri* Benth (28.1%) [13], *P. percica* (32.5%) [3], *P. fruticosa* (21.4 %) [4, 10], *P. ferruginea* (8.9 %) [15], *P. nissolii* (33.9%) [16], *P. cancellata* Bunge (25.6%) [17], *P. bruguieri* Desf. (23.6%) [18], and *P. cretica* C. Presl (34%) [10]. Whereas, β-caryophyllene is present in the essential oils of *P. chorassanica* (25%) [3], *P. fruticosa* (8.7%) [4, 10], *P. olivieri* Benth (16.1%) [13], *P. persica* Boiss (5.1%) [3], *P. samia* (5.3%) [10], *P. bruguieri* Desf. (6.7%) [18], and *P. ferruginea* (15.6%) [16]. The similarity, in essential oil composition in the same genus can be attributed to their common genotype. However, differences can be attributed to gene expression that is activated differently in organs, in response to environmental conditions.

Both oils did not inhibit *Escherichia coli* and *Salmonella enteritidis* proliferation, whereas they exhibited an antibacterial effect against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Salmonella typhimurium* (Table 2). The flower oil had an antibacterial action with MIC values varying from 39 to 625 μg/ml while the oil from leaves had MIC values varying from 156 μg/ml to 2.5 mg/ml.

The observed antibacterial effect against Gram-negative bacteria supports previous reports [4, 12]. The investigation of the antibacterial activity of *P. mauritanica* leaf and flower essential oils demonstrated growth inhibitory effect on the same bacterial strain. It have been reported that *trans*- and β-caryophyllene have an antibacterial effect [19, 20]. In addition to its antibacterial effect, *trans*-caryophyllene has also been reported to exhibit cytotoxic activity against solid tumor cell lines [19]. In addition, germacrene-D is known to have a strong effect on insect behavior [21] and has significant antibacterial and antifungal activities [22]. Consequently, we hypothesize that the antibacterial effect of the tested essential oils may be ascribed in part, but not exclusively, to their important content of *trans*- and β-caryophyllene and germacrene-D. Essential oils always represent a complex mixture of different chemical components, thus it is very difficult to reduce the antibacterial effect of the total oil to a few active principles. In general, it cannot be excluded that, in addition to the main compounds, other minor compounds contribute to the oil's activity. This difference can possibly be explained by the different methodologies (the most frequently used methods included paper disc agar diffusion, agar well diffusion, and incorporation of essential oil in agar media prior to inoculation) used to assess the antibacterial activity. In addition, a number of factors hamper the evaluation of antimicrobial activity of essential oils, namely, their volatility at room temperature, their water insolubility, and their complexity [7].

Compared to the positive control ampicillin, the essential oil was 60–250 orders of magnitude less active. Generally, the use of pure components such as antibiotics gives a more potent antimicrobial activity when compared to a complex mixture of components such as essential oils. Indeed, we believe that qualitative and quantitative differences detected in the composition of leaf and flower essential oils, may explain their different inhibitory effects on bacterial strain growth. The efficiency of essential oils differed and depended on their concentrations, the complexity of their compositions and the tested bacterial strain.

*Phlomis mauritanica* essential oils were better inhibitors of Gram-positive bacteria proliferation than of Gram-negative bacteria. In general, Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria [10, 23, 24]. The weak antibacterial activity against Gram-negative bacteria was ascribed to the

**Table 2** Antibacterial activity of *P. mauritanica* essential oils

| Bacterial strain (Gram) | Minimal inhibitory concentration (μg/ml) | | |
|---|---|---|---|
| | Flower essential oil | Leaf essential oil | Ampicillin |
| *Staphylococcus aureus* (+) | 39 | 156 | 1.5 |
| *Enterococcus faecalis* (+) | 625 | 156 | 2.5 |
| *Escherichia coli* (−) | >10[a] | >10[a] | 6 |
| *Salmonella enteritidis* (−) | >10[a] | >10[a] | 1.9 |
| *Salmonella typhimurium* (−) | 625 | 2.5[a] | 3.9 |

+, −: Gram-positive and negative bacteria, respectively

[a] Value in mg/ml

presence of an outer membrane, which possessed hydrophilic polysaccharide chains as a barrier to hydrophobic essential oils and phospholipids, and more pores in cell envelope [24]. While, the cell membrane of Gram-positive bacteria contains mucopolysaccharides, proteins, and a lesser amount of phospholipids. So, the permeability, entrance and reaction of the most antibiotics and/or antimicrobial agents through cell envelope (the outer and cytoplasmic membrane) are highly efficient for Gram-positive bacteria depending on reaction with the protein layer (mucopolysaccharides or peptidoglycans) [25]. Accordingly the high degree of susceptibility of *Salmonella typhimurium* was unexpected. Parekh et al. [26] observed that *Salmonella typhimurium* was one of the most resistant Enterobacteriaceae to plant extracts. Thus, we believe that *P. crinita* Cav. ssp. *mauritanica* Munby essential oil would be of interest as far as it was active toward this strain. To our knowledge, this is the first qualitative and quantitative phytochemical analysis of *P. crinita* Cav. ssp. *mauritanica* Mumby.

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## References

1. Katayoun MS, Mohammed A, Afsaneh G (2004) The essential oils composition of *Phlomis herba-venti* L. leaves and flowers of Iranian origin. Flav Fragr J 19:29–31
2. Couladis M, Tanimauidis A, Tazakou O, Chinou IB, Harvaia C (2000) Essential oil of *Phlomis lanata* growing in Greece: chemical composition and antimicrobial activity. Planta Med 66:670–672
3. Parisa S, Gholamerza A, Abbas S (2004) Composition of the essential oil of *Phlomis persica* Boiss and *Phlomis chorassanica* Bunge from Iran. Flav Fragr J 19:538–540
4. Risti MD, Duletic-Lausevic S, Knezevic-Vukcevic J, Marin PD, Simic D, Vukojevic J, Janackovic P, Vajs V (2000) Antimicrobial activity of essential oil and ethanol extract of *Phlomis fruticosa* L. (Lamiaceae). Phytother Res 14:267–271
5. Gürbüz I, Üstün O, Yesilada E, Sezik E, Kutsal O (2003) Anti-ulcerogenic activity of some plants used as remedy in Turkey. J Ethnopharmacol 88:93–97
6. Zargari A (1960) Medicinal plants, vol 4. Clarendon, Oxford, p 449
7. Mangena T, Muyima NYO (1999) Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. Lett Appl Microbiol 28:291–296
8. Pottier-Alapetite G (1976) Flore de la Tunisie, Imprimerie officielle de la république. Tunisienne, Tunis, 789 p
9. Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64:711–713
10. Kilani S, Abdelwahed A, Ben Ammar R, Hayder N, Ghedira K, Chraief I, Hammami M, Chekir-Ghedira K (2005) Chemical composition, antibacterial and antimutagenic activities of essential oil from (Tunisian) *Cyperus rotundus*. J Essent Oil Res 17(6):695–700
11. Ben-Sghaier M, Chraief I, Skandrani I, Bouhelel I, Boubaker J, Kilani S, Neffati A, Mahmoud A, Hammami M, Chekir-Ghedira L, Ghedira K (2007) Chemical composition and antimicrobial activity of the essential oil of *Teucrium ramosissimum* (Lamiaceae). Chem Biodivers 4(7):1480–1486
12. Aligiannis N, Kalpoutzakis E, Kyriakopulou I, Mitaku S, Chinou IB (2004) Essential oils of *Phlomis* species growing in Greece: chemical composition and antimicrobial activity. Flav Fragr J 19:320–324
13. Mirza M, Baher NZ (2003) Volatile constituents of *Phlomis olivieri* Benth from Iran. Flav Fragr J 18:131–132
14. Sezgin C, Suleyman G, Guido F, Pier LC, Ivano M (2005) Essential oils of *Phlomis leucophracta*, *Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora* from Turkey. Biochem Syst Ecol 33:617–623
15. Formisano C, Snatore F, Bruno M, Bellone G (2006) Chemical composition and antimicrobial activity of the essential oil of *Phlomis ferruginea* Ten. (Lamiaceae) growing wild in Southern Italy. Flav Fragr J 21(5):848–851
16. Kirimer N, Baser KHC, Kürkcüoglu M (2006) Composition of the essential oil of *Phlomis nissolii* L. J Essent Oil Res 18(6):600–601
17. Morteza-Semnani K, Moshiri K, Akbarzadeh M (2006) The essential oil composition of *Phlomis cancellata* Bunge. J Essent Oil Res 18(6):672–673
18. Morteza-Semnani K, Saeedi M (2005) The essential oil composition of *Phlomis bruguieri* Desf. from Iran. Flav Fragr J 20(3):344–346
19. Hector RJJ, Fernando B, Adolfina RK, Oliva MM, Mirta SD, Victorio ST, Julio AZ (2002) Chemical constituents and antimicrobial activity of the essential oil of *Lantana xenica*. Planta Med 68:762–764
20. Deba F, Xuan TD, Yasuda M, Tawata S (2007) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var *radiata*. Food control 19(4):346–352
21. Picaud S, Olsson ME, Brodelius M, Brodelius PE (2006) Cloning, expression, purification and characterization of recombinant (+)-germacrene D synthase from *Zingiber officinale*. Arch Biochem Biophys 452:17–28
22. Sahin F, Güllüc M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H (2004) Biological activities of the essential

oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the eastern Anatolia region of Turkey. Food Control 15:549–557
23. Outtara B, Simard RE, Holley RA, Piette GJD, Bégin A (1997) Antimicrobial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol 37:155–162
24. Shigeharu I, Toshio T, Hideyo Y (2001) Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother 47:565–573
25. Ihsan Edan AS, Sundus SB, Bassam YK, Yass KA (2007) Efficiency of antibacterial agents extracted from *Thymus vulgaris* L. (Lamiaceae). Int J Nutr Wellness 4(1). http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijnw/vol4n1/thymus.xml
26. Parekh J, Chanda S (2007) In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr J Microbiol Res 1(6):92–99