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Chemical Composition and Antimicrobial Activity OF Essential Oil from the Leaves of *Satureja hortensis* L.

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Abstract: Interest in essential oils was recently revived with their popularity increasing in medicine, pharmacy, and aromatherapy.the present study is aimed to determine the volatile composition of *Satureja hortensis* L.essential oil (EO) by means of gas chromatography coupled to flame ionization/mass spectrometry detection. Moreover, the antimicrobial activities of the EO on same food borne pathogens were tested. The results showed that the essential oils was analyzed by GC/GC-MS and resulted in the identification of twenty compounds representing 97.52 % of the oil were identified. The major constituents of the isolated oils were the hydrocarbon and oxygen compounds are: Cadinol 24.02%, thymol 19.57%, Himachalene 10%, Selinene8.20%.The antimicrobial effect of *Satureja hortensis L.*:essential oil "in vitro" condition was determined using the agar diffusion method and it was found that it was active The present study suggests that the essential oil can be considered as new and potential source of natural antimicrobial agents.

Keywords: *Satureja hortensis* , leaves, Essential oil, GC/SM, antimicrobial activity.

I. Introduction

Nowadays, there is a renewed interest in medicinal plants because of the potentially important sources of bioactive substance that may be very important in the field of medicine. Essential oils could be obtained from roots and rhizomes (such as ginger), leaves (mint, oregano and eucalyptus), bark and branches (cinnamon, camphor), flowers (jasmine, rose, violet and lavender) and fruits and seeds (orange, lemon, pepper, nutmeg). In general, essential oil represents less than 5% of the vegetal dry matter. Although all parts of the plant may contain essential oils, their composition may vary with the part of the plant employed as raw material. Other factors such as cultivation, soil and climatic conditions and harvesting time can also determine the composition and quality of the essential oil [1].

One of the most interesting chemical aspects, emerging from all these searches, is their high chemodiversity, i.e., different composition, sometimes even dramatic, among phyto complex from plants belonging to the same species, but grown in different geographical conditions and Biodiversity contexts. The quality of medicinal and spice plants raw materials and their products has to fulfill the requirements of safety, efficacy and stability [2].

Numerous species of medicinal plants from Algeria are important aromatic and ornamental plants, as well as being medicinal. [3]. *Satureja hortensis* L., commonly known as summer savory, belongs to family Lamiaceae. It is an annual herbaceous shrub growing approximately 30 to 60 cm in height,

with tiny purple flowers and slender bronze-green aromatic leaves on hairy stems. [4]. the essential oil and oleoresin are used in the food industry. In addition, the essential oil of S. hortensis has been used in the perfume industry, either alone or with other essential oils [5]
In the traditional medicine, *Satureja hortensis L*.:leaves and flowering aerial parts of species have been used extensively like, antimutagenic,antidiabetic, antibacterial, antifungal and other biological activities.
The aim of our research was to evaluate the chemical composition and antimicrobial of essential oil extracted from fruit of *Satureja hortensis.* The best of our stady is the valorization of medicinal and aromatic plants of the Algerian floral.

I. Experimental Section

II.1 Plant material collection

The leaves of *Satureja hortensis L.* specimens were collected in March 2015 from collected from jedioua Relizane situated in the North West of Algeria. This plant was identified by botanists of Faculty science a voucher specimen was deposited in the Herbarium of the Department of Ecology at the Agronomic Institute under code number 2015-52520.
The leaves of *Satureja hortensis L.:* were shades, dried, and stored in a tightly closed container for further use. The essential oils were obtained by hydro-distillation from the plant material using a Clevenger –type

apparatus for 3h. The essential oil was dried over anhydrous Na2SO4 and stored in a scaled vial in the dark; at 4°C [6]. The essential oil yield was calculated on a dry weight by gravimetric method.

II.2. Analysis of the essential oils

Following physicochemical analyses are determined: the refractive index, density, polarimeter deviation; point of freezing, solubility in ethanol at 9^{o}C; and the acidity. The oil was analysed by GC on a Perkin- Elmer 8500 gas chromatograph equipped with a FID, fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32 mm; film thickness, 0.25 µm). The column temperature was programmed from 75 ^{o}C to 200 ^{o}C at a rate of 2.5 ^{o}C/min. The injector and detector temperatures were programmed at 230 ^{o}C and 300 ^{o}C, respectively. Helium was used as carrier gas at flow rate of 0.6 mL/min. The GC-MS analysis was carried out using two different GC-MS systems. The first was a Hewlett Packard 5973-6890 GC-MS operating on EI mode (equipped with a HP 5MS 30 m x 0.25 mm x 0.25 m film thickness capillary column). Helium (1 mL/min) was used as carrier gas. Temperature program: initial temperature of the column was 60

°C (for 5 min), then raised to 280 ^{o}C at 3 °C/min, and held there for 30 min (total time: 93.33 min). The compounds were identified by comparison of their retention indexes (RI) [7]. retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, NIST02, Wiley 575 libraries spectra and the literature [8]. The percentage composition of the essential oil is based on peak areas obtained without FID factor corrections. The second GC-MS system analysis was a Finnegan Trace GC Ultra system, operating on EI mode and equipped with AT™ Aquawax 30 m x 0.32 mm x 0.25 µm film thickness capillary column. Helium was used as the carrier gas, at a flow rate of 1.5 mL/min (constant flow) and a 1:10 split ratio. Temperature program: initial temperature of the column 60 °C (for 5 min), then raised to 235 ^{o}C at 3°C/min, retention indices (RI) determination, a hydrocarbon series was analyzed on GC together with the essential oil on a polar columns, and their linear retention indices were determined and compared with those reported in the literature [9]. And also by computer matching them with the NIST/EPA/NIH Mass Spectral Library data .

II.3. Microbial strains

Antimicrobial activity was carried out according to the disc diffusion assay, tested in vitro against *Escherichia coli, Bacillus cereus*, *Salmonella typhimurium,* and *S.pneumonia* suspensions were

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adjusted to1×10^7 CFUmL− (equivalent to 0.5 McFarland). Antimicrobial tests were carried out using the disc diffusion

Method. The Muller-Hinton mutriet agar and dimethyl sulfoxide (DMSO) solutions (in ratio 1:25 v.v-1) were vortexed for 2 min and immediately 20 ml were poured into sterile Petri dishes (90 mm diameter) and left to set for 30 min. Paper discs (6 mm diameter) were impregnated aseptically with 3 µl of essential oil at final concentrations of 1-20 µg/ml and placed on the inoculated agar surfaces. After aerobic incubation for 24 hours at 37°C, the antimicrobial activity was estimated by measuring the diameters of inhibition zone [9]. The control test by aqueous DMSO alone showed no toxicity in the concentrations used for these bacteria. The antibacterial mini- mum inhibitory concentrations (MICs) were per- formed according to the Mueller- Hinton broth microdilution method in 96 multiwell microtiter plate. The essential oils were dissolved in the aqueous DMSO and the initial concentration was 25 *Satureja hortensis* is /ml. The initial test concentration was serially diluted two fold. Each well was inoculated with 5 µg/ml of suspension containing 10^7 CFU/ ml of bacteria and incubated for 24 hours at 37°C. The MIC of the tested material was determined as the lowest concentration at which no visible growth of the microorganism had occurred. Each test was carried out in triplicate.

III. Results and Discussion

Complete knowledge of the chemical constituents of *Satureja hortensis L.:*will facilitate synthesis of other chemicals and compounds for their potential applications in the cosmetics, food, and pharmaceutical sectors, [10]. Essential oils are a mixture of various compounds characterized by aromatic smell, generally liquid colorless to slightly yellowish color and insoluble in water, but soluble in organic solvents [11]. The essential oil obtained by hydrodistillation of aerial parts *Satureja hortensis L.:* slightly yellowish color and insoluble in water but soluble in organic solvents and possessed a distinct sharp odor. The yields were 0.69%.Determination of density was done by double weighing d = 0.810, the Specific rotation = -0.65 by polarimetry and the refractive index n = 1.4660 by an interferometric method. (Table 1).

Table 1. Physicochemical composition of Satureja hortensis L

Specification	Density D20	Refractive index	Optical activity N20	Solubility in ethanol 90(%)	Freezing Point (°C)
Satureja hortensis	0.810	1,4660	-6.5	1:3	-19

The chemical composition of the extracted *Satureja hortensis* was analyzed by GC-MS, which resulted in identification of twenty volatile compounds. The analysis of the volatile constituents was carried out using GC-MS systems, equipped with columns of different polarities (HP-5 and Aquawax, respectively). The chemical compositions are summarized in Tables 2. The identified components represented 97.04% of all the components found in the oil samples. These percentages were based on normalization of peak areas without application of the response correction factor. The major components included: resulted in the identification of Twenty compounds representing 97.52 % of the oil were identified. The major constituents of the isolated oils were the hydrocarbon and oxygen compounds are: Cadinol 24.02%,myrtenal 19.57% , Himachalene 10%, Selinene8.20%: Pentcosane 19.04% , sabinene hydrate 11.10%.Other components were present with smaller percent. By other finding the chemical composition of S. hortensis was analyzed using GC-MS and 63 different compounds were identified. The compounds identified represented in total 95.57% of the essential oil. The major constituents of this oil were: carvacrol (73.24%), o-cymene (7.37%), γ-terpinene (6.13%) and thymol (1.70%). Of 63 compounds, 13 have not been identified. The percentage composition of the remaining 59 compounds ranged from 0.59% to 0.003% [12] -Variation in essential oil content and composition of S. hortensis L. from different origins has been reported by several authors. Thymol (29–43%) was the major component of wild accessions of S. hortensis from

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Turkey and oil content of the accessions varied between 1.28% and 4.75% [13]

This is the first report of the chemical composion of *Satureja hortensis from algeria*.Thus, further investigations are necessary to study the potential of the essential oil leaves.

The essential oil of *Satureja hortensis* exhibited strong antibacterial activity against *Staphylococcus aureus, Listeria grayi ,Escherichia coli ,Pseudomonas aeruginosa ,Staphylococcus epidermidi.*
The antibacterial effects depended on its concentration and action time. Kill-time assays also confirmed the essential oil had a significant effect on the growth rate of surviving[14]. We hypothesized that the essential oil may interact with the cell wall and membrane first. On the one hand it destroys cell wall and membranes, next causing the losses of vital intracellular materials, which finally result in the bacterial death[15]. Besides,essential oil penetrates to the cytoplasmic membrane or enters inside the cell after destruction of cell structure, and then inhibits the normal synthesis of DNA and proteins that are required for bacterial growth. These results suggested that the effects of the clove essential oil on the growth inhibition of all bacteria positive gram and negative may be at the molecular level rather than only physical damage[16]. Findings in this study supported the observations of some other researchers about *Satureja hortensis* containing some substances with antibacterial properties [17]. Since only the essential oils from Satureja hortensis have been evaluated in terms of antimicrobial activity against a limited number of microorganisms up to now [18].
This study revealed that leaf essential oil exhibited the highest potency of antimicrobial among all bioactivity tests, suggesting the potential application of this essential oil in skin care. However, further study is required to confirm the antimicrobial activity of this essential oil on a significant strain number of the same

bacteria. In addition, the safety of this essential oil including cytotoxicity and skin irritation and allergy requires further investigation

Table 2. *The major identified components in essential oil from Satureja hortensis L.:analyzed by GC-MS technique with retention indices on HP-5MS capillary Column*

Volatile compounds	**RI[a]**	**RII[b]**	**Area %**
. α-Pinene	939	939.5	0.6
sabinene	939	941	0.13
Verbenene	968	970	6.5
thymol	1188	1189	12.57
Citronellol	1199	1201	1.22
carvone	1236	1239	0.88
geraniol	1253	1256	0.85
geranial	1268	1271	2.16
caryophyllene	1409		1.5
Farnesene	1459	1460	03.50

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-Himachalene	1493	1498	10.73
remophilene	1504	1508	0.12
Selinene	1514	1521	8.20
Myristicin	1532	1533	0.12
Spathulenol	1550	1566	0.77
Piperitone	1578	1590	4.10
ryophyllene oxide	1580	1588	8.19
-Cadinol	1628	1634	24.30
Octacosane	1900	1909	5.4
Total			97.52

a= retention indices on MetSil column;b= retention indices on CP-Sil 88 column.

Table 3. *Inhibition zone (mm) using direct contact technique in agar medium and MIC (g/mL) for the essential oil using microdilution method in 96 multiwall microliter plate ATCC 14990*

Microorganism	**Strain**	**Diameter of inhibition**	**MIC (g/mL)**
Staphylococcus aureus	ATCC 29230	18.5±0.20	16.60
Listeria grayi	DSM 2060	12.0±0.34	13.50
Escherichia coli	ATCC 4350	16.3±0.10	15.50
Pseudomonas aeruginosa	ATCC 27853	11 ±0.02	10.00
Staphylococcus epidermidi	ATCC 14990	12.9±0.7	00.18

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IV. Conclusion

The chemical composition of the essential oil from *Satureja hortensis*L. Leaves was analyzed by GC/GC-MS and resulted in the identification of 20 compounds. The main constituents of the essential oil were Cadinol 24.02%, thymol 19.57% , Himachalene 10%, Selinene8.20%, Pentcosane 19.04% , sabinene hydrate 11.10%. The antimicrobial effect of *Satureja hortensis* essential oil "in vitro" condition was determined using the agar diffusion method and it was found that it was active which may find its application in food.

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V. References

[1] Sonam Chouhan, Kanika Sharma and Sanjay Guleria, Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives.medecine ,(2017),46(2): 446-475.
[2] Burt S.oils: their antibacterial properties and potential applications in foods--a review. , (2004) 1; 94(3):223-53.
[3]
[4] Clark, A.M., Natural products as resource for new drugs. Pharmaceutical Research 13,(1996) 1133–1141.
[5] Sefidkon, F., Jamzad, Z., & Mirza, M.. Chemical variation in the essential oil of *Satureja sahendica* from Iran. Food Chemistry, (2004) 325–328
[6] Chouitah o, Essential Oil from the Leaves of Ajuga iva: Chemical Composition and Antimicrobial Activity" that has just been published recently on "Journal of Essential Oil Bearing Plants" Volume 20, (2017) Issue 3.
[7] DWINJ. A. VELDHUIZEN OHANNAL. M. TJEERDSMA-VANB Requirements for the Antimicrobial Activity of Carvacrol OKHOVEN, Health & Food Safety (2006) 417.
[8] Joulain, D. and Koenig, W.A.,the Atlas of Spectral Data of Sesquiterpene Hydrocarbons. Hamburg, Germany: EB- Verlag,(1998) 658.
[9] Phlomis bovei De Noé subsp. Bovei,Chemical Composition and Antimicrobial Activity of the Essential Oil of Algerian(2007) 144.
[10] Mann1, J.L Markham1 , H.C. Bell2 , J.E. Gustafson3 , J.R. Warmington3 and S.G. Wyllie1,The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil) S.D. Cox1 , C.M.(1999)122
[11] Mohamed Sabry, Abeer Mohamed El Sayed1 and Salmin Khalid Alshalmani2 , Analysis and Potential Cytotoxic Activity of Haplophyllum tuberculatum Essential Oils Against Lung and Liver Cancer Cells, Journal, Vol 8, Issue 1, (2016) 233-240 .
[12] TROMBETTA, D., CASTELLI, F., SARPIETRO, M.G., VENUTI, V., CRISTANI, M., DANIELE, C., SAIJA, A.,
[13] MAZZANTI, G. and BISIGNANO, G.,. Mechanisms of Antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy (2005) vol. 49, no. 6, 2474-2478.
[14] Klaudia Magierowicza , Edyta Górska-Drabika,*, Cezary Sempruchb; The insecticidal activity of Satureja hortensis essential oil and its active ingredient - carvacrol against Acrobasis advenella (Zinck.) (Lepidoptera, Pyralidae)(2018).
[15] Tepe, B., Cilkiz, M.,. A pharmacological and phytochemical overview on Satureja. Pharm. Biol. (2016)54, 375-412.
[16] Keniche A., Mezrai A., Kajima Mulengi J.,Novel class of phosphonoaziridines as antibacterial agents, The Open Conference Proceedings Journal, Dubai, 2 (2011) 28-36.
[17] Deans, S.G., Svoboda, K.P., Antibacterial activity of summer savory (*Satureja hortensis L.)* essential oil and its constituents. Journal of Horticulturel Science (1989). 64, 205–210.
[18] Zargari, A. Medicinal Plants, 4th ed. Tehran University Publications, Tehran, (1990). 42–45.

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