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I J N R E

First on the chemical characterization and antimicrobial activity of the essential oil from *Satureja candidissima* (Munby) Briq. native plant from west Algeria

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Abstract:

The present work reports the in vitro antimicrobial activitiy of the essential oil from *Satureja candidissima* (Munby) Briq. and its GC/MS analysis. To our best knowledge, this is the first study on this aromatic plant in Algeria, due to its rarity or its conformity with other plants known by the same common name. The main compounds of the hydrodistilled essential oil were pulegone (47.62%), menthone (24.50%) and terpinen-4-ol (11.63%). The essential oil of exhibited a good antimicrobial activity against 5 bacteria and a yeast (*Candida albicans*) with MBC values ranging from 6.25 to 100 µl/ml. Very weak activity was observed against *Klebsiella pneumoniae* and no activity against *Pseudomonas aeruginosea*.

Keywords: Essential oil, aromatic plant, antimicrobial activity, *Satureja candidissima* (Munby) Briq.

Résumé:

Le présent travail porte sur l'activité antimicrobienne in vitro de l'huile essentielle de *Satureja candidissima* (Munby) Briq. Et son analyse GC/MS. À nos connaissances, il s'agit de la première étude réalisée sur cette plante aromatique en Algérie, en raison de sa rareté ou de sa conformité à d'autres plantes connues sous le même nom. Les principaux composés de l'huile essentielle obtenue par hydrodistillation étaient le pulegone (47,62%), la menthone (24,50%) et le terpinène-4-ol (11,63%). Cette huile essentielle est caractérisée par sa bonne activité antimicrobienne contre 5 bactéries et une levure (*Candida albicans*) avec des valeurs de MBC allant de 6,25 à 100 µl / ml. Une très faible activité a été observée contre *Klebsiella pneumoniae* tandis que *Pseudomonas aeruginosea* est résistante.

Mots clés : Huile essentielle, plante aromatique, activité antimicrobienne, *Satureja candidissima* (Munby) Briq.

1. Introduction

Medicinal Plants have formed the basis of sophisticated traditional medicine practices that have been used for thousands of years (Salim *et al.,* 2008), and the interest in their use by the general public over the last twenty years has been phenomenal. This, in turn, has encouraged healthcare

professionals to take a more active interest in acquiring knowledge about these remedies (Sandberg and Corrigon, 2001).

So, the term Phytotherapy is now accepted in Europe in English, French and German. It means simply a therapy using processed or crude plant material. There are several sub-disciplines involved: botany for the identity of the plant used; agricultural knowledge for the cultivation of the drug plant; phyto-chemistry for the extraction and structure elucidation of active ingredients, pharmacology for the mode of action of active substances; internal medicine for the clinical use of the product (Sandberg and Corrigon, 2001).

It should be remembered that before the introduction of synthetic chemistry, all remedies were natural products, and the corresponding drugs were included in the various pharmacopoeias. The difference between modern and old phytotherapy is that nowadays extracts of the crude drugs can be standardized to a certain content of active ingredients thus, guaranteeing the correct and reproducible dosage (Sandberg and Corrigon, 2001; Salim et *al.*, 2008). Since, many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form, especially plant secondary metabolites (Salim et *al*., 2008).

Essential oils are the volatile liquids that are distilled from plants, and for their power in the realm of personal, holistic healthcare, it becomes important to evaluate their activities for further medicinal uses (Higley & Higley, 2005). They contain a mixture of many organic compounds which include alcohols, aldehydes, esters, ketones, terpenes, oxides, coumarins, lactones, acids, aromatic aldehydes, and phenols. The relative amount of each compound contained in each oil denotes its therapeutic value (Stevensen, 1998).

Satureja candidissima (Munby) Briq. is an aromatic medicinal plant belonging to family Lamiaceae, it is a covered plant except in inflorescence, with a thick whitish velvety tomentum, ovoid leaves and pinkish flowers long 8-12 mm (Quezel and Santa, 1963).

Harvested between rocky lawns (400 m.) in west Algeria (Oran and its surroundings, it is not dominant compared to the other species of the genus, but still used by locals to to flavor culinary dishes and also to treat flu and fever, intestinal worms and painful periods. It's called by locals " Nabta beda" or "Zaater cheleuh", and is a synonyme of *Melissa candidissima* (Munby) (Quezel & Santa, 1963), and *Calamintha candidissima* (Munby) Benth. var. *laxiflora* (mnhn.fr/fr).

2. Materials and methods

2.1. Plant material

The aerial parts of the plant were collected at flowering season from west Algeria (wilaya Ain Temouchent), identify in the Laboratory of Botanic, University of Tlemcen, Algeria. The plant was dried at room temperature for two weeks.

2.2. The extraction of essential oil

It was done by hydrodistillation of the aerial part of the plant, where 100 g of dry plant is introduced into a flask bi collar and moistened with water; the mixture is brought to a boil for 2-3 hours. The water laden vapours of essential oil, the refrigerant passing through, condense and drop into a separator funnel, water and oil separate by density difference. The essential oil is stored in dark vials at 4°C until use.

2.3. GC/MS analysis

Gas chromatography, type Perkin Elmer CLARUS 500 was used, with Flame Ionization Detector (FID), coupled with mass spectrometry Perkin Elmer Clarus 600, two fused silica capillary columns (30mx0.25mm, 0.25µm film thickness) are used: the Elite-WAX is a polar column with Polyethylene Glycol as stationary phase and the Elite 1 column of 100% Dimethyl Polysiloxane stationary phase as the nonpolar column. Carrier gas is Hydrogen with a flow of 45 ml/min. the initial temperature is 50°C and rise with 3°C/min and then held isothermally at 230°C (20min). Injection volume: 0.2µl of pure oil.

Identification of the components was done by the comparison of their relative retention times and mass spectra with NIST Atomic Spectra Database, and personal collection of the Rosier Davenne's laboratory.

2.4. Antimicrobial activity

The antimicrobial activity of *Satureja candidissima* essential oil was evaluated against laboratory reference strains (Table 1).

Table 1: Laboratory strain references and origin.

	Microorganisms	Gram	Reference	Origin
Bacteria	*Pseudomonas aeruginosa*	Negative	ATCC 27853	MNHN
	Escherichia coli		ATCC 8739	MNHN
	Klebsiella pneumoniae		IBMC Strasbourg	MNHN
	Staphylococcus aureus	Positive	ATCC 6538	MNHN
	Listeria monocytogenes		ATCC 19111	MNHN
	Bacillus cereus		ATCC 25921	MNHN
	Micrococcus luteus		ATCC 9341	MNHN
Yeast	*Candida albicans*		ATCC 10231	MNHN

2.4.1. Disc diffusion method

According to the NCCLS 2001, 100µl of microorganisms suspension, containing $2x10^8$ CFU/ml is inoculated on Mueller–Hinton agar previously sterilized and solidified in Petri dishes, then filter paper discs (6 mm in diameter) impregnated with 10µl of oil are placed. After an incubation at 37°C for 24 h for bacteria and at 30°C for 24 and 48 h for *Candida albicans*., the diameters of the inhibition zones (mm) were measured.

2.4.2. MIC/MBC assay

For bacteria and *Candida albicans*, a micro-dilution method was used to determine the MIC. All tests were performed in Muller Hinton Broth. The essential oil was dissolved in 1% dimethylsulfoxide (DMSO). Serial dilutions of oil were prepared in well microtiter plate over the range of 0.78–100µl/ ml. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to $5x10^5$ CFU/ml for bacteria and to $2.5x10^6$ CFU/ml for *Candida albicans*, incubated for 24h. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not

demonstrate visible growth (no visible turbidity). MBC is calculated by sub-culturing some liquid from the concentrations showing no turbidity, on solid media and then incubating, the MBC will be the lowest concentration where no positive culture (no growth) is observed (NCCLS, 2001).

3. Results and Discussions

3.1. Antimicrobial activity

The essential oil yield from aerial parts of *Satureja candidissima* harvested from west Algeria is **0.90%** (w/w), it's an important yield compared to other species of Satureja genus, as *Satureja calamintha. Nepeta* L. (called by the same name by locals: Nabta) from Algeria (0.45%) or *Satureja Calamintha* (L.) *Scheele* from Morocco with a yield of 0.082% (Ech-Chahad et *al.*, 2013) or several satureja species from Turkey (0.4 to 1.1%) (Azaz et *al.*, 2002).

3.2. GC/MS analysis

Table 2 indicates the chemical components of the essential oil obtained from the aerial parts of *Satureja candidissima* collected from west Algeria. Twelve constituents were identified, representing 90.61% of the total oil fraction. The major constituents of the oil were pulegone (47.62%), menthone (24.50%) and terpinen-4-ol (11.63%).

Satureja species are widely used in foods as flavour condiments and in folk medicine, earlier investigations studied their essential oils composition and show to be rich in components such as carvacrol,γ-terpinene, thymol, p-cymene and borneol (Dardioti et *al.*, 1997; Slavkovska et *al.*, 1997; Azaz et *al.*, 2002; Ghannadi, 2002; Adiguzel et *al.*, 2006; Ech-Chahad et *al.*, 2013). But *Satureja candidissima* has a different profil.

Table 2: Chemical composition of the essential oil of *Satureja candidissima* growing in west Algeria (RT: retention time).

Components	Aire (%)	RT (min)
α-pinene	0.53	10.03
camphene	0.53	10.52
sabinene	0.24	11.46
β-pinene	0.56	11.62
myrcene	0.99	12.19
p-cymene	0.06	13.42
limonene	0.90	13.48
linalool	0.25	16.74
menthone	**24.50**	18.86
iso-menthone	**2.80**	19.23
terpinen-4-ol	**11.63**	19.65
pulegone	**47.62**	22.56
Total	**90.61**	/

3.3. Antimicrobial activity

The antimicrobial activity of *Satureja candidissima* essential oil against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, MIC and MBC values. The results are given in Table 3. The data of the study clearly indicated that the essential oil has a good antimicrobial activity against both bacteria and the yeast. Regarding the bacteria tested, the oil could not inhibit the growth of *Pseudomonas aeruginosa* and has a very weak activity against *Klebsiella pneumoniae*.

For the other microorganisms, the inhibition zone ranges from 13 to 26 mm, MIC values from 0.78 to 6.25 µl/ml, and MBC values from 6.25 to more than 100 µl/ml. the results show also that *Candida albicans* is the most sensitive to the essential oil.

Table 3: Antimicrobial activitiy of *Satureja candidissima* essential oil.

Microorgamisms	Inhibition diameter (mm)	MIC (µl/ml)	MBC (µl/ml)
Pseudomonas aeruginosa	6± 0.0	-	-
Klebsiella pneumoniae	7.5 ± 0.5	-	-
Escherichia coli	13 ± 0.1	3.125	25
Staphylococcus aureus	17.5 ± 0.5	0.78	100
Listeria monocytogenes	14 ±0.0	6.25	50
Bacillus cereus	13.5 ±0.5	3.125	>100
Micrococcus luteus	18 ± 1	6.25	50
Candida albicans	25 ± 1	0.78	6.25

Many of the previous studies demonstrated that the members of the genus Satureja show a high antimicrobial activity due to the presence of thymol, carvacrol, and their precursors (Azaz et *al.*, 2002; Adiguzel et *al.*, 2006) in this study the richness in monoterpenes of the essential oil of *Satureja candidissima* give it a good antimicrobial potential, this means that antimicrobial activities are not only due to the presence of phenols and alcohols, but also the synergy between all the constituents of an essential oil can display activities.

On the other hand, the resistance of *K. pneumoniae* is due to the possession of a capsule composed of complex acidic polysaccharides, protects the bacteria from serum bactericidal factors and transports complex enzymatic systems, which seems to prevent essential oils from accessing the fragile internal membrane (Fournomiti et *al*., 2015)

For *P. aeruginosa*, its resistance may be due to the rich hydrophobic lipopolysaccharide (LPS) present in the outer membrane, which can provide protection against different agents, thus limiting the diffusion of hydrophobic compounds through it, while this extra complex membrane is absent in Gram-positive bacteria (Peterson and Shanholtzer, 1992).

4. Conclusion

Resistance to antimicrobials acquired by microbes is a serious public health issu, so searching for new active molecules in nature becomes necessary.

The use of essential oils as potential replacement to synthetic antibiotics, is one of the solutions to this problem, in this study, we found that the aromatic oil from the endemic plant "*Satureja candidissima* (Munby) Briq. has an excellant activity against Gram negative and Gram positive bacteria that cause the most important infections such *Escherichia coli* and *Staphylococcus aureus,* and the yeast *Candida albicans* with MIC ranging from 0.78 to 6.25 µl/ml of essential oil.

However, we need more studies to establish the relation "composition-activity" and identify the mode of action of our essential oil against bacteria and yeasts, so that in future, we will be able to formulate new antimicrobials based on *Satureja candidissima* essential oils.

References

Adiguzel A, Ozer H, Kilic H and Cetin B (2006). Screening of Antimicrobial Activity of Essential Oil and Methanol Extract of *Satureja hortensis* on Foodborne Bacteria and Fungi. Czech J. Food Sci 25 (2), pp 81-89.

Azaz D, Demircib F, Satıla F, Kürkcüoglu M and Hüsnü Can Baser K (2002). Antimicrobial Activity of Some Satureja Essential Oils. Z. Naturforsch 57c, pp 817-821.

Dardioti A, Cook CM, Kokkini S and Lanaras T (1997). Composition of *Satureja horvatii* subsp. *macrophylla* oil isolated by hydrodistillation and micro-simultaneous distillation/extraction. J. Essent. Oil Res. 9, pp 663–666.

Ech-Chahad A, Farah H and Bouyazza L (2013). Composition chimique de l'huile essentielle de *Satureja calamintha* (L.) Scheele du Maroc. Afr. sci. 9 (3), pp 77-81.

Fournomiti M, Kimbaris A, Mantzourani I, Plessas S, Théodoridou I, Papaemmanouil V, Kapsiotis I, Pan opoulou M, Stavropoulou E, Bezirtzoglou E and Alexopoulos A (2015). Antimicrobial activity of essential oil of cultivated Oregano (*Oreganum vulgare*), Sage (*Salvia officinalis*) and Thyme (*Thymus vulgaris*) against clinical isolates of *Escheriachia coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*. Microb. Ecol. Health Dis. **26**, pp 1-7.

Ghannadi A (2002). Composition of the essential oil of *Satureja hortensis* L. seeds from Iran. J. Essent. Oil Res. **14**, pp 35–36.

Herbarium P00083221, In : mnhn.fr/fr . Available via. https://science.mnhn.fr/institution/mnhn/collection/p/item/p00083221 , [10/03/2016].

Higley A and Higley C (2005). Quick Reference Guide for Using Essential Oils. 9th Edition, Abundant Health,USA, pp 2-3.

NCCLS (2001). Performance Standards for Antimicrobial Susceptibility Testing, 11th Informational Supplement. M100-S11. Wayne, PA, USA.

Peterson LR and Shanholtzer CJ (1992), Tests for bactericidal effects of antimicrobial agents: technical performance and clinical relevance. Clin. Microbiol. Rev., vol. 5, no. 4, pp 420–432.

Quezel P and Santa S (1963). Nouvelle Flore de l'Algérie et des Régions Desertiques Méridoniales. Tome II, CNRS, Paris, pp 810.

Salim AA, Chin YW and Kinghorn AD (2008). Drug Discovery from Plants. In: Ramawat KG and Merillon JM. Bioactive Molecules and Medicinal Plants. Springer-Verlag, Berlin Heidelberg, pp 1-2. DOI: 10.1007 / 978-3-540-74603-4_1.

Sandberg F and Corrigon D (2001). Natural Remedies, their origins and uses. Taylor & Francis, London and New York, pp 1-3.

Slavkovska V, Jancic R, Milosavljevic S and Djokovic D (1997). Variability of the essential oil composition of the species *Satureja montana* L. (Lamiaceae). J. Essent. Oil Res.9, pp 629–634.

Stevensen CJ (1998). Aromatherapy in Dermatology. Clin.Dermatol. 16, pp 689-694.