Chemical composition and essential oil antimicrobial activity of four grazed plants growing wild in northeastern Tunisia

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Chemical composition and essential oil antimicrobial activity of four grazed plants growing wild in northeastern Tunisia

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Abstract. The aim of this work was to study the chemical composition and the antibacterial activity of essential oils of *Rosmarinus officinalis; Thymus vulgaris; Myrtus communis; Artemisia herba-alba* from northeastern Tunisia (Zaghouan). Leaves and twigs from these species were collected in spring and the essential oils (EO) were extracted by hydrodistillation and then analysed using GC/MS. The antibacterial activity was studied by diffusion in agar against four strains: *Staphylococcus aureus* ATCC29213; *Staphylococcus aureus* 6816; *Listeria monocytogenes* ATCC19195 and *Escherichia coli* ATCC35218.

Results showed that *Rosmarinus officinalis* EO are characterized by the presence of 1,8-cineole (39.0%) as chemotype. The *Thymus vulgaris* EO are composed mainly of thymol (60.0%). The *Myrtus communis* EO are composed of 29.5% α-Pinene, 20.4% limonene and 11.5% linalool. *Artemisia herba-alba* EO are mainly composed of thujone (42%) and camphor (17.5%).

Rosmarinus officinalis EO did not show any activity at 1/100 dilution. Whereas at this level *Myrtus communis* showed anti-bacterial activity only against *Escherichia coli.* The cited concentration for *Artemisia herba-alba* was sufficient to inhibit the growth of all strains, except *Listeria monocytogenes*. *T. vulgaris* EO showed the highest anti-bacterial effect against all the tested bacteria. This bioactivity is mainly because these EO are rich in thymol, known for its effectiveness against the microbial agents.

It was concluded that the studied species have an important antibacterial activity that can be tested to modulate ruminant fermentation in order to improve the efficiency of digestion and reduce the production of greenhouse gases.

Keywords: Essential oils – *Rosmarinus officinalis* – *Thymus vulgaris* – *Myrtus communis* – *Artemisia herba-alba* – Antimicrobial activity.

Composition chimique et l'activité antibactérienne de l'huile essentielle de quatre plantes sauvages broutées par les ruminants dans le nord-est de la Tunisie

Résumé. *Le but de ce travail était d'étudier la composition chimique et la activité antibactérienne des huiles essentielles de* Rosmarinus officinalis ; Thymus vulgaris ; Myrtus communis ; Artemisia herba-alba *du Nord-Tunisie (Zaghouan). Les feuilles et les brindilles de ces espèces ont été collecté au printemps et HE ont été extraites par hydrodistillation, puis analysées par GC/MS. L'activité antimicrobienne a été réalisée par diffusion dans un milieu gélose contre quatre souches* Staphylococcus aureus *ATCC29213 ;* Staphylococcus aureus *6816 ;* Listeria monocytogene *ATCC19195 and* Echerichia coli *ATCC35218.*

Les résultats ont montré que les huiles essentielles de Rosmarinus officinalis sont caractérisées par la présence de 1,8-cinéol (39,0%) en tant que composants chimiques principaux. Les huiles essentielles de Thymus vulgaris *sont composées principalement de thymol (60,0%). Les huiles essentielles de* Myrtus communis *étaient composées principalement de 29,5%* α*-pinène, limonène 20,4% et 11,5% linalol alors qu'*Artemisia herba-alba *sont principalement composées de thujone (42%) et le camphre (17,5%).*

Les huiles essentielles de Rosmarinus officinalis *n'a pas montré une activité à la dilution 1 / 100. Par contre, à ce niveau,* Myrtus communis *a une activité antibactérienne seulement contre* Escherichia coli. *Pour* Artemisia herba-alba *la concentration citée était suffisante pour inhiber la croissance de toutes les souches, à l'exception de* Listeria monocytogene. Pour les huiles essentielles de *T. vulgaris*, *elles montré le plus grand effet antibactérien contre toutes les bactéries testées. Cette bioactivité est principalement due à leur richesse en thymol, connu pour son efficacité contre les agents microbiens.*

Il a été conclu que les espèces étudiées ont une activité antibactérienne importante qui peut être testé pour moduler la fermentation des ruminants afin d'améliorer l'efficacité de la digestion et de réduire la production de gaz à effet de serre.

Mots-clés. *Huiles essentielles* – Rosmarinus officinalis – Thymus vulgaris – Myrtus communis – Artemisia herba-alba – *Activité antibactérienne.*

I – Introduction

Since ancient times, the essential oils (EO) obtained from the aromatic species have been used in food, pharmaceuticals and cosmetics thanks to their antimicrobial and antioxidant properties. Many researchers have reported EO as potential alternative antimicrobials (Solórzano-Santos & Miranda-Novales, 2012). These biological activities are directly correlated with the presence of bioactive volatile components (Mahmoud & Croteau, 2002). The aim of this work is to characterize the chemical composition of the essential oils obtained from *Rosmarinus officinalis, Thymus vulgaris, Myrtus communis, Artemisia herba-alba* and to study their antimicrobial activity against 4 strains: *Staphylococcus aureus* ATCC29213; *Staphylococcus aureus* 6816; *Listeria monocytogenes* ATCC19195 and *Escherichia coli* ATCC35218.

II – Material and methods

1. Plant material

For the studied plants: *Rosmarinus officinalis; Thymus vulgaris; Myrtus communis; Artemisia herba-alba,* leaves and thin twigs (less than 4 mm in diameter) were collected from the region of Zaghouan (North of Tunisia, semi-arid) in spring 2011. The plant material was left to dry at ambient temperature for 7 days before extracting EO.

2. Essential oil extraction

Extraction of the essential oils was carried out by hydrodistillation. For each plant, three distillations were carried out by boiling 1 kg of dried plant material for 1 hour. The EO were stored at 0° C in the dark before conducting the analyses.

3. Chromatography analysis

The GC/MS analysis was performed on an HP 7890 mass spectrometer (Agilent technologies, Palo Alto, California, USA), using an HP-5MS capillary column (30 m, 0.25 mm coated with 5% phenyl methyl silox, 0.25 lm film thickness). Oven temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C/min. The carrier gas was He with a flow rate of 1.2 ml/min and a split ratio of 60:1. The volatile components were identified by comparing their retention indices (RI) relative to (C8-C22) n-alkanes with those of the literature or with those of compounds available in the authors' laboratory. In addition, their recorded mass spectra was matched with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams, 2001). The percentage composition was determined by peak area normalization. Analyses were performed in triplicate.

4. Microbiological procedure

For the studied species, the EO, which are non-miscible with water, were emulsified in a 0.2% agar solution (Remmal *et al*., 1993, Satrani *et al*., 2001) in order to obtain a homogeneous distribution of the EO in the medium and maximize the microbe / EO contact. 13.5 mL of sterilized autoclaved TSA (Tryptic Soy Agar) medium was placed in test tubes and heated for 12 min at 121°C then cooled to 45°C, when 1.5 mL of Agar solution diluted to the final concentrations: 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v / v) was added aseptically. After stirring, the solution was inoculated using a calibrated platinum loop to ensure the same volume of inoculum at 37°C for 24 h. Analyses were performed in triplicate.

III – Results and discussion

1. Chemical composition of essential oils

The ten most abundant EO compounds and their classification are listed in Table 1. For rosemary, the main compound was 1,8-cineole: 39%. Several other studies have mentioned 1,8-cineole as chemotype (Celiktas *et al*., 2007, Wang *et al*., 2008). Hussain *et al*. (2010) reported very similar values of major compounds with an increase in camphor depending on borneol. Moreover, variation in the chemical composition of rosemary EO (3 rosemary chemotypes) has been reported according to the geographical origin (Lahlou and Barrada, 2003, Zaouali *et al*., 2013). The 1,8-cineole rosemary contains more than 40% of this chemotype, characteristic of Tunisia, Morocco, Italy, France, Turkey, Greece and Yugoslavia (Pintore *et al*., 2002). Rosemary with camphor-borneol chemotype is characteristic of Spain and the α-Pinene-verbenone rosemary is characteristic of Algeria and Corsica (Guazzi *et al*., 2001).

As for *thymus vulgaris*, we mentioned a thymol chemotype (60%) with the presence of β-cymene (7.6%) and γ-terpinene (7.6%). Several thyme chemotypes have been mentioned. In several cases, the reported chemotype is thymol with major anti-infectious properties (Ivanovic *et al*., 2011, Stojkovic *et al*., 2013, Nikolic *et al*., 2014). However, other chemotypes are also cited. Carvacrol *Thymus vulgaris* (Imelouane *et al*., 2006), linalool *thymus vulgaris* (Jukic *et al*., 2003), α-terpineol *thymus vulgaris* (Imelouane *et al*., 2006) and o-cymene and β-cymene *Thymus vulgaris (*Klaric *et al*., 2007, Guerra-Boone *et al*., 2015).

Concerning *Artemisia herba-alba*, thujones, which gives the specific odour and taste to white sagebrush (Mohsen *et al*., 2009), are present at 42% (30.1% β-thujone and 11.9% α-thujone). For sagebrush grown in southern Tunisia, Mghiri *et al*. (2010) identified 4 chemotypes of EO: β-thuyone (58%); α-thuyone: 49.3%; α-thujone (24.1%) and β-thujone (24.3%) and 1,8-cineole (18.4%), β-thujone (14.1%), camphor (8%) and α-thujone (10.7%).

For *Myrtus communis*, we mentioned the α-pinene chemotype (29.5%) with high amounts of limonene (20.4%); linalool (11.5%) and linalyl acetate (10.0%). The most reported Tunisian myrtle chemotype is α-pinene. According to Aidi-Wannes *et al*. (2010), the α-pinene content during the vegetative cycle of *Myrtus communis* ranges from 28.3 to 58%. The other major compounds are 1,8-cineol (12.7-30.7%), linalool (2.4-21.5%) and limonene (0.1-13.3%).

Table 1. The EO main compounds

Rosmarinus officinalis	*Thymus vulgaris*	*Myrtus communis*	*Artemisia herba-alba*
α-pinene: 39.0%	thymol: 60%	α-pinene: 29.5%	α-thujone: 30.1%
borneol: 9.8%	β-cymene: 7.6%	limonene: 20.4%	camphor: 17.5%
α-pinene: 9.0%	γ-terpinene: 7.6%	linalool: 11.5%	α-thujone: 11.9%
camphor: 8.4%	β-caryophyllene:2.8%	linalyl acetate: 10.0%	1,8 cineol:7.6%
β -pinene: 7.7%	β-linalool: 2.7%	geranyl acetate: 5.6%	chrysanthenone: 5.3%
camphene: 4.2%	α-terpinene: 2.1%	1,8 cineol: 2.3%	camphene: 4.9%
borneol acetate: 3.5%	β-cymene: 1.8%	p-cymene: 1.4%	endo-borneol: 3.9%
α-terpineol: 3.4%	α-thujone : 1.8%	α-humulene1.4%	sabinyl acetate: 1.5%
β-caryophyllene: 2.0%	α-pinene: 1.7%	α-terpineol: 1.3%	α-pinene:1.1%
β-cymene: 1.7%	α-silinene : 1.5%	neryl acetate: 1.1%	γ-terpinene:1.1%

2. Antimicrobial activity of essential oils

Table 2 presents the essential oil antibacterial activity of *Rosmarinus officinalis; Thymus vulgaris; Myrtus communis* and *Artemisia herba-alba. Thymus vulgaris* (thymol: 60%) exerted the strongest activity against the tested strains. A concentration level of 1/2000 was sufficient to inhibit all strains. Other reports mentioned the strong activity of thymol against *Escherichia coli* O157:H7 (Friedman *et al*., 2002; Mathela *et al*., 2010, Abu-Darwish *et al*., 2012), *Listeria monocytogenes* (Friedman *et al*., 2002, Al-Mariri *et al*., 2013, Mith *et al*. 2014) and *Staphylococcus aureus* (Rota *et al*. 2007, Palaniappan & Holley, 2010). EO from *Artemisia herba-alba* (thujone: 42%) showed moderate activity. At 1/100 concentration, only *Listeria monocytogenes* was sensitive to *Artemisia herba-alba* derived EO and was inhibited at 1/250. Similar results were obtained by Mghiri *et al*., (2010) for *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 35218.2010. EO from *Rosmarinus officinalis* (1.8-cineol: 39%) and *M. communis*, showed the weakest activity. At 1/100 level, all strains were inhibited except *Escherichia coli* which was inhibited at 1/250 by *M. communis* EO. Similar results were obtained for *Thymus algeriensis* whose main EO compounds are α-pinene (20,5%), β-pinene (8,02%) and limonene (4,85%) reported for their weak activity (Chalchat *et al*., 2000).

Table 2. EO antibacterial activity

	Dilution				Dilution				Dilution	Dilution	Dilution	Dilution	Dilution
	1/100	1/100	1/100	1/100	1/250	1/250	1/250	1/250	1/500	1/1000	1/2000	1/3000	1/5000
	RO	TV	MC	AHA	RO	TV	MC	AHA	TV	TV	TV	TV	TV
Staphylococcus aureus ATCC29213	+	–	+	–	+	–	+	+	–	–	–	+	+
Staphylococcus aureus 6816	+	–	+	–	+	–	+	+	–	–	–	+	+
Listeria monocytogenes ATCC19195	+	–	+	+	+	–	+	+	–	–	–	+	+
Escherichia coli ATCC35218	+	–	–	–	+	–	+	+	–	–	–	+	+

RO: Rosmarinus officinalis; TV: Thymus vulgaris; MC: Myrtus communis; AHA: Artemisia herba-alba. – : inhibition, + : development.

IV – Conclusion

The EO chemotypes were 1,8-cineol (39,0%), thymol (60,0%), α-pinene (29,5%) and thujone (42%) for rosemary, thyme, myrtle and sagebrush, respectively. In addition, *T. vulgaris* EO showed the highest anti-bacterial effect against all the tested bacteria. This bioactivity is mainly because these EOs are rich in thymol, known for its effectiveness against microbial agents. *Artemisia herba-alba* has shown a moderate activity, whereas *Rosmarinus officinalis* and *Myrtus communis* EO has shown the weakest activity against the tested strains. It was concluded that the studied species have antibacterial activity that can be tested to modulate the ruminant fermentation in order to improve the efficiency of digestion and reduce the production of greenhouse gases.

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