



Chemical composition and antioxidant, antibacterial, allelopathic and insecticidal activities of essential oil of *Thymus algeriensis* Boiss. et Reut.



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ABSTRACT

The present study describes chemical composition, antioxidant, antibacterial, allelopathic and insecticidal properties of essential oil of *Thymus algeriensis* (Lamiaceae), a North African endemic species. The essential oil from all organs of *T. algeriensis* were extracted by hydrodistillation and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). A high variation among organs for the majority of compounds was shown. Caryophyllene oxide (21.1%) and veridiflorol (17.2%) were the main constituents in roots, while elemol (10.2%) and caryophyllene oxide (17.8%) were identified as the main constituents for stems. Leaves were characterized by high levels of α -pinene (19.5%), 1,8-cineole (11.6%) and camphor (10.4%). Based on the determination of the diameter of inhibition and the determination of the minimum inhibitory concentration, a moderate to high antibacterial activity according to oils was revealed against five bacteria strains. However, oils from Dj. Jdidi locality showed higher bactericidal effect (MICs = 1–3.25 μ l/ml) than those from other samples. The level of antioxidant activity estimated by DPPH (IC₅₀ = 4.31–9.23 mg/ml) and ABTS (11.69–28.23 μ gTE/mg DW) test was moderate. The obtained results also showed that the leaf oils inhibited the shoot and root growth of *Medicago sativa* and *Triticum aestivum* seedlings. Thus, the *T. algeriensis* oils may be used as a natural herbicide. Furthermore, these oils were found to possess strong insecticidal activity (LC₅₀ = 44.25–112.75 μ l/l air) against cotton leafworm larvae. Thus, the essential oil of this plant could be used as a potential source of natural antioxidants and bioactive molecules in pharmaceutical as well as in food industries.

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1. Introduction

Thymus algeriensis Boiss. et Reut. (Synonym *Thymus hirtus* Willd. subsp. *algeriensis* Boiss. et Reut.) is the most widespread North African species. It's one of the four *Thymus* species that grows wild in different regions of Tunisia. It is a short lived and gynodioecious shrub (Ben El Hadj Ali et al., 2010; Morales, 1996). It reproduces by seeds and can reach 20–50 cm in height. The leaves are opposite and linear/lanceolate (6–12 mm). The flowers, with ovate bracts and pink purplish or whitish purple corolla, are small (5–7 mm). Flowering takes place between April and June (Ben El Hadj Ali et al.,

2010). In Tunisia, the species grows wildly on poor fertile calcareous soils and occurs in scattered and small populations in different bioclimatic zones extending from the sub-humid to the lower arid, in five isolated areas: the Northwestern part of the country, the Cap Bon, the Tunisian Dorsal mountain, the Sahel and the arid areas (Ben El Hadj Ali et al., 2010).

T. algeriensis is an herbaceous fragrant plant and aromatic spice largely used, fresh or dried, as a culinary herb (Le Floc'h and Boulous, 2008). Furthermore, this plant is also widely used in folk medicine and known for its antiseptic, antispasmodic, antibacterial and antifungal properties (Hazzit et al., 2009). Essential oils from aerial parts were used as flavour ingredients in a wide variety of food, beverage and confectionery products, as well as in perfumery (Hazzit et al., 2009).

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Studies on the chemical composition and biological activity of *T. algeriensis* oils from leaves remains have been performed (Dob et al., 2006; Hazzit et al., 2009), while the variation in the chemical composition (individual compounds and/or compound classes) from different plant parts were assessed for the first time. Then, the phytotoxic and insecticidal activities of this species from Tunisia have not been reported before.

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Thus, there are two basic categories of antioxidants namely synthetic and natural ones. A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively used in food industry, although their use has begun to be questioned because of their toxicity (Scalbert et al., 2005). Therefore, the development and utilization of more effective antioxidants of natural origin, obtained from botanical sources especially herbal plants, are desired (Scalbert et al., 2005). In recent decades, the essential oils of plants has been of great interest as sources of natural products and biologically active compounds. Furthermore, the antioxidant property of essential oils also has been verified in vitro to promote their use as natural food additives (Ruberto and Baratta, 2000).

The aim of this work is (i) to assess the essential oil of roots, stems and leaves of Tunisian *T. algeriensis* and compare their composition between samples, and (ii) to investigate the antibacterial, antioxidant, allelopathic and insecticidal activities of leaf oils. Therefore, these results can be used for further application of this plant in food and pharmaceutical industries as natural valuable products.

2. Materials and methods

2.1. Plant material

T. algeriensis was gathered at the vegetative stage in three different localities from the Eastern region of Tunisia belonging to different bioclimates according to Emberger's pluviothermic coefficient Q2 (Emberger, 1966): Korbous from the sub-humid (Coordinates; Latitude: 36°50' N; Longitude: 10°35'E), Jdidi Jebel Montain located in the upper semi-arid bioclimate (Coordinates; Latitude: 36°25' N; Longitude: 10°28'E) and Hammem Sousse from the lower semi-arid (Coordinates; Latitude: 35°11' N; Longitude: 10°27'E). From each locality, samples were collected and transported to the laboratory for the essential oil analysis. The fresh plants were separated into roots, leaves and stems. Specimens were air-dried at 30 °C in a shady place at room temperature. Voucher specimens are kept at the herbarium of the Higher Institute of Biotechnology of Monastir (ThA. 88, 89 and 90).

2.2. Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

2.3. Essential oil extraction and identification

The essential oils have been extracted from (100 g) air-dried roots, stems and leaves by hydrodistillation for 3 h, using a Clevenger-type apparatus. Oil yields were then estimated on the basis of the dry weight of plant material. Oils were recovered directly, from above the distillate without adding any solvent, and stored in dark vials at 4 °C.

Gas chromatography (GC) analyses were carried out following the method of Ben El Hadj Ali et al. (2010). Gas chromatography-mass spectrometry (GC-MS) analyses were performed with an Agilent 7890A gas chromatograph equipped with a HP-5MS fused silica column (30 m × 250 µm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 µm film thickness), interfaced with an Agilent mass selective detector 5975C inter MSD. Oven temperature was programmed to rise from 60 to 240 °C at a rate of 4 °C/min; transfer line temperature was 250 °C. The carrier gas was Helium with a flow rate of 0.8 ml/min and a split ratio of 50:1. Mass scan range from 50 to 550 m/z at 70 eV and scan velocity was 2.91 scans/s.

1 µl of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. The identification of oil components was assigned by comparison of their retention indices (RI) determined with reference to a homologous series of C₉–C₂₈ n-alkanes and with those of authentic standards. Further identification was confirmed by comparison of their mass spectra with those recorded in NIST08 and W8N08 libraries of the GC-MS data system. Determination of the percentage composition was based on GC peak area normalization without correction factors.

2.4. Antibacterial activity

The antibacterial activity of oils was determined only for those from leaves because of their low yields in roots and stems. For each sample, the antibacterial activity was tested against two Gram-negative bacteria: *Escherichia coli* ATCC 25.922 and *Pseudomonas aeruginosa* ATCC 9027 and three Gram-positive bacteria: *Saphylococcus aureus* ATCC 25.923, *Listeria monocytogenes* ATCC 7644 and *Bacillus cereus* ATCC 11.778. Microorganisms were graciously supplied from the culture collection of the Microbiology Laboratory of the Department of Biology from the Higher Institute of Biotechnology of Monastir, Tunisia. The disk agar diffusion method was made according to Hazzit et al. (2009). Microorganisms were maintained in tryptic soy agar (TSA). 100 µl of suspension of the tested microorganisms, containing 10⁶ colony forming units (CFU/ml) of bacteria, spread on TSA, were placed in 9 cm Petri dishes (15 ml). The sterile paper discs (Watman disc of 6 mm diameter) were impregnated with 10 µl of each oil and then distributed on to the surface of inoculated plates (90 mm). Before incubation, all Petri dishes were kept in the refrigerator (4 °C) for 2 h and incubated after at 37 °C for 24 h for bacteria growth. The antimicrobial potentials were estimated according to indices reported by Rodriguez Vaquero et al. (2007). Diameters (mm) of growth inhibition zones, including the diameter of discs, were measured after incubation at 37 °C for 24 h. In each assay, DMSO was used as the negative control, whereas the antibiotic gentamicine (10 µg/ml) was used as positive control. The minimum inhibitory concentration (MIC) represents the lowest concentration of the total essential oil at which the microorganism does not demonstrate visible growth after incubation, was determined as described previously (Okeke et al., 2001). Serial dilutions of 1/2, 1/4, 1/6, 1/8 and 1/10 were made with 5% DMSO. 10 µl from each dilution of essential oil were added to 5 ml of TSA broth tubes then incubated at 37 °C for 24 h in an incubator shaker. All tests were performed in triplicate.

2.5. Antioxidant activity evaluation

The antioxidant activity of *T. algeriensis* oils was assessed using free radical-scavenging activity with DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) radical assay.

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