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Original Research Article

Phytochemical screening and antibacterial activity of a medicinal plant (Salvia Officinalis)

Lamia Benhalima[1], Sandra Amri[1]*, Saber Belhaoues[2], Mourad Bensouilah[3]

[1]Dept. of Biology, 8 Mai University, Guelma, Algeria
[2]National Center for Environmental Research (CRE), Alzone Annaba, Algeria
[3]Badji Mokhtar University, Annaba, Algeria

Abstract

Introduction: Medicinal plants are a natural source of many bioactive molecules, which fulfill various functions.
Aim and Objectives: The study was to research investigates the phytochemical screening and antibacterial activity of the medicinal plant *Salvia officinalis* against reference bacterial strains.
Materials and Methods: *Salvia officinalis* extract was prepared by maceration of the plant with distilled water and then evaporation. The search for different classes of secondary metabolites was carried and antibacterial activity was evaluated by diffusion method and determination of minimum inhibitory and bactericidal concentration. Three reference strains were used: *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC25923.
Results: Phytochemical analysis revealed the presence of alkaloids, reducing compounds, flavonoids, polyphenols, saponins, sterols, tannins and terpenoids. The *Salvia officinalis* extract showed antibacterial effect from 40 mg for *Escherichia coli* specie and 60 mg/ml for *Pseudomonas aeruginosa* and *Staphylococcus aureus* species. The minimum inhibitory concentration was determined to be 40 mg/ml for *Escherichia coli* and 60 mg/ml for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The minimum bactericidal concentration were 50, 80 and 140 mg/ml for *Escherichia coli*. *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. The calculation of the minimum bactericidal concentration/ minimum inhibitory concentration ratio had indicated that the extract exerts a bactericidal effect against all three tested strains.
Conclusion: Study highlights the potential of the medicinal plant *Salvia officinalis* as a rich source of bioactive molecules and has a bactericidal effect against the reference strains.

Keywords: Plant, Screening, Antibacterial activity, Reference strains.

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1. Introduction

Infectious diseases have accounted for one-third of global mortality[1] and their incidence is on the rise.[2] In Algeria, as in many developing countries, infectious and parasitic diseases present a significant public health challenge due to their prevalence and severity.[3] The introduction of antibiotic therapy in the 1940s profoundly revolutionized the medical field, resulting in a substantial reduction in mortality associated with infectious diseases. Unfortunately, bacterial resistance to traditional antibiotics has rapidly emerged as a significant global health concern. The European Commission (2005) estimates that between one and ten million tons of antibiotics have been released into the biosphere over the past 60 years.[4] The rise of antibiotic-resistant bacteria has prompted researchers to explore the plant kingdom. Particularly medicinal and culinary plants. In search of new, effective natural molecules.[5] Aromatic and medicinal plants are rich in bioactive compounds with specific biochemical and organoleptic properties. Making them valuable for use as bio-pesticides and in the pharmaceutical. Cosmetic, and food industries. Currently, the valorization of lignocellulosic

*Corresponding author: Sandra Amri
Email: amrisandra@ymail.com

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material by-products is a major focus in the field of green chemistry.[6] According to the World Health Organization (2002). 80% of the African population still relies on traditional medicine. With most therapies involving the use of active principles derived from medicinal plants.[7] Phytotherapy has developed into a sophisticated medical science that involves the use of pure essential oils extracted from plants. In Algeria, plants play a crucial role in traditional medicine, which is extensively used across various health sectors.[3] The practice of using plants has been passed down through generations to the present day. In this context, the primary objective of this research is to examine the phytochemical profile and antibacterial activity of an aqueous extract of medicinal plant *Salvia officinalis* against three reference strains: *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853 and *Staphylococcus aureus* ATCC 25923.

2. Materials and Methods

2.1. Preparation of extract

The extraction process was conducted at the EMMAL laboratory (Eco-biology of Marine and Coastal Environments - University of Badji Mokhtar-Annaba). The plant material utilized in this study comprises the aerial parts of *Salvia officinalis*, it was collected from the Annaba region (Algeria) on March 2022. The *Salvia officinalis* extract was prepared according to the method of Mariod et al.[8] The freshly harvested plants were washed and dried in the shade in a dry, ventilated area before being ground into powder for use. The powdered plant material underwent maceration with distilled water under agitation for 24 hours. The resulting filtrate was evaporated using a rotary evaporator to remove the extraction solvent, lyophilized and stored in sterile hermetically sealed vial at - 25°C in the dark.

2.2. Screening of secondary metabolites

The dried powder of *Salvia officinalis* was used for the search for different classes of secondary metabolites. The colorimetric methods described by Edeogal et al,[9] and Karumi et al,[10] were used. The presence of secondary metabolites is indicated by precipitation. Turbidity or colour change reactions.

2.3. Antibacterial activity

The antibacterial activity was evaluated by the method of diffusion, as described by Celiktas et al.[11] The extract was deposited at 20, 40, 60 and 80 mg in the wells, DMSO was used as negative control and antibiotic discs of penicillin (10 µg) as a positive control.

2.4. Determination of the minimum inhibitory and bactericidal concentration

The determination of the minimum inhibitory concentration (MIC) and of the minimum bactericidal concentration (MBC) was carried out according to the method described by Benhalima et al.[12] Non inoculated broth Mueller-Hinton was used as the negative control and broth Mueller-Hinton without the addition of extract as the positive control.

3. Results and Discussion

3.1. Phytochemical screening

The extraction yield refers to the percentage of crude extract obtained from a dried plant sample using an extraction procedure. The aqueous extract was characterized by its color and yield, which varied depending on the harvest period. The aqueous extract was brown in color. It exhibited a relatively high yield of approximately 25.50% from 250 g of powdered plant material. The results of the phytochemical screening (**Table 1**) indicate that *Salvia officinalis* contains alkaloids, reducing compounds, flavonoids. Polyphenols, saponins, sterols, tannins, anthraquinones, anthocyanins, leuco-anthocyanins, mucilages and terpenoids. The abundance of active chemical compounds in this extract may explain its use as a medicinal plant with antioxidant properties.

Table 1: Screening phytochimiques of *Salvia officinalis.*

Screening	Results	Screening	Results
Alkaloïds	+	Free anthraquinc	-
Flavonoids	+	Catechic tannins	+
Polyphenol	+	Gallic tannins	-
Saponins	+	Leuco-anthocyanins	+
Combined anthraquinones O-Hetroside	+	Mucilages	+
Combined anthraquinones C-Heteroside	-	Reducing compound	+
Anthocyanins	+	Triterpenoids and stéroids	+

3.2. Antibacterial activity

The antibacterial activity was assessed by measuring the diameter of the inhibition zones. The results are summarized in **Table 2-3.**

Table 2: Diameters of the inhibition zones of the reference strains tested against the positive and negative control (Mean ± SD).

	Escherichi a coli	***Pseudomonas aeruginosa***	***Staphylococcus***
Penicilli n (10µg)	14.20 ± 0.55	19.00 ± 0.5	10 ± 0.23
DMSO (2%)	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00

Table 3: Diameters of the inhibition zones of the reference strains tested by the extract of *Salvia officinalis* (Mean ± SD).

Reference strains	Diameters of inhibition zones (mm)			
	20 mg	**40 mg**	**60 mg**	**80 mg**
Escherichia coli ATTC 25922	00.00	20.33 ± 0.57	26.66 ± 0.57	32.20 ± 0.54
Pseudomonas aeruginosa ATTC 27853	00.00	00.00	20.16 ± 0.57	27.20 ± 0.15
Staphylococcus aureus ATTC25923	00.00	00.00	08.33 ± 0.57	12.36 ± 0.36

Table 4: MIC and MBC (mg/ml) of the Salvia officinalis extract of the species.

Reference strains	MIC	MBC	MBC/MIC		Activity	MBC/MIC
Escherichia coli ATCC25922	40	50	1,25		Bactericidal	4
Pseudomonas aeruginosa ATCC27853	60	80	1,33		Bacteriostatic	8-16
Staphylococcus aureus ATCC25923	60	140	2,33		Tolerant	32

The 2% DMSO results demonstrated that DMSO is appropriate for use as a solvent, as it did not affect the normal growth process of the reference strains. Additionally, given the potential critical nature of bacterial resistance. Penicillin (10 µg) was tested as a positive control, exhibiting inhibition zones ranging from 10 ± 0.23 to 19 ± 0.5 mm. It was observed that the antibacterial effect of the extract is concentration-dependent. At 10 mg, the extract exhibited no effect on the three bacterial strains. At 20 mg, the extract showed antibacterial activity against *Escherichia coli* only. However, at 40 mg the extract inhibited the growth of all three bacterial strains, suggesting that Gram negative bacteria were most susceptible than Gram positive to our extract. This fact contrasts with other work done by Sanusi et al.[13] Also, these findings suggest that the inhibition of bacterial growth is influenced by the bacterial species, the nature of the extract and its concentration. Given that the extract is a complex mixture of many compounds, the active portion may be relatively low.[14] The minimum inhibitory concentration and minimum bactericide concentration is represented in **Table 4**. The MIC of the extract of *Salvia officinalis* was determined to be 40 mg/ml for *Escherichia coli* and 60 mg/ml for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The lowest minimum bactericidal concentration was also noted for *Escherichia coli* at 50 mg/ml. followed by *Pseudomonas aeruginosa* at 60 mg/ml and *Staphylococcus aureus* at 140 mg/ml. The comparison of the MBC/MIC ratio for the reference strains to the values of Marmonier[15] the bioactive molecules indicates that this extract exhibits a bactericidal effect against the three reference strains. Several studies have demonstrated that bacterial resistance is generally higher in Gram-negative bacteria than in Gram-positive bacteria,[16] due to the presence of the external phospholipid membrane that is almost impermeable to hydrophobic compounds[17] However, our study did not observe this trend, as the extract demonstrated similar effects on Gram-negative and Gram-positive bacteria. This result suggests the presence of bioactive molecules with broad-spectrum antibacterial activity. Also, this suggests that antibacterial activity of *Salvia officinalis* extract may be attributed to other classes of secondary metabolites. According to many reports Belhaoues et al,[18] and Olivier et al,[19] the presence of alkaloids, terpenoids, steroids, flavonoids, polyphenol and tannins may be responsible for the antibacterial properties of extracts.

4. Conclusion

This study highlights the potential of the medicinal plant *Salvia officinalis* as a rich source of bioactive molecules, phytochemical screening results highlighted the presence of alkaloids, reducing compounds, flavonoids, polyphenols, saponins, sterols, tannins and terpenoids. The antibacterial effect of the extract is concentration dependent with inhibition of all three bacterial strains observed at 40 mg. The minimum inhibitory concentration of the extract was determined to be 40 mg/ml for *Escherichia coli* and 60 mg/ml for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the minimum inhibitory concentration was of the order of 50, 80 and 140 for *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. The MBC/MIC report indicated that *Salvia officinalis* extract has a bactericidal effect against the three reference strains.

5. Author Contributions

All authors contributed to the study conception and design, all authors read and approved the final manuscript. Material preparation, data collection and analysis were performed by Dr. Lamia Benhalima and Dr. Sandra AMRI, Dr. Saber Belhaoues. The first draft of the manuscript was written by Dr. Lamia Benhalima and all authors commented on previous versions of the manuscript. Conceptualization: Dr. Lamia Benhalima; Methodology: Dr. Lamia Benhalima and Dr. Sandra AMRI; Writing - original draft preparation: Lamia Benhalima; review: Mourad Bensouilah, Dr. Sandra AMRI and Dr. Saber Belhaoues; Supervision: Mourad Bensouilah.

6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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