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AROMATHÉRAPIE EXPÉRIMENTALE

Chemical Composition, Antibacterial, and Antifungal activities of the Essential Oil of *Thymus numidicus* Poiret from Algeria

Composition chimique, activités antibactérienne et antifongique de l'huile essentielle de *Thymus numidicus* Poiret d'Algérie

Y. Messara · F. Fernane · R. Meddour

Abstract The present study has been carried on Numidian thyme (*Thymus numidicus*), a medicinal plant belonging to the Lamiaceae family. Numidian thyme is an Algero-Tunisian endemic species. The essential oil was extracted from the leaves of the plant harvested in wild habitat at Tifrit (Tizi Ouzou, Algeria), by steam hydrodistillation with 1.58% yield. Analysis by GC–MS (Gas chromatography-mass spectrometry) of the essential oil helped to identify more than 70 compounds and to classify it as a thymol chemotype. The major components of the oil were: thymol (40.40%) and carvacrol (13.37%). Antibacterial and antifungal activities of *Thymus numidicus* essential oil was studied in vitro on three bacterial (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) and one fungal strains (*Candida albicans*). The results showed that this essential oil has a wide spectrum of antimicrobial activity on Gram-negative and Gram-positive bacteria and possesses strong antifungal activity on Candida. This activity is probably due mainly to the predominant presence of thymol, and it is more important on fungi than bacteria. Results of this study look promising for the valuation of medicinal plants with the aim of promoting the Algerian pharmaceutical industry.

Mots clés *Thymus numidicus* · Huile essentielle · Activité antibactérienne · Activité antifongique · *Candida albicans* · Analyse phytochimique

Résumé La présente étude a porté sur le thym de Numidie (*Thymus numidicus*), une plante médicinale appartenant à la famille des *Lamiaceae*. Cette espèce endémique algéro-tunisienne a été récoltée dans un habitat sauvage à Tifrit (Tizi Ouzou, Algérie). Son huile essentielle a été extraite des feuilles de la plante par hydrodistillation à la vapeur et a donné un rendement de 1,58 %. L'analyse par GC-MS (chromatographie en phase gazeuse couplée à la spectrométrie de masse) de l'huile essentielle a permis d'identifier plus de 70 composés et de la classer en chémotype thymol. Les principaux constituants de l'huile étaient : le thymol (40,40 %) et le carvacrol (13,37 %). Les activités antibactériennes et antifongiques de l'huile essentielle de *Thymus numidicus* ont été étudiées in vitro sur trois souches bactériennes (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) et une souche fongique (*Candida albicans*). Les résultats montrent que cette huile essentielle a un large spectre d'activité antimicrobienne sur les bactéries Gram+ et Gram– et possède une forte activité antifongique sur *Candida albicans*. Cette activité est probablement due à la présence prédominante de thymol, et elle est plus importante sur les champignons que les bactéries. Les résultats de cette étude semblent prometteurs pour l'évaluation des plantes médicinales dans le but de promouvoir l'industrie pharmaceutique algérienne.

Keywords *Thymus numidicus* · Essential oil · Antibacterial activity · Antifungal activity · *Candida albicans* · Phytochemical analysis

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Introduction

During the last few years, the interest in the use of medicinal plants, as well as their essential oils, has grown in leaps and bounds [1]. Medicinal plants are widely used for their antibacterial, antifungal, and insecticidal activities. Currently, about 300 essential oils are sold to industries such as pharmaceutical, agricultural, food, and cosmetics industries [2].

The interest in natural products, especially the use of medicinal plants and their essential oils, is due to several reasons [3], such as the high cost of synthetic drugs, the use of organic products devoid of any harmful effects or side effects related to chemicals products, environmental protection, and the effectiveness of therapeutic plants, which has been proved by science for many plants.

Essential oils of medicinal plants are highly complex mixtures of organic compounds with very diverse structures and chemical functions. They are recognized by two or three major components present at relatively high concentrations compared to others. Usually, pure essential oils are divided into two biosynthetic distinct groups. The first group consists of terpenes and terpenoids and the second one has aromatic and aliphatic components [1].

Thyme is widely used in traditional medicine in several forms: leaves and flowers are used as infusion against cough; decoction as an anti-inflammatory agent to cure headaches, hypertension, gastritis (carminative), and for pest control; and for external use as an antiseptic. Thyme leaves are rich in essential oil, and its antiseptic action is known for treating lung infections and regulating the digestive system, mainly in cases of diarrhea [2,4]. In Kabylia, *Thymus numidicus* was specifically used in folk medicine to treat respiratory problems (colds, flu, tonsillitis, pharyngitis, asthma, etc.) [5].

The chemical composition has been previously established for *Thymus numidicus* [6–10].

As *Thymus numidicus* growing wild in Algeria is frequently used in traditional medicine, the aim of the present work is to evaluate the antifungal and antimicrobial activities of its essential oils and relate it to its chemical composition for further application in food and pharmaceutical industries as a natural valuable product.

Material and methods

Origin of plant material

The aerial parts of *Thymus numidicus* Poiret, growing wild in a maquis, were collected in April 2012, at Djebel Tamgout (altitude 700 m above sea level), near Tifrit locality, Tizi Ouzou Province, Algeria. One of us (RM) identified this species (Fig. 1) and a voucher specimen is conserved at the herbarium of the Faculty of Biological and Agricultural Sciences, University of Tizi Ouzou, Algeria.

Extraction and determination of essential oil composition

The leaves were washed and dried in the laboratory, for 10 days, in open space and protected from light. Extraction of essential oil was performed through a steam distillation process. Thirty three grams of dry leaves and 1 liter of water were put into a flask. The mixture was boiled for 2 h. The steam charged with essential oil was condensed in the refrigerant and the distillate collected in a separator funnel. The essential oil extracted was dried with anhydrous sodium sulfate and kept at a cool temperature of +4 °C in glass bottles away from light. The yield of essential oil content (in %) is defined as the ratio between the mass of essential oil

Fig. 1 *Thymus numidicus* Poiret (Zaâtar, Thizathrin, thym de Numidie): a. flowers, b. leaves (photos Y. Messara)

obtained by extraction and the total mass (in grams) of dried plant material treated. It is represented by the following formula:

$$YEO = \frac{MEO}{MD} \times 100$$

YEO: yield of essential oil.

MEO: mass of essential oil.

MD: mass of dried plant material.

Characterization of essential oils

The analyzes of the organoleptic characteristics of the essential oil (appearance, color, and odor) and physicochemical parameters (specific gravity, peroxide index, acid index, refractive index, pH) were performed according to the standards of the European Pharmacopoeia [11].

The essential oil was analyzed using a Hewlett Packard 6890 gas chromatograph coupled to the Agilent MSD mass spectrometer model 5973, with helium as a carrier gas having a flow rate of 0.5 ml/min. The compounds were separated on an HP-5 ms capillary column (30 m × 0.32 mm, film thickness 0.25 µm). The amount of sample injected in the split mod was 0.2 µl with split ratio 1/20, the injector temperature was 250 °C. The CG oven temperature was kept at 45 °C for 5 min and programmed to 250 °C for 10 min at a rate of 2 °C/min.

The mass spectrometer operated in the electron impact with ionization voltage of 70 eV, and the source temperature was 280 °C. The mass scanning range was 25–350 m/z. The components of the essential oil were identified by comparing their retention indices relative to (C7 and C28) *n*-alkanes either with those of published data or with authentic compounds.

Antimicrobial activity of essential oil

Microbiological analyzes were conducted to investigate the antimicrobial and antifungal activity of *Thymus numidicus* essential oil on the growth of microorganisms (bacteria and yeasts) using the method of diffusion on agar [12–14]. The microbial strains used were namely *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* clinically isolated on CHROMagar orientation, and *Candida albicans* clinically isolated on CHROMagar candida.

From a culture of bacteria, 18–24 h old, and yeasts after 48 h, bacterial suspensions in physiological saline to 0.9% were made at a concentration of 10^7 to 10^8 CFU/ml. The latter was obtained using a spectrophotometer by measuring the optical density at 620 nm. This concentration corresponds to an optical density of 0.08–0.1.

Mueller-Hinton culture medium was used for bacteria and Sabouraud base for yeasts. First, the two media was melted over a waterbath at 95 °C and cooled to 45 °C. Then they were poured into Petri dishes at the rate of 15 ml per box. The dishes were stored at 4 °C for 24 h (to allow the culture medium to dry) [15]. The seeding was done by means of a sterile swab, previously soaked into bacterial suspensions. The sterile filter disks (6 mm of Ø) were separately impregnated with essential oil and placed on the agar previously inoculated with the microorganism test.

To evaluate the antimicrobial and antifungal activity qualitatively, we used the method of aromatogram [16]. Here, the inhibition of growth of microorganisms in contact with the essential oil was estimated and the minimum inhibitory concentration (MIC) of essential oil was determined. The MIC is defined as the lowest concentration of essential oil inhibiting 90% of the microbial growth [17]. The evaluation of the antimicrobial activity is obtained by measuring the diameter of the inhibition zone (mm).

The scale for estimating the antimicrobial activity was given by Giordani et al. [18]. They classified the diameter of inhibition zones (*D*) of the microbial growth into five classes:

- very strong inhibitory: $D \geq 30$ mm;
- strongly inhibitory: $21 \leq D \leq 29$ mm;
- moderately inhibitory: $16 \leq D \leq 20$ mm;
- light inhibitory: $11 \leq D \leq 16$ mm;
- no inhibitory: $D \leq 10$ mm.

Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in millimeters (including disk diameter of 6 mm) for the microorganisms test, and calculation of % inhibition according to the following formula [19]:

$$\%\,Inhibition = \left(\frac{D_{test}}{D_{Petri\ dishes}}\right) \times 100$$

D_{test} : Diameter of inhibition zone.

$D_{Petri\ dishes}$: Diameter of Petri dishes.

Results and discussion

Analysis and composition of the essential oil

The essential oil of *Thymus numidicus* is viscous, yellow-orange in color and its very strong odor indicates the presence of thymol. According to Saidj et al. [7], the Numidian thyme essential oil has a movable liquid aspect, yellow color, and a pleasant aromatic odor.

This essential oil has a pH of 6–7, a density of 0.891, a refractive index at 20 °C of 1.488, an acid index of 4.52, and peroxide index of 56. Due to lack of index values of *Thymus numidicus* essential oil, we compared our results with those given in the literature [20] for the oils of other species of Thymus with the same chemotype, such as *Thymus vulgaris,* *Thymus fontanesii*, and *Thymus longiflorus*. The results obtained were comparable, except for the acid index, which was lower than the *Thymus vulgaris* acid index (8.4).

The yield of essential oil of *Thymus numidicus* is 1.58% in our study. This yield is comparable to those obtained (1.1–2.06%) for the same species in other regions of Algeria [6,7,9,21,22].

Gas chromatography–mass spectrometry (GC–MS) analysis of *Thymus numidicus* essential oil identified more than 70 compounds, of which 9 with a relative content greater than 2%, represented 87.83% of the total oil content (Table 1). This essential oil is rich in monoterpenes (82.07%), thymol and carvacrol are the major compounds (monoterpenic phenols), with a rate of 40.40% and 13.37%, respectively, followed by thymol-methyl-ether (8.3%), *p*-cymene (7.18%), *γ*-terpinene (6.41%), and linalool (4.06%).

The predominance of phenolic compounds (thymol, carvacrol) in the essential oil of *Thymus numidicus* is in agreement with previous results reported by several authors in Algeria [6–8,10,18,19,21]. The most abundant compound in the essential oil of Numidian thyme (collected anywhere) is thymol, which can represent up to 51–68%, and even more (77.5%) in some areas [9]. It is thus of thymol chemotype. However, the essential oil of *Thymus numidicus* sample collected from Constantine (North-east of Algeria) contains thymol with a rate of only 23.92%, and a higher content of *p*-cymene 11.41% [3]. This is probably related to the later sampling period (June instead of March–May). According to Hadef et al. [6] and Kouch et al. [9], the end of the life cycle (July) of *Thymus numidicus* is characterized by high relative content of *p*-cymene and low content of phenols. Given this, *γ*-terpinene and *p*-cymene are the precursors of the biosynthesis of thymol and carvacrol by hydroxylation.

Table 1 Yield and composition of essential oil of *Thymus numidicus* at Tifrit (Tizi Ouzou, Algeria)

Compounds	**%**
α-thujene	0.47
α-pinene	0.63
Camphene	0.13
1-octene-3-ol	0.46
Octane -3-one	0.03
β-myrcene	**2.37**
Octane-3-ol	0.1
α-phellandrene	0.06
β-pinene	0.17
α-terpinene	0.59
p-cymene	**7.18**
Limonene	0.74
1,8-cineol	0.65
γ-terpinene	**6.41**
Linalool	**4.06**
Cis-sabinene hydrate	0.28
Camphor	0.05
Borneol	0.02
Terpineol-4	0.14
α-terpineol	0.14
Thymol methyl ether	**8.30**
Carvacrol methyl ether	0.34
Thymoquinone	0.04
Thymol	**40.40**
Carvacrol	**13.37**
Eugenol	0.04
Trans-carvyl acetate	0.02
Ylangene	0.03
α-copaene	0.09
β-bourbonene	0.09
Cis-jasmone	0.04
α-gurjunene	0.10
β-caryophyllene	**2.48**
α-muurolene	0.04
α-humelene	0.10
Aromadendrene	0.13
Γ-muurolene	0.10
β-bisabolene	**3.26**
δ-cadinene	0.85
Cadina1-4-diene	0.03
α-cadinene	0.05
α-calacorene	0.09
Viridiflorol	0.01
β-sesquiphellandrene	0.52
Ledol	0.01
α-epi-cadinol	0.23
β-eudesmol	0.01
α-cadinol	0.07
Total	95.52

Antimicrobial activity

The average diameter of the inhibition zone observed around disks impregnated with pure essential oil, after 24 h of incubation at 37 °C for bacteria and 48 h for yeasts, as well as the percentage of inhibition zone is summarized in Table 2.

The essential oil of *Thymus numidicus* has proven effective against the strains studied. *Pseudomonas aeruginosa* has a smaller zone of inhibition (17 mm, moderately inhibitory class), unlike *Escherichia coli* and *Staphylococcus* with 33 mm and 39 mm (very strong inhibitory) diameter respectively. The most outstanding effect is against *Candida albicans* (Fig. 2), since the essential oil has practically inhibited 90% of the colony present in the Petri dish with a diameter of 80 mm (very strong inhibitory). The other strains also perform differently with diameters between 33 and 80 mm. Hence, the essential oil of *Thymus numidicus* has a broad spectrum of antibacterial activity on Gram-positive and Gram-negative bacteria and on the yeast *Candida albicans*.

Table 2 Values of average diameter (D) and percentage (%) of inhibition zone (I %) of *Thymus numidicus* essential oil (EO) on different bacterial strains			
Strains		**EO** *Thymus numidicus*	
		D **(mm)**	***I*** **(%)**
Gram+	*Staphylococcus aureus* ATCC 25923	39 ± 3	43
Gram−	*Escherichia coli*	33 ± 2	36
	Pseudomonas aeruginosa ATCC 27853	17 ± 2	19
Yeast	*Candida albicans*	80 ± 5	90

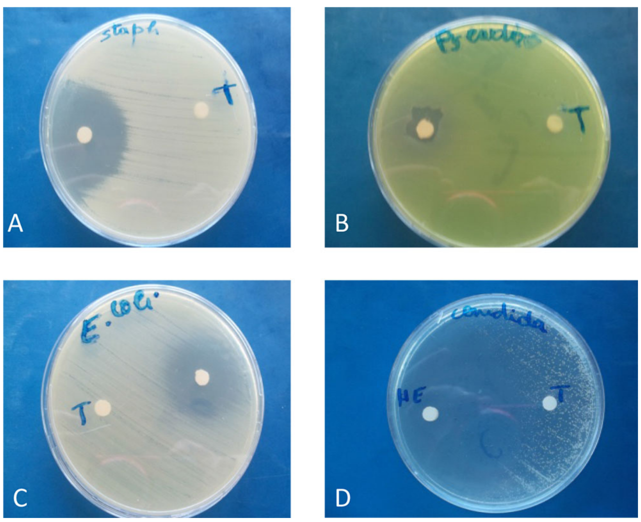

Fig. 2 Antibacterial and antifungal activity of essential oil of *Thymus numidicus* on
A: *Staphylococcus aureus*, B: *Pseudomonas aeruginosa*, C: *Escherichia coli*, D: *Candida albicans*, T: witness (sterile disk)

The biological activity of these oils is often attributed to the occurrence of such bioactive compounds as terpenes, thymol, carvacrol, α-terpinene, linalool, and β-caryophyllene [3]. This activity may be due to the synergistic effect of the main components.

These results are confirmed by the low MIC values (Table 3). Even at very low concentrations in some cases, despite the use of Tween 80, which considerably reduces the antimicrobial activity of essential oils [13], *Pseudomonas aeruginosa* ATCC 27853 was the most resistant because it is inhibited at a concentration about 0.4% (4.55 mg/ml), while *Escherichia coli* and *Staphylococcus aureus* were inhibited from a minimum concentration of 0.05% (0.44 mg/ml). A minimum concentration of 0.03% (0.27 mg/ml) was needed to inhibit the yeast *Candida albicans*.

Recently, Giordani et al. [18] have also observed a very strong antifungal effect against *Candida albicans*. Moreover, Zeghib et al. [22] found that the essential oil of *Thymus numidicus* from Constantine presents inhibitory activity with low MIC values against tested bacterial strains, such as *Staphylococcus aureus* and *Escherichia coli*. Finally, Kouch et al. [9] have showed an interesting sensitivity, with only 0.4 mg/ml, of several strains of *Pseudomonas aeruginosa* against the essential oil of *Thymus numidicus* that varies according to strains (inhibition zones ranging from 17.5 to 55 mm).

Conclusion

This study allowed us to determine the chemical composition of the essential oil of *Thymus numidicus* Poiret from Algeria (Tifrit, Tizi Ouzou), extracted by steam distillation and to evaluate its antimicrobial activity. Analysis of the essential oil by GC-MS made it possible to identify nine components whose rate is higher than 2%, representing 87.83% of the oil. The major components were thymol (40.4%) followed by carvacrol (13.37%). Thus, GC-MS analysis of *Thymus numidicus* essential oil confirms its thymol chemotype. Microbiological tests show that its essential oil has an antimicrobial activity on both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria and a very strong antifungal activity on *Candida albicans*, with low MIC values. But, this essential oil was more active against tested strains of *Staphylococcus aureus* and *Escherichia coli*, than *Pseudomonas aeruginosa*. This interesting activity is mostly attributed to the high concentration of terpenes and phenolic compounds in this essential oil, including thymol, which is the major component [9].

Table 3 Antimicrobial activity of *Thymus numidicus* essential oil at different concentrations

Dilution%		**1**	**0.4**	**0.2**	**0.1**	**0.05**	**0.03**	**0.02**	**W**
Concentration of essential oil (mg/ml)		8.91	4.55	1.78	0.89	0.44	0.27	0.21	0
Gram−	*Pseudomonas aeruginosa*	−	−	+	+	+	+	+	+
	Escherichia coli	−	−	−	−	−	+	+	+
Gram+	*Staphylococcus aureus*	−	−	−	−	−	+	+	+
Yeast	*Candida albicans*	−	−	−	−	−	−	+	+

Practical applications of the results obtained herein are expected, such as the cultivation of some endemic species for extraction of secondary metabolites.

Conclusion

Cette étude nous a permis de déterminer la composition chimique de l'huile essentielle de *Thymus numidicus* Poiret d'Algérie (Tifrit, Tizi Ouzou), extraite par distillation à la vapeur et d'évaluer son activité antimicrobienne. L'analyse de l'huile essentielle par CG-SM a permis d'identifier neuf composants dont le taux est supérieur à 2 %, ce qui représente 87,83 % de l'huile. Les principales composantes étaient le thymol (40,4 %) suivi par le carvacrol (13,37 %). Ainsi, l'analyse CG-SM de l'huile essentielle de *Thymus numidicus* confirme son chémotype « thymol ». Les tests microbiologiques montrent que son huile essentielle a une activité antimicrobienne sur les bactéries Gram– (*Escherichia coli*) et Gram+ (*Staphylococcus aureus*) et une activité antifongique très forte sur *Candida albicans*, avec de faibles valeurs de CMI. Elle semble plus active sur les souches testées de *Staphylococcus aureus* et *Escherichia coli*, que sur *Pseudomonas aeruginosa*. Cette activité intéressante est principalement attribuée à une forte concentration de terpènes et de composés phénoliques dans cette huile essentielle, y compris le thymol qui est le composant principal [9].

Les applications pratiques des résultats obtenus dans ce travail sont prometteuses, telles que la cultures de certaines espèces endémiques pour l'extraction de métabolites secondaires.

Liens d'intérêts

Les auteurs déclarent ne pas avoir de liens d'intérêts.

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