Evaluate the beneficial effects of *Salvia officinalis* L. extracts by assessing their activity

Messaouda Rezagui[1*] and Nadia CHIAHI[2]

1. Toxicology Laboratory - Faculty of Medicine, Department of Pharmacy, UBM Annaba, Algeria.

2. Laboratory for Terrestrial and Aquatic Ecosystems - Mohamed-Cherif Messaadia, University -of Souk Ahras, Algeria

Abstract

Bioactive natural compounds are found in medicinal plants through secondary metabolites, which are commonly referred to as bioactive natural compounds. The use of antioxidants and antimicrobials has become increasingly popular in recent years. Our objective is to assess the antibacterial and antioxidant effects of *Salvia officinalis* extracts from the Nechmaya region (Wilaya Guelma - ALGERIA) in this study. This study aims to understand the organoleptic characteristics of essential oils and to obtain different yields depending on the harvest periods, as well as the polyphenol and flavonoid content of the plant. The antiradical activity results from the DPPH method indicate that the methanolic extract has a stronger antioxidant activity than the essential oil.

Keywords: antibacterial activity, antioxidant activity, methanolic extract, essential oil, *Salvia officinalis*

Rezagui, M. and N. CHIAHI. 2024**.** Evaluate the beneficial effects of *Salvia officinalis* L. extracts by assessing their activity. *Iranian Journal of Plant Physiology 14* (4), 5307-5313.

Introduction

Algeria's privileged biogeographic location between the Mediterranean basin and sub-Saharan Africa allows for a wide range of climates, leading to great ecological and floristic diversity. Over 3,000 species from different botanical families are present, 15% of which are endemic and have not been fully explored from phytochemical and pharmacological perspectives (Bouasla et al., 2021).

Our study investigates the antioxidant activity of *Salvia officinalis* extracts and essential oil (HE), as well as the antibacterial activity of this aromatic plant (Srivastava et al., 2012). The *Lamiaceae* family is one of the most widely utilized plant families globally and serves as a source of spices and extracts with significant medicinal properties. The selection of *Salvia officinalis* is motivated by its popularity as one of the most commonly used aromatic plants worldwide (Raja, 2012).

The use of essential oils in the food, pharmaceutical, and cosmetic industries, as well as in culinary and traditional medicine, has become a popular topic of scientific research (Sharma et al., 2023). This study focuses on extracting *Salvia* essential oil through hydro distillation, assessing its yield, quantifying its total polyphenol and flavonoid content, and examining its antibacterial and antioxidant properties, which

** Corresponding Author*

E-mail Address: m.rezagui23dz@gmail.com

Received: August, 2024

Accepted: September, 2024

may offer potential applications as a natural preservative and aromatic agent.

Materials and Methods

The plant was cultivated in December 2021 in the city of Nechemaya, Wilaya of Guelma, Algeria, and was harvested on April 9, 2022, in the same location. Harvesting was carried out carefully to avoid deteriorating the organic and mineral elements present. Maceration was achieved by soaking the powdered plant material in a solvent for an extended period to extract its active ingredients, a process performed at room temperature (Colvin, 2018).

The dry plant material (2 g) is subjected to extraction by cold maceration using a methanolic solvent (40 ml) for 1 hour at room temperature. The extract is then filtered using filter paper, and the filtrate is concentrated using a rotary evaporator under reduced pressure at 45°C. Methanol is added to the dry residue obtained, and the mixture is placed into a refrigerant.

Essential oil extraction

The extraction of essential oils was carried out in the analytical chemistry laboratory (Pharmacy Department of Annaba) by hydrodistillation, which is the most commonly used process for extracting essential oils. The device used was a Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia, 6th edition (Baj et al., 2013). The essential oil obtained is then recovered and stored in opaque bottles tightly closed with foil before the cap is placed. The bottles are kept in a refrigerator (4°C) and are protected from light and air to prevent alterations of the essential oil until use.

The yield is defined by the ratio between the mass of the essential oil obtained after extraction (M) and the mass of the plant material used (M_o).

$$YEO = (M / M_o) \times 100$$

where YEO refers to the essential oil extraction yield in %, M refers to the mass of the essential oil in grams, and M_o refers to the mass of plant material used in grams. The analytical chemistry laboratory at Annaba Medical School conducted the chemical study of *Salvia officinalis* extract.

Determination of total polyphenols

Secondary metabolites are a diverse group of plant molecules with varying chemical natures and levels from one species to another. Several analytical methods can be used to quantify total phenols, with the Folin-Ciocalteu reagent analysis being the most commonly used. Phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) form the Folin-Ciocalteu reagent, which is yellow in colour. It is reduced during the oxidation of phenols to a mixture of blue oxides of tungsten and molybdenum (Ribéreau-Gayon, 1968). The colour produced, with maximum absorption at 760 nm, is proportional to the amount of polyphenols present in the plant extracts (Boizot and Charpentier, 2006).

To perform the calibration curve, a standard substance, such as Gallic acid, is used at a concentration of 5-20 µg/ml. The total polyphenol concentrations of each extract are calculated from the regression equation of the calibration curve with Gallic acid. Results are expressed in micrograms of Gallic acid equivalents per milligram of extract (µg EAG/mg) or (mg EAG/g extract). The total polyphenol content is calculated as follows:

$$C = (c \times V) / m$$

where C is the total phenol content (mg EAG/g extract), c is the extract concentration equivalent to Gallic acid established from the calibration curve (mg/ml), V is the volume of extract (ml), and m is the dry weight of the extract (g).

Total Flavonoid (TF) Determination

The aluminium chloride colorimetric method developed by Chang et al. (2002) was used to measure total flavonoids. First, 0.5 ml of the methanolic extract with 1.5 ml of 80% methanol, 0.1 ml of 10% aluminium chloride in ethanol, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were mixed in a tube. The resulting mixture was kept in the dark for 30 minutes. Afterward, a spectrophotometer at 415 nm immediately

measured the absorbance rate. The total flavonoid content was measured using quercetin equivalents in one gram of dry plant.

Antioxidant Activity (DPPH Assay)

Bartoz (Sadowska-Bartosz and Bartosz, 2022) method was used to calculate the total antioxidant capacity (TAC). First, 1 ml of the methanolic extract and 1 ml of the 0.1 mM DPPH reagent were added to the 20 ml tube. The test tubes containing the solution were then kept in the dark for 30 minutes to activate radical inhibition by the DPPH. After the required time, the absorbance rate was measured by a spectrophotometer at 517 nm. For measurement, the device was first calibrated with 80% methanol, and then the DPPH solution was read, followed by the rest of the samples. The readings were calculated using the final antioxidant calculation formula:
DPPH free radical scavenging percentage:

$$(Ac\text{-}As)/Ac \times 100$$

Where Ac is the control sample's absorbance rate, and As is the absorbance rate of the other samples.

Antibacterial Properties of Salvia officinalis Essential Oil

Testing the antibacterial properties of *Salvia officinalis* L. essential oil in an in vitro setting. To evaluate the antibacterial activity of sage essential oil, we conducted this study in the microbiology laboratory at Dorban Hospital (Annaba University Hospital) to determine the rate of inhibition of bacterial growth when exposed to the essential oil of *Salvia officinalis*. An aromatogram was produced by the diffusion method on an agar medium (solid medium). Etymol is used in the aromatogram. The term "Etymol" is derived from the Greek word "Arôma" and the Latin word "aroma," meaning "aroma," and from the Greek "gramma," meaning "letter" or "writing." It is an in vitro method for measuring the antibacterial, antiviral, and antiparasitic effects of essential oils (Goetz and Ghedira, 2012).

Preparation of the inoculum

From a 24-hour young culture, 3 to 5 well-isolated, identical colonies are collected using a sterile swab and placed in a tube containing 5 to 10 ml of sterile 0.9% physiological saline, then mixed using a vortex. The suspension is standardized to be equivalent to 0.5 McFarland, with an optical density reading of 0.08 to 0.10 at 625 nm (McFarland, 1907).

Seeding bacteria

Insert a sterile swab into the bacterial suspension. To remove excess liquid, wring it out by pressing firmly and rotating it against the tube wall. Rub the swab over the entire dry agar surface, starting from the top and moving downward in tight streaks. Repeat the process twice, rotating the plate 60° each time, ensuring to rotate the swab as well. The inoculation can be completed by running the swab around the periphery of the agar. When inoculating multiple Petri dishes, reload the swab each time (McFarland, 1907). After seeding, sterile forceps are used to place Whatman paper disks (6 mm in diameter) on the surface of the MH medium in Petri dishes. The disks are then saturated with 10 µL of *Salvia officinalis* L. essential oil.

Operating mode

To examine the effectiveness of *Salvia officinalis* extract against bacteria, three ATCC-type bacterial strains were selected in the microbiology laboratory at Dorban Hospital using the diffusion method on agar medium.

Transplanting bacterial strains

The different bacterial strains were seeded using the streak method (four quadrants) on nutrient agar and then incubated for 18 to 24 hours at 37°C to obtain pure and young colonies.

Incubation

To ensure that the oils applied to the inoculated medium distribute evenly, the plates are left at laboratory temperature for 30 minutes to 1 hour. The plates are then incubated in an oven at 37°C for 24 hours.

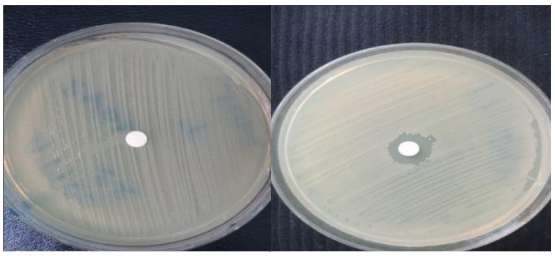

Fig. I. *Escherichia coli ATCC 22*

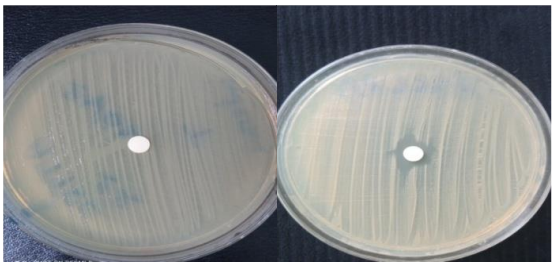

Fig. II. *Staphylococcus aureus ATCC 23*

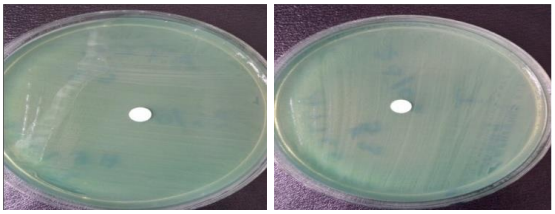

Fig. III. *Pseudomonas aeruginosa ATCC 53*

Results

Extraction of the essential oil of *Salvia officinalis*

The evaluation of organoleptic properties is a common part of studies that aim to analyze the factors that influence the characterization of essential oils. The yield of the essential oil is expressed as a percentage relative to 150g of dry plant material, after the extracted oil is weighed using a balance, the weight found is related to the total mass of the plant material (150g). The yield for oil essential (OE) extraction in winter and spring (0.8% and 1.05% respectively) experienced an evolution, with a slight increase during the flowering stage, while the average (0.925) met AFNOR: (French association of standardization.) (0,5<0,575<2). The synthesis and storage of active ingredients in the leaves and flowering tops during the flowering period could explain these results.

Determination of total polyphenols

The total phenol dosage was estimated by the spectrophotometric method of Singleton with the Folin-Ciocalteu reagent. The total phenol content of *Salivia officinalis* is determined from the linear regression equation of the calibration curve, expressed in mg gallic acid equivalent per g dry extract (mg EAG/gES). The calibration curve indicates that *Salvia officinalis* methanolic extract contains 223.45 mg EAG/g in total polyphenols.

Determination of flavonoids

The determination of flavonoids was carried out using aluminum trichloride (AlCl3). Quercetin was used as a standard. From the calibration curve, the flavonoid content of the methanolic extract of *Salvia officinalis* is 3.60 mg EQ/g.

Antioxidant activity:

The DPPH test (1,1-diphenyl-2-picrylhydrazyl) was utilized to evaluate the antioxidant activity of the methanolic extract of *Salvia officinalis*, which measures the antiradical activity of polyphenolic compounds. The reference for this test is ascorbic acid. IC50 Determination: The methanolic extract of *Salvia officinalis* is effective against free radicals at 0.023 mg/ml, while the standard antioxidant (ASC) has an IC50% of approximately 0.024 mg/ml.

Antioxidant activity of the essential oil of *Salvia officinalis*

The antioxidant capacity of *Salvia officinalis* EO was assessed by means of the DPPH test, which is a stable free radical with an absorbance band of 517 nm, which was used to evaluate its anti-radical activity. In this test ascorbic acid is used as a reference.

IC50 determination.

Salvia officinalis essential oil has moderate antiradical activity. With an IC50 of 0.104 mg/ml, it transformed the purple stable DPPH radical into yellow DPPH-H, which is much more effective than the reference antioxidant (vitamin C), which has an IC50 of 0.024 mg/ml.

The essential oil of *Salvia officinalis* exhibits moderate antiradical activity. It has a significantly higher IC50 of 0.104 mg/ml and can transform the purple stable DPPH radical into yellow DPPH-H than the reference antioxidant (vitamin C), which has an IC50 of 0.024 mg/ml.

Antibacterial activity of the essential oil of *Salvia officinalis*

The aromatogram method was utilized in vitro to evaluate the antibacterial potential of the pure essential oil of *Salvia officinalis* L. and to estimate the diameter of the inhibition zones.T hree strains were tested for their antibacterial activity, which included *Staphylococcus aureus* ATCC 23, *Escherichia coli* ATCC 22, and *Pseudomonas aeruginosa* ATCC 53. These results are interpreted according to the previous scale (Table 1).

The data gathered indicates that *Salvia officinalis* L.'s essential oil has a moderate antibacterial effect on the tested germs. Both *Staphylococcus aureus ATCC 23* and *Escherichia coli ATCC 22*, which have a diameter range of 12 and 15 mm, were sensitive to *Rosmarinus officinalis* L. (EO) among their bacterial strains. The strain *Pseudomonas aeruginosa* ATCC 53 is able to resist this essential oil without a zone of inhibition. The evaluation of the in vitro antibacterial activity of *Salvia officinalis* crude essential oil showed that *E. coli* was the most sensitive to this oil with an inhibition diameter of 15mm followed by S. aureus with a diameter of 12mm while P. aeruginosa showed no sensitivity to with respect to this oil.

Discussion

The organoleptic qualities of the essential oil we extract from *'Salvia officinalis* L.' through hydro distillation meet AFNOR 2000 standards (Adrar et al., 2016). Lu and Foo (2001)approved our results, demonstrating that sage leaves contain a significant amount of polyphenols. The total phenol content is not constant and can vary from plant to plant and between species within the same genus. The polyphenolic content differs qualitatively and quantitatively depending on several factors:

Table 1- Antibacterial activity of essential oil against pathogenic germs.

	Strain tested	**Inhibition diameter mm)**	**Degrees of sensitivity**
Gram(+)	*Staphylococcus aureus* ATCC23	12mm	Sensitive(+)
Gram(-)	*Escherichia coli* ATCC22	15 mm	Very Sensitive (++)
	Pseudomonas aeruginosa ATCC53	6mm (absence of inhibition zone)	Resistant (-)

- Geographical area, drought, soil, aggressions, and diseases are examples of climate and environmental factors.
- Genetic heritage, harvest period and stage of plant development. (Miliauskas et al., 2004).
- The extraction method and the quantification method, the solvent used (water, methanol, etc.) can also influence the estimation of the total phenol content (Lee et al., 2003).

Since flavonoids account for the majority of polyphenol compounds, the high content of phenolic compounds compared to flavonoids is reasonable, as per (Djeridane et al., 2006). Additionally, there is a correlation between adverse climate conditions and collection conditions, such as high temperatures, the soil's nature, and the stage of plant evolution.

It's logical that the high content of phenolic compounds compared to flavonoids is due to the fact that flavonoids are the main polyphenol compounds, as per (Djeridane et al., 2006). The reason for this is an increase in the plant's phenolic metabolism, along with a connection to adverse climatic conditions and collection conditions like high temperatures, soil quality, and plant evolution stage.

It is crucial to note that there is a positive correlation between antioxidant activity and the concentration of polyphenols in plant extracts. (Rice-Evans et al., 1996) have shown that phenolic

compounds and flavonoids are responsible for the plant's antioxidant activity. According to (Erhan et al., 2012), species of the Lamiaceae family, particularly *M. pulegium* and *S. officinalis*, are rich in phenolic compounds.

The antioxidant activity of an essential oil can be influenced by changes in its composition, either due to one of its major or minor constituents, or through a synergy between them(Basavegowda and Baek, 2021).

The antibacterial activity of essential oils from different aromatic plants is explained by the lysis of bacterial membranes(Wang et al., 2020). Essential oils, flavonoids, alkaloids or even tannins could induce a leakage of potassium ions at the membrane and by way of consequences of irreversible damage to this membrane(Álvarez-Martínez et al., 2021). The lack of antibacterial effect on the different strains tested could be caused by their inefficiency, or by the insufficient volume and concentration of extracts used, as well as by the acquired resistance developed by the germ itself.

Salvia officinalis essential oil's antimicrobial properties were studied by Salah Benkhrara et al. (2021)using a method that is not aromatogram-based (well agar diffusion) against some pathogenic enterobacteriaceae. The results showed that this oil is very active on strains of *E. coli* (Benkherara et al., 2021). Similarly some researchers found that the essential oil of sage exerted strong inhibition against *Escherichia coli* with 13mm diameter of the inhibition zone compared to *Pseudomonas aeruginosa* which exhibited resistance to this essential oil (Abhadionmhen and Imarenezor, 2024).

However, there are studies that contradict our study and prove otherwise. Rathore et al. (2023)demonstrated that *S. aureus* is sensitive to different essential oils from different aromatic plants. Researchers explained its results by explaining the difference in chemical composition of the bacterial wall between Gram + and Gram - (Tavares et al., 2020).

Conclusion

Natural antioxidants and antimicrobials have gained significant attention due to their therapeutic potential. In this study, the essential oil and methanolic extract of *Salvia officinalis L.* demonstrated promising antioxidant and antibacterial activities. The plant exhibited a high polyphenol content, which contributes to its strong antioxidant properties, potentially useful in preventing diseases like cancer and cardiovascular conditions. Antibacterial evaluations revealed activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli*, highlighting its potential as a natural antimicrobial agent. These findings encourage further exploration to identify and characterize the bioactive compounds responsible for these beneficial effects.

References

Abhadionmhen, O. and E. Imarenezor. 2024. Antimicrobial Efficacy of Ethanolic Extracts of *Salvia officinalis* Leaves on Proteus mirabilis. *Covenant Journal of Health and Life Sciences,* 12-12.

Adrar, N., N. Oukil and F. Bedjou. 2016. Antioxidant and antibacterial activities of Thymus numidicus and *Salvia officinalis* essential oils alone or in combination. *Industrial Crops and Products,* 88, 112-119.

Álvarez-Martínez, F., E. Barrajón-Catalán, M. Herranz-López and V. Micol. 2021. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine,* 90, 153626.

Baj, T., A. Ludwiczuk, E. Sieniawska, K. Skalicka-Woźniak, J. Widelski, K. Zieba and K. Głowniak. 2013. GC-MS analysis of essential oils from *Salvia officinalis* L.: comparison of extraction methods of the volatile components. *Acta poloniae pharmaceutica,* 70, (1) 35-40.

Basavegowda, N. and K.-H. Baek. 2021. Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications. *Biomolecules,* 11, (9) 1267.

Benkherara, S., O. Bordjıba, S. Harrat and A. B. Djahra. 2021. Antidiabetic potential and chemical constituents of *Haloxylon scoparium* aerial part, an endemic plant from Southeastern Algeria. *International Journal of Secondary Metabolite,* 8, (4) 398-413.

Boizot, N. and J.-P. Charpentier. 2006. Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. *Le Cahier des Techniques de l'INRA, In: Numéro spécial,* 79-82.

Bouasla, I., T. Hamel, C. Barour, A. Bouasla, M. Hachouf, O. M. Bouguerra and M. Messarah. 2021. Evaluation of solvent influence on phytochemical content and antioxidant activities of two Algerian endemic taxa: *Stachys marrubiifolia* Viv. and *Lamium flexuosum* Ten.(Lamiaceae). *European Journal of Integrative Medicine,* 42, 101267.

Colvin, D. M. 2018. A review on comparison of the extraction methods used in licorice root: Their principle, strength and limitation. *Med Aromat Plants,* 7, (6) 323.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food chemistry,* 97, (4) 654-660.

Erhan, M., Ş. Bölükbaşı and H. Ürüşan. 2012. Biological activities of pennyroyal (*Mentha pulegium* L.) in broilers. *Livestock Science,* 146, (2-3) 189-192.

Goetz, P. and K. Ghedira. 2012. *Phytothérapie anti-infectieuse*. Springer

Lee, K. W., Y. J. Kim, H. J. Lee and C. Y. Lee. 2003. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *Journal of agricultural and food chemistry,* 51, (25) 7292-7295.

Lu, Y. and L. Y. Foo. 2001. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food chemistry,* 75, (2) 197-202.

Mcfarland, J. 1907. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association,* 49, (14) 1176-1178.

Miliauskas, G., P. Venskutonis and T. Van Beek. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry,* 85, (2) 231-237.

Raja, R. R. 2012. Medicinally Potential Plants of Labiatae (Lamiaceae) Family: An. *Research journal of medicinal plant,* 6, (3) 203-213.

Rathore, S., S. Mukhia, R. Kumar and R. Kumar. 2023. Essential oil composition and antimicrobial potential of aromatic plants grown in the mid-hill conditions of the Western Himalayas. *Scientific Reports,* 13, (1) 4878.

Ribéreau-Gayon, P. 1968. *Les Composés phénoliques des végétaux: par Pascal Ribéreau-Gayon*. Dunod

Rice-Evans, C. A., N. J. Miller and G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine,* 20, (7) 933-956.

Sadowska-Bartosz, I. and G. Bartosz. 2022. Evaluation of the antioxidant capacity of food products: Methods, applications and limitations. *Processes,* 10, (10) 2031.

Sharma, A., V. Kumar, C. Mittal, V. Rana, K. Dabral and G. Parveen. 2023. Role of Essential Oil Used Pharmaceutical Cosmetic Product. *Journal for Research in Applied Sciences and Biotechnology,* 2, (3) 147-157.

Srivastava, A., L. J. M. Rao and T. Shivanandappa. 2012. A novel cytoprotective antioxidant: 4-Hydroxyisophthalic acid. *Food chemistry,* 132, (4) 1959-1965.

Tavares, T. D., J. C. Antunes, J. Padrão, A. I. Ribeiro, A. Zille, M. T. P. Amorim, F. Ferreira and H. P. Felgueiras. 2020. Activity of specialized biomolecules against gram-positive and gram-negative bacteria. *Antibiotics,* 9, (6) 314.

Wang, X., Y. Shen, K. Thakur, J. Han, J.-G. Zhang, F. Hu and Z.-J. Wei. 2020. Antibacterial activity and mechanism of ginger essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Molecules,* 25, (17) 3955.