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Chemical Composition, Antioxidant and Antibacterial Activity of *Thymelaea microphylla* Essential Oil from Algeria

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ABSTRACT

The chemical composition of essential oil, isolated from Thymelaea microphylla by hydrodistillation, was analysed by GC and GC/MS. A total 30 compounds representing 99.91% of the oil were identified in Dreaat population. The essential oil of T. microphylla is Characterized by a high rate of Tridecanal (31.24%), Nonanal<n-> (11.43%), Pentadecen 2-one (6Z) (7.93%) and the Citronellol (6.80%). Antioxidant capacity was determined using the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The extracts essential oil showed a good antioxidant activity. Disc diffusion method was used to evaluate the antibacterial activity on five different strains. The oil showed a significant effect against Gram-negative and Gram-positive bacteria.

Keywords: Essential oil, Chemical composition, Antioxidant activity, Antibacterial activity, *Thymelaea microphylla*, Algeria

INTRODUCTION

Most of the natural products reported and listed in the genus *Thymelaea*, belong to the large group of polyphenolic compounds and more precisely to flavonoids [1-6]. Compounds of the sterol family are reported in *Thymelaea microphylla* [5] and in *Thymelaea hirsuta* [1]. *T. microphylla*, *T. hirsuta*, *Thymelaea lythroides* and *Thymelaea tartonraira* are very rich in coumarines, [1,7,5]. The microphybenzimidazole compound was isolated from *T. microphylla* [8].

The essential oils of the genus *Thymelaea*, have been the subject of several phytochemical studies. *T. hirsuta* oils are characterized by high amounts of sesquiterpenes and monoterpenes [9,10]. The *T. microphylla* essential oil composition is dominated by the monoterpenes (67.84%) [11], and the study of its ethanolic extracts showed the presence of monoterpenes glycosides [12].

T. hirsuta is known for its powerful antiseptic, hypoglycemic and anti-inflammatory properties and for the treatment of hypertension, such as antimelanogenesis, antidirehyde and purgative and in the treatment of influenza [1,2,5,6,10,13-17]. In M'Sila region, *T. hirsuta* is recommended by herbalists for the treatment of human diseases (Leishmanicide, dewormer and eczema) [18]. In Algeria anticancer and antiinflammatory effects of *T. micrphylla* methanol extracts have been tested [5,19,20]. *T. lythroides* extracts have strong antifungal activity [2].

T. microphylla essential oil showed a high antibacterial activity against *Escherchia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and a weak inhibitory effect against *Pseudomonas aeruginosa* [21]. The essential oil of *T. hirsuta*, shows antioxidant, antimicrobial and antifungal activities [9,16,19]. The cytotoxicity of the oil of this species is demonstrated by Felhi et al. [16].

The objective of this study is to determine the chemical composition of *T. microphylla*, species endemic to the Sahara, and to evaluate the antibacterial and antioxidant activities of its essential oil.

MATERIALS AND METHODS

Plant material

T. microphylla Coss. & Dur., commonly called "Methnane Ghazal or Methnane Labiadh", is a plant endemic to North Africa. It is a woody plant with much branched stems whose leaves are small, ovoid, scattered and distant on the branches. Flowers are glomerulate 2-5 in the axils of leaves, yellowish in colour with very short lobes. This plant is found in arid and desert pastures (Figure 1) [22].

Figure 1: *Thymelaea microphylla* from the M'Sila region

T. microphylla is collected from the Dreaat forest (Hammam Dalaa, M'Sila). Aerial parts were collected during the flowering stage in Mai 2017 (Figure 2).

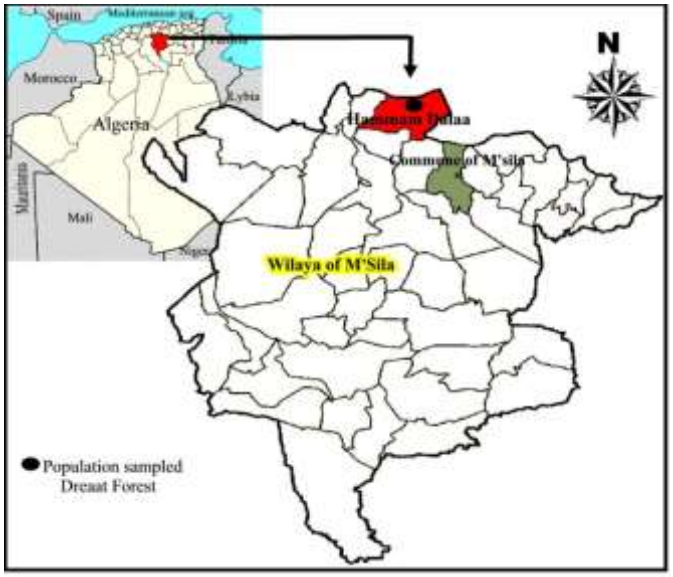

Figure 2: Sampling area of *Thymelaea microphylla* Coss. and Dur.

The air dried materials were subjected to hydro-distillation for 3 h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph CPG/FID 7890, coupled to a gaz chromatograph: CPG/MS 7890/5975C, equipped with a Colonn Apolar: DB5 MS: 40 m, 0.18 mm, 0.18 µm, programming from 50°C for 5 min-5°C/min until 300°C. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1: 30), injector and detector temperature is 280°C with split 1/100. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the *m/z* range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library [23,24] and those described by Adams as well as on comparison of their retention indices either with those of authentic compounds or with literature values [25].

Antibacterial activity

The Extract Essential oil was tested against the following bacteria; 2 Gram-negative bacteria: *E. coli* ATCC 25922; and *P. aeruginosa* ATCC 27853, and 3 Gram-positive bacteria; *B. subtilus* ATCC 6633, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 51299. The *in vitro* antibacterial activity of the examined extract was assessed the determination of the activity by the micro dilution method, according to recommendations of the Clinical and Laboratory Standards Institute (NCCLS). The bacterial inoculums was prepared from overnight broth culture in physiological

saline (0.8% of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5% sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm) were placed on inoculated agars, by test bacteria, filled with 10 l of mother solution and diluted essential oil (1: 1, 1: 2, 1: 4, 1: 8 and 1: 16 v:v of Dimethyl Sulfoxide (DMSO)). DMSO was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24 h aerobically (Bacteria). After incubation, inhibition zone diameters were measured and documented.

Antioxidant activity

The free radical-scavenging activity of *T. microphylla* crude extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine [26]. The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decreases at 517 nm until the absorbance remains constant. 4.0 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 2.9 ml of extract solution in methanol at different concentrations (37.5 to 300 µl). Thirty minutes later, the absorbance was measured at 517 nm. BHT (Butelated hydroxytoluen) was used as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$I\% = \left(\frac{A.blank - A.sample}{A.blank}\right) * 100$$

Where (A. blank) was the absorbance of the control (blank, without essential oil) and (A. sample) was the absorbance in the presence of the essential oil. All the tests were performed in triplicate and the graph was plotted with the mean values [27].

RESULTS

Chemical analysis

The hydro-distillation of *T. microphylla* essential oil gave a viscous liquid with pale yellow oil. The average yield of essential oil of the sample is 0.14%. The analysis and identification of the components of the essential oil of this species was performed using the GC-GC/MS (Figure 3). The compound identified in these oils and the abundances are presented in order of their appearance (Table 1).

This analysis led to the identification of 30 components representing 99.93% of the total oil of *T. microphylla*. According to our results the chemical composition of the species, *T. microphylla* is dominated by the presence of major compounds, the Tridecanal (31.24%), Nonanal<n-> (11.43%), Pentadecen 2-one (6Z) (7.93%) and the Citronellol (6.80%). As well as other component with lower percentages, Tetradecanal (4.43%), Caryophyllene 14, 6-hydroxy 4-5 dihydro (3.65%), Lavandulyl acetate tetrahydro (3.50%), Dodecanal (3.27%), Undecanal (3.09%) and the presence in trace of other compounds.

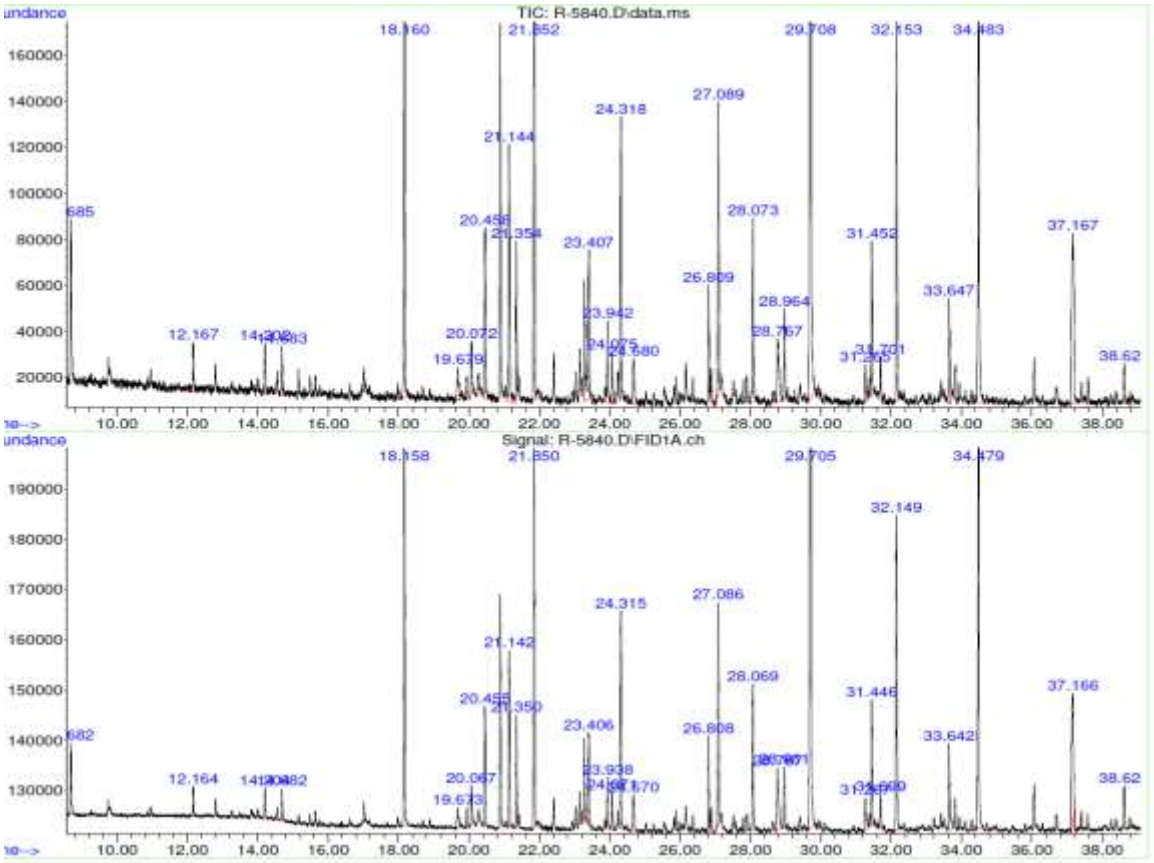

Figure 3: GC/Masse and GC/FID profiles of *Thymelaea microphylla*

Table 1: Chemical composition of *Thymelaea microphylla* essential oil

Yield %	**KI**	**0.14**	**Yield %**	**KI**	**0.14**
Number of compound		**30**	**Number of compound**		**30**
Total		**99.91**	**Total**		**99.91**
Pentanone-4-hydroxy-4-methyl-2	838	1.76	Citronellyl acetate	1322	0.89
α-pinene	935	0.55	Tetradecane (C14)	1399	1.55
Pentyl furan-2	989	0.62	Dodecanal	1410	3.27
Octanal	1002	0.58	Neryl acetone	1449	2.27
Nonanal-n	1102	11.43	Dodecen 1-ol (2E)	1476	1.41
Camphor	1151	0.56	β-ionone (E)	1483	1.11
Lavandulol	1164	0.57	Tridecanal	1513	31.24
Naphthalene	1191	1.94	Hexenyl benzoate (3Z)	1577	0.54
Dodecane	1200	2.8	Tridecen 1-al (2E)	1585	1.74
Decanal-n	1207	1.64	Sesquisabinene hydrate Trans	1595	0.57
Citronellol	1224	6.8	Tetradecanal	1614	4.43
Lavandulyl acetate tetrahydro	1273	3.5	Isomenthone 2(3-oxobutyl)	1679	1.35
Isopulrgyl acetate iso	1295	1.06	Pentadecen-2-one (6Z)	1716	7.93
Tridecane	1300	0.55	Caryophyllene 14, 6 hydroxy 4-5 dihydro	1841	3.65
Undecanal	1309	3.09	Farnesyl acetone (5E, 9Z)	1912	0.51

Antibacterial activity

The results of the experiments assessing the bacteriostatic effects of *T. microphylla* essential oil of the study on Gram-negative and Gram-positive bacteria are presented in (Table 2).

Table 2: Antibacterial activity of *Thymelaea microphylla* essential oil

	Antibiotic*		**Dilution**				
Bacteria	Gen	Amo	1/1	1/2	1/4	1/8	1/16
Bacillus subtillus ATCC 6633	26	-	29	19.5	18.5	14	11.5
Echerichia coli ATCC 25922	26	-	26.5	26	16.5	15.5	13.5
Enterococcus faecalis ATCC 51299	-	26	18.5	17.5	15.5	15	14.5
Pseudomonas aureiginosa ATCC 27853	28	-	-	-	-	-	-
Staphyllococcus aureus ATCC 25923	30	-	-	-	-	-	-

*Gen= Gentamicine; Amo= Amoxicilline

Effectively, the essential oil from *T. microphylla* leaves were demonstrated antibacterial activity against the gram negative clinical pathogens bacteria tested, *B. subtillus, E. coli and E. faecalis, bat no activity against the bacteria P. aureiginosa and S. aureus.*

Antioxidant activity

The antiradical activity of *T. microphylla* essential oil is evaluated by their inhibitory capacity of a methanolic solution of DPPH; it's measured by spectrophotometer at 517 nm. The standard used was BHT (Butylated hydroxytoluen) (Table 3). The change in the colour of the solution containing *T. microphylla* essential oil proves that the oil has antioxidant activity.

Table 3: Antioxidant activity of essential oil of *Thymelaea microphylla*

Concentrations	**300 µl (1/1)**	**150 µl (1/2)**	**75 µl (1/4)**	**37.5 µl (1/8)**
Absorbance of EO (nm)	0.057	0.119	0.317	0.515
Absorbance de BHT (nm)	0.068	0.14	0.33	0.64
Inhibition (%) of EO	91.53	82.31	52.89	23.47
Inhibition (%) of BHT	89.89	79.19	50.96	4.9

The inhibition percentages of different concentrations of *T. microphylla* essential oil as well as the BHT standard fluctuate between 4.90% and 89.89% for BHT, 23.47% and 91.53% for *T. microphylla* oil (Figure 4). It should be noted that there is a certain similarity in the percentages of inhibition, for the oil and for the BHT standard, except for the 1/8 dilution.

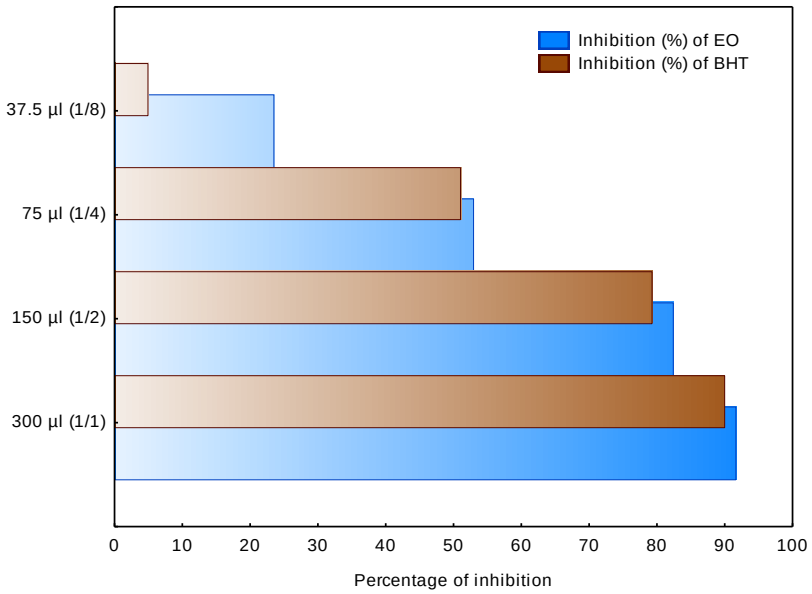

Figure 4: Inhibition percentage of *Thymelaea microphylla* essential oil

The highest inhibition value is 91.53% for oil and 89.89% for BHT, indicating a possibility that *T. microphylla* oil contains a greater amount of free radical accepting compounds, as well as that the greatest antioxidant potential. The percentage of inhibition of the free radical increases with the increase of the concentration, either for the BHT or for the essential oil. IC_{50} is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the free radical concentration by 50%.

DISCUSSION

Many works have shown a great heterogeneity in the chemical composition of the genus *Thymelaea*. In Tunisia, *T. hirsuta* is characterized by heptane, germacrene-D, γ-eudesmol and citronellyl formate [9,28]. The *T. hirsuta* essential oil, cultivated in Tunisia, is dominated by hexadecanoic acid, 4, 8-and 13- dimethylhecosane methylhexacosane [10].

Chemical analysis of *T. microphylla* essential oils allowed us to identify tridecanal (31.24%), nonanal <n-> (11.43%), pentadecen (7.93%) and citronelloll (6.80%) as major components. The chemical results differ from those cited in the literature. *T. microphylla* samples from the Ourgla region (Algeria) show the predominance of D-menthone, 2-undeconeone, pulegone and perillal [11]. This difference in composition is probably due to various conditions including the environment, geographical origin, harvest period, temperature and drying time.

T. hirsuta essential oil shows significant antioxidant activity [17,29]. This oil reduces the formation of DPPH radicals in a dose-dependent manner [9]. The investigation of the antioxidant activity of the essential oil of *T. micropylla* has shown that this species has antioxidant activity. The various studies of aqueous and methanolic extracts of *T. microphylla* prove the presence of antioxidant activity [3,6,12,20,21,30].
Species of the genus *Thymelaea* exhibit important biological activities. The essential oil of *T. hirsuta* has moderate antimicrobial activity against all microorganisms tested [16,19]. *T. lythroides* has a strong antifungal activity [2] whereas the methanolic extracts of *T. micropylla* showed no bacteriological effect [20].

Our investigation shows a high activity of *T. micriphylla* essential oil on *B. subtillus, E. coli, E. faecalis* bacteria and no effect against *P. aureiginosa and S. aureus.*

The studies of Noman et al. [31] showed high antibacterial activity of *T. microphylla* essential oil against *E. coli* and *S. aureus, K. pneumoniae* and a weak inhibitory effect against *P. aeruginosa*. The absence of antibacterial activity can be explained by the developed resistance of some strains that react differently to several essential oils; this is the case of *P. aeruginosa* [32,33].

CONCLUSION

This study aims to identify the chemical composition of *T. microphylla* essential oils and the evaluation of their antioxidant and antibacterial activities. The analysis of the chemical composition of the essential oil by GC/MS has allowed the identification of 30 compounds. The Tridecanal is the major component of the chemical composition, although the study has identified the other natural products as minor components. The results exhibited antioxidant activity, which are stronger than the positive control BHT. An additional characteristic of *T. microphylla* essential oil was its prominent antibacterial activity against *B. subtillus, E. coli, E. faecalis* and no effect against *P. aureiginosa and S. aureus.* The Essential oil may be a promising alternative treatment of localized infections even with severe hospital acquired strains. Further investigations must be made to determine the active constituent(s) for their application in medical research.

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