

The Antimicrobial Activity of the Essential Oil of *Salvia officinalis* Harvested in Boumerdes

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Abstract—The Algeria by its location offers a rich and diverse vegetation. A large number of aromatic and medicinal plants grow spontaneously. The interest in these plants has continued to grow in recent years. Their particular properties due to the essential oil fraction can be utilized to treat microbial infections. To this end, and in the context of the valuation of the Algerian flora, we became interested in the species of the family *Lamiaceae* which is one of the most used as a global source of spices. The plant on which we have based our choice is a species of sage "*Salvia officinalis*" from the Isser localized region within the province of Boumerdes. This work focuses on the study of the antimicrobial activity of essential oil extracted from the leaves of *Salvia officinalis*. The extraction is carried out by essential oil hydrodistillation and reveals a yield of 1.06%. The study of the antimicrobial activity of the essential oil by the method of at aromatogramme shown that Gram positive bacteria are most susceptible (*Staphylococcus aureus* and *Bacillus subtilis*) with a strong inhibition of growth. The yeast *Candida albicans* fungus *Aspergillus niger* and have shown moderately sensitive.

Keywords—Aromatogram, anti-microbial activity, essential oil, *Salvia officinalis*.

I. INTRODUCTION

A large number of plants, aromatic, medicinal, which have very interesting biological properties, which find application in various fields namely medicine, pharmacy, cosmetics and agriculture. The medicinal plants included in the pharmacopoeia are considered drugs. A medicinal plant is a plant whose one of the organs, such as leaves or bark has curative virtues, and sometimes toxic as its dosage. However, the evaluation of herbal antioxidant and antimicrobial properties as remains a very interesting and useful task, especially for plants of rare or less common or not known in medicine and medicinal traditions use. These plants represent a new source of active compound. Indeed, secondary metabolites and are still the subject of much research in vivo and in vitro, including the search for new natural constituents such as phenolic compounds, saponins and essential oils [1].

Sage is native to the edges of the Mediterranean. [2]. This is a sub-shrub that was introduced in Eastern Europe and Northern Middle Ages and in America in the seventeenth century. *Salvia officinalis* is common throughout Algeria particularly at scrub and woodland [3]. It is found in Oran (Dahra) bridge Cheliff in Saida, the Aures and Oued Abdi. It is also referred to Ghazaouet and gardens [4] are smaller and

sessile upper leaves [5]. Sage is by its astringent tannin, catechol nature that transforms storage phlobaphene inactive. It also has bactericidal properties associated with the presence of a diterpene acid, salvine and its monomethyl ester [6]. *Salvia officinalis* is used to estrogenic properties [7]. In Algeria little study is conducted on *Salvia officinalis* is in this context that the present work is performed. The objective of this work is to demonstrate the antimicrobial activity of essential oil of sage. This activity was measured in bacteria, yeasts and fungi.

II. MATERIALS AND METHODS

A. Plant Material Used

The leaves of *Salvia officinalis* used for this study come from the Isser region in the province of Boumerdes. The plant was harvested in May 2012.

Botanical classification of the sage: [3]

Kingdom: *Plantae*

Phylum: *Spermatophytes*

Sub - Phylum: *Angiosperms*

Class: *Dicotyledonous*

Family: *Labiatae*

Genre: *Salvia*

Species: *Salvia officinalis* L.

This species is divided into several sub-species, but modern taxonomy regards them as full species. We received more than 600 different species of *Salvia* [10]

The leaves are greenish-gray color aggrieved surface oblong, lanceolate (3-8 × 3-4 cm) to the lower leaves stalked (Fig. 1). The upper leaves are smaller and sessile. [5] The fruit is smooth lingering long in the form of the chalice; black to dark brown color, each achene, globular shape, about 2 mm in diameter, has a white scar and contains only a single exalbuminous seed. Flowering lasts from June to October. [10]

This species is naturalized in northern and central Spain, southern France, in the western Balkans, Central and Southern Europe and the Near East. While the main growing regions of former Yugoslavia, Albania, Hungary, the Czech Republic, Bulgaria, Romania, Spain, Italy, Turkey, Algeria, Germany, Austria, India, Indonesia, Tanzania, south Africa, the Caribbean and the United States. [10]

The sage is common throughout Algeria particularly in scrubland and woodland. [3] Sage is used since ancient times. It has stimulant properties, tonic, digestive, febrifuge and vulnerary. It is therefore a high degree cure. Sage tea is effective to aid digestion, identifies strengths of the stomach and intestine, quiet spasmodic vomiting. It activates the blood circulation in the skin. It is advantageously used against

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diarrhea, bloating, and night sweats. Sage also shows restorative, reconstructive circulatory disorders. The fresh leaves or powder of dried leaves friction, preserve teeth caries in mouthwash. Gargles of vinous infusion destroyed fungi thrush and ulcer scorbutic gums [4].

The aerial parts intended for use in the dry state are harvested in early flowering, which corresponds generally to a maximum content of aromatic constituents, unlike dill leaves, wormwood and peppermint, which are harvested before flowering [10].

Sage likes dry soils with good calcium intake, located in hot, sunny and protected from the wind. Indeed, sage is grown from seed in spring. The leaves are harvested in the summer [10].



Fig. 1 Sage leaves

1. Microbial Strains

The antibacterial and antifungal activity of the essential oil extracted from the leaves of *Salvia officinalis* was evaluated on different microorganisms. These six bacteria (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Serratia rubidaea*, *Pseudomonas aeruginosa* and *Salmonella enterica*), yeast (*Candida albicans*) and fungus (*Aspergillus niger*) selected for their high frequency to contaminate food and for their pathogenicity.

2. Harvesting, Drying and Grinding Leaves

The harvested leaves are dried in the shade for a week in a dry and ventilated place, then ground using a grinder. The powder is placed in airtight jars and stored dry (room temperature) and to protect from moisture (Fig. 2).



Fig. 2 The powder of sage

3. Extraction of Essential Oil by Steam Distillation

Operating Procedure

The plant material, consisting of leaves of *Salvia officinalis* (50 g) was introduced into a glass flask of 1000 ml, filled with distilled water (600 ml). All boiled for 2 hours and 30 minutes to 3 hours of time. During this operation, the charge of water vapor and essential oil condense refrigerant passing through a tilted. The distillate drips and is collected in a funnel. The

separation of essential oil (organic phase) and the aromatic water (aqueous phase) is done by adding petroleum ether. Finally, the organic phase was recovered and placed in a device (rotavapor) to evaporate the solvent (petroleum ether) and then retained the essential oil in the refrigerator at 4° C in sealed vials small dark to preserve the light.

III. RESULTS

A. Yield Calculation

After the execution of extraction by the use of an amount of 250 g of dried plant material, a mass of 2.66 g is obtained of essential oils. The value of performance of essential oil is 1.06%.

The yield of *Salvia officinalis* picked Isser in the region is 1.06%. This performance is comparable to those obtained in other studies. The extraction of the essential oil of common sage obtained by distillation for four hours in a Clevenger apparatus performance is based on the origin of the plant: France (2.05%), Hungary (2.50%), Portugal (2.90%), Romania (2.30%). [8] These yields are higher than those calculated in this study. Sage contains 1.5 to 2.5% essential oil [4]. This variation in performance can be attributed not only to the origin of the plant and the extraction technique but also the period of collection of plant material and drying method [9, 10].

B. Organoleptic and Physicochemical Characteristics

TABLE I
ORGANOLEPTIC

Organoleptic Characteristics	Results
Aspect	Liquid, Clear
Color	yellow
Odor	Camphor

The characteristics of essential oils obtained from the leaves of the sage are identical to those of the French AFNOR standards.

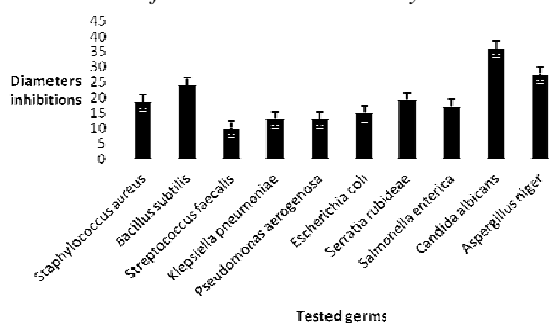
C. Physicochemical Characteristics

TABLE II
PHYSICAL AND CHEMICAL CHARACTERISTICS

Physical and Chemical Characteristics	Results
Refractive index	1.456
Acid	0.305

The essential oil derived from the leaves of the common sage to Isser is characterized by a refractive index of 1.46. The value of the acid number is 0.305 obtained Isser says freshness and stability essential oil of sage in this area.

D. Evaluation of the Antimicrobial Activity

Fig. 3 Results of the Antimicrobial Activity of Essential Oils of *Salvia officinalis*

1. Bacteria

TABLE III

RESULTS OF THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS ON BACTERIA

Germs tested	Diameter(mm)	1st test	2nd test	Average Diameter
	<i>Serratia rubidaea</i>		24	22
<i>Escherichia coli</i>		25	27	26±1
<i>Salmonella enterica</i>		32	30	31±1
<i>Pseudomonas aeruginosa</i>		13	13	13
<i>Staphylococcus aureus</i>		35	35	35
<i>Bacillus subtilis</i>		45	40	42.5±2.5

2. Yeast and Fungus

TABLE IV

RESULTS OF THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL YEAST AND MUSHROOMS

Germs tested	Diameter(mm)	1st test	2nd test	average diameter
		yeast <i>Candida albicans</i>	15	17
Fungi <i>Aspergillus niger</i>		17	17	17

The inhibitory effect of essential oil of *Salvia officinalis* harvested Isser applies on different bacterial strains

For Gram⁺ bacteria, two strains were the most susceptible namely *Bacillus subtilis* ($d = 42.5 \text{ mm} \pm 2.5$) and *Staphylococcus aureus* ($d = 35 \text{ mm}$) (Table III and Fig. 3). Some authors have worked on the antimicrobial activity of essential oils of the aerial part of *S. officinalis* in Marsa (Tunis) claiming a sensitivity of *Staphylococcus aureus* ($d = 12 \text{ mm}$) with the use of a disk of 6 mm [11] the results of the present study are in agreement with them. In Italy, the minimum concentration for inhibition of *Bacillus subtilis* 0.4 mg/ml. [12]

Tunisia Sfax more precisely some authors report a minimum inhibitory concentration ranging from 0.062 to 0.125 mg/ml [13].

Serratia rubidaea has proved moderately sensitive ($d = 23 \pm 1 \text{ mm}$) and *Pseudomonas aeruginosa* is slightly sensitive ($d = 13 \text{ mm}$). It is important to note that the Gram⁺ bacteria are more sensitive to the activity [14]. Indeed, the phenolics develop their action against bacteria through interaction with the cellular membrane. This activity is due to the hydrophobic nature of these compounds which can interact with cell membranes and accumulate in the lipid bilayer of bacteria, thus occupying the space between the fatty acid chains [15].

Candida albicans reveals a resistance to the effect of the essential oil *S. officinalis* [16] it is noted that in the present work this yeast has shown moderately sensitive ($d = 16 \pm 1 \text{ mm}$) (Table II). It is important to note that the Gram⁺ bacteria are more sensitive to the activity of the essential oil the sage that a gram negative bacterium [14]. The growth of resistant and multi-antibiotic resistant bacteria can be inhibited by certain essential oils. Citrus oils, lavender, mint, juniper, tea tree, thyme and eucalyptus reveal themselves particularly effective against *Staphylococcus aureus* resistant to methicillin and enterococci resistant to vancomycin. The essential oils isolated from two species of Korea thyme *Thymus magnus* and *Thymus quinquecostatus*, are also capable of inhibiting the growth of resistant bacteria such as *Streptococcus pneumoniae*, *Samonella typhimurium*, *Staphylococcus aureus* and *Salmonella entereditis* [17].

IV. CONCLUSION

All microbiological results obtained in this study show that the essential oil of *Salvia officinalis* has a significant antibacterial activity and antifungal. In which some strains seem to be distinguished by a very high compared to other strains sensitivity. Otherwise it would be desirable prospects to test the healing and anti-inflammatory activity of essential oils of *Salvia officinalis*

REFERENCES

- [1] T. Bahorun, B. Gressier, F. Trotin, C. Brunet, T. Dine, M. J. Luyckx Vasseur, M. Cazin, J.C. Cazin, and M. Pinkas, "Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations," *Arzneimittelforschung*, Drug Resear. 46 II (11), 1997, pp. 1086–1089.
- [2] B. L. Bezanger, M. Pinkas et M. Torck, "Les plantes dans la thérapeutique moderne." Ed. Maloin, Paris, 1975, p. 469.
- [3] P. Quezel, et S. Santa, "Nouvelle flore de l'Algérie et des Régions Désertiques Méridionales," Ed. CNRS, Vol.2, 1963, p. 1170.
- [4] A. Beloued, "Plantes médicinales d'Algérie." Ed. of. pub. Univ., Alger, 2005, p. 284.
- [5] J. Bruneton, "Pharmacognosie. Phytochimie et plantes médicinales," Ed. tec et doc, Lavoisier, Paris, 1999, p. 1120.
- [6] B. L. Bezanger, M. Pinkas, et M. Torck, "Plantes médicinales des régions tempérées." Ed. Maloin, Paris, 1990, p. 395.
- [7] P. Schauenberg, "Guide des plantes médicinales. Analyse, description et utilisation de 400 plantes," Ed. Delachaux. Paris, 2006, p. 396.
- [8] J. K. Chalchat, L. P. Carry, C. Menut, G. R. Lamaty, M. Malhuret, and J. Chopineau, "Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils," *J. Essent. Oil Res.*, 1997, 9, pp. 67–75.
- [9] S. Maric, M. Maksimovic, and M. Milos, "The impact of the Locality Altitudes and Stages of Developpement on the Volatile Constituants of *Salvia officinalis* L. from Bosnia and Herzegovina," *Journal of Essentielles Oil Research*, 18(2), 2006, pp. 178–180.
- [10] E. Teuscher, R. Anton, et A. Lobstein, "Plantes aromatiques. Épices aromates condiments et huile essentielle," Ed. Lavoisier, Paris, 2005, p. 522.
- [11] E. A. Hayouni, I. Chraief, M. Abedrabba, M. Bouix, J. Y. Leveau, M. Hammami, and M. Hamdi, "Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat," *INSAT, Tunisia*, 2008, p. 251.
- [12] A. Delamare, T. Ivete, M. Pistorello, L. Artico, A. Luciana, and E. Sergio, "Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil," *Food Chemistry*, 2005, 100, pp. 603–608.

- [13] M. Bouaziz, T. Yangui, S. Sayadi, and A. Dhouib, "Disinfectant properties of essential oils from *Salvia officinalis* L. cultivated in Tunisia," *Food and Chemical Toxicology*, 2009, 47, pp. 2755–2760.
- [14] M. Marino, C. Bersani, and G. Comi, "Impedance measurements to study the antimicrobial activity of essential oils from lamiaceae and compositae," *Int. J. Food microbial*, 2001, 6(7), pp. 187–195.
- [15] S. Griffin, S. Wyllie, J. L. Markham, and N. Leachd, "The role of structure and molecular properties of terpenoides in determining their antimicrobial activity," *Flavour Frager J.*, 1999, 14, pp. 322–332.
- [16] D. Miladinović, and L. J. Miladinović, "Antimicrobial activity of essential oil of sage from Serbia," *Facta universitatis Series: Physics Chemistry and Technology*, 2000, 2(2), pp. 97–100.
- [17] E. Guinoiseau "Molécules antibactériennes issues d'huiles essentielles séparation, identification et mode d'action." Thèse Doct; Univ. Corse, 2010, 272p.