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Phytochemical screening and biological activity of essential oils and flavonoïds of aromatic plant *Salvia officinalis* L. in north-eastern Algeria

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Abstract *This study focuses on the evaluation of antibacterial biological activity of natural products of an aromatic plant in the north-eastern Algeria: Salvia officinalis L. or officinal Sage. To do this and after a phytochemical screening, essential oils and flavonoïds are extracted respectively by hydrodistillation and by organic solvents according to the method of Charaux and Paris 1954. These compounds are thereafter identified by GC/MS and TLC. Their antibacterial effect was determined, in vitro on Mueller-Hinton medium, towards several bacterial strains responsible for certain infectious diseases. The results showed that the isolated essential oil is characterized by a Chemotype with nineteen (19) terpene compounds with a prevalence of a-Thujone (about 37 %). Moreover, The TLC analysis of the obtained flavonoïque extract and the revelation by Aluminum chloride ($AlCl_3$) of the observed different tasks showed the presence of three compounds or groups of flavonoïque compounds having frontal ratios between 0.128 and 0.928. The tests of antibacterial activity showed that growth inhibition varies according to the bacterial species and the concentration of the extracted natural product. In general, the essential oils have a very important inhibitory power against studied multiresistant germs. Pseudomonas aeruginosa ATCC 27853 strain proved to be very sensitive face to different concentrations of the obtained essential oils and flavonoïds with a maximum of inhibition of 37.08 and 45.92 mm of diameter respectively, followed by the Escherichia coli 1429 strain, Escherichia Coli 1554, Proteus mirabilis and Klebsiella pneumonia whose recorded inhibition zones usually exceed 30 mm in diameter. These results are promising and provide a scientific validation as for the massive use of the plant Salvia officinalis L.*

***Key Words*:** Biological activity, essential oils, flavonoïds, *Salvia officinalis*, bacterial strains.

1. Introduction

In the plant kingdom, the secondary metabolites play an important ecological roles, in particular while contributing to the phenomena of communication and defense .
Since antiquity, some characteristics of the active ingredients were known for the man and certain spices were used for their characteristics of perfume, their savor and their conservative effect [4]. The exploitation of these compounds or active ingredients was carried out in the form of oils extracted from plants (essential oils) by the means of distillation, this technique

being employed in India and Persia there are more than 2000 years [15]. These compounds are endowed with antimicrobial properties to differing degrees, they are antifungal properties [2, 1, 16, 23], antiviral [5], antiparasitic [31] and also insecticidal properties [17, 18]. The essential oils which present a good antibacterial activity are also a good antifungal [28, 29].
In this study, we were interested in a plant species very widespread in the Mediterranean basin and widely used for its many therapeutic virtues. It is about *Salvia officinalis* L. or officinal Sage. It is a species of the Lamiaceae family, from 30 to 60 cm of height, stems forming of the drawn up and hairy quadrangular branches, with oval and elongated leaves, greenish gray because of a cottony hairs on the lower face, have a characteristic aromatic odor and small flowers blue violets which open out in June or July [20, 13]. The objective is to check the specificity of this species on the antiseptic plane and in particular the antibacterial activity of essential oils and flavonoïds.

2. Materials and methods

2.1 Materials

The plant material having been the subject of this study is composed of leaves of the plant species *Salvia officinalis* L. The harvest is carried out in December 2009 at the National park of El-Kala (P.N.E.K) in North-eastern of Algeria. The samples were deposited in the laboratory of Plant Biology and Environment (LBVE), Faculty of Science; University BADJI Mokhtar - Annaba.

The microbiological material consists of nine bacterial strains: *Escherichia coli* 12, *Escherichia coli* 1554, *Escherichia coli* 1429, *Escherichia coli* ATCC 25922, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* 7244, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* They come from a medical analysis laboratory located at the state of Annaba (Algeria).
The Mueller-Hinton agar (MH) (pH = 5.7) is the principal used culture medium.
The rifampin (Rif with 5µg): An antibiotic of the Rifamycines family, it has an excellent activity against Gram-positive germs: *Staphylococci* and *Enterococci* [14, 9, 34]. This antibiotic was used in order to compare the antibacterial activity of the essential oils and flavonoïds with that of antibiotics.

2.2 Methods

2.2.1 Phytochemical screening

Preliminary tests are carried out in a tube, on the leaves of the plant species *Salvia officinalis* L. according to the usual techniques of laboratory [26] for the detection of the presence or absence of the different compounds or active principles of the plant.

2.2.2 Extraction, separation and identification of the obtained extracts

2.2.2.1 Essential Oils

The extraction of essential oils (EO) is done by stripping with steam. According to [8], it is carried out with 50 g of plant drug boiled during 3 hours with a speed of distillation from 2 to 4 ml per minute.
The analysis of extracted essential oils is carried out using a gas phase chromatograph coupled to a mass spectrometer of the Schimadzu type equipped with a flame ionization detector (FID) and provided with a silica capillary column type QP20 10C25 FS-OV1701

with 25 m length, 0.25 mm in internal diameter and 0.25 µm film thickness. The column temperature is programmed from 60 to 200°C at a rate of 3°C/ min during 13 minutes. The injector temperature is fixed at 240°C and that of detector (FID) at 250°C. The flow rate of carrier gas (helium) is fixed at 1.5 ml/ min. The injected sample volume is 0.1µl of pure oil diluted at 10 % in hexane.
The compounds of essential oil are identified by their retention indices on the column (retention index is calculated in relation to the retention time of a series of linear alkane (C9 - C28)) and by comparison between their mass spectra and those of the chemical compounds indexed in a commercial library (ov.17/GCMS Rcal time analysis). The percentage of each compound is determined from the peak areas without taking account of correction factors by supposing that all the components have nearby answer coefficients.

2.2.2.2 Flavonoïds
The extraction of flavonoïds (Flav) is made according to the standard technique of Charaux-Paris [25]: exhaustion by boiling alcohol, concentration to dryness, taken up by boiling water; the aqueous solution is treated successively by ether, ethyl acetate and butanol.
The analysis or separation of the obtained flavonoïds is done by thin layer chromatography (TLC).

2.2.3 Antibacterial Activity
The evaluation of the antibacterial activity of the essential oils and flavonoïds extracted from leaves of *Salvia officinalis* L. is conducted using the agar diffusion method.

A disc (6 mm of diameter) impregnated of the tested product (pure EO, EO diluted to ½, EO diluted to ¼, pure Flav, Flav diluted to ½, Flav diluted to ¼) is placed on the agar (4 mm thickness; in Petri dishes of 90 mm of diameter) previously inoculated with the strain, gets wet and the product diffuses radially from the disc on the agar forming a concentration gradient. After incubation of 18 to 24 hours at a temperature of 37°C, if the product is toxic for the bacterial species, it forms a zone around the disc. If this zone is larger, the species is more sensitive. Control discs (sterile distilled Water) and comparison discs (Antibiotic) are included in the trials.

Bacterial suspensions
From the bacterial cultures previously reactivated on Mueller-Hinton medium (MH) at a temperature of 37°C during 24 hours, the bacterial suspensions are prepared in physiological water and homogenized in order to obtain a concentration of 10^6 to 10^8 C.F.U/ ml. After sowing of the bacteria, the discs impregnated of the product to be tested are applied.

3. Results and Discussion

3.1 Results
During preliminary tests carried out on the leaves of *Salvia officinalis* L., we noted the presence of five secondary metabolism compounds (gallic tannins, saponins, flavonoïds, cardinolids, terpenes and sterols) and the absence of three other important compounds: anthocyanins, leuco anthocyanins and alkaloids (Tab.1).

Table 1: Different chemical constituents of leaves of *Salvia officinalis* L.

ACTIVE PRINCIPLE	LEAVES
Gallic Tannins	(+)
Saponins	(+)
Anthocyanins	(-)
Leuco anthocyanins	(-)
Alkaloids	(-)
Flavonoïds	(+)
Cardinolids	(+)
Terpenes and Sterols	(+)

(+) detected, (-) not detected

An average content of 1.52 ml in 100 g of plant material corresponding to a rate of 1.52 % of essential oils. These oils are denser than water, with a fluid and mobile limpid liquid aspect, of pale yellow color and pleasant odor.
During the analysis by gas chromatography coupled to mass spectrometry (GC / MS), the obtained chromatogram profile revealed the presence of nineteen (19) terpene compounds with a prevalence of α-Thujone (36,74 %) followed by Cineole (22.97 %), Camphor (11,34 %) and β-Thujone (8.81 %) (Tab.2).

Table 2: Terpene Compounds of the obtained essential oils.

Peak n°	**Identified Compound**	**RT (mn)**	**A (%)**	**peak n°**	**Identified compound**	**RT (mn)**	**A (%)**
1	α-Pinène	3.460	0.22	11	Camphre	12.247	11.34
2	Camphène	3.822	0.41	12	L-Camphre	12.435	3.81
3	Ocimène	3.951	1.06	13	β-Linalool	12.816	1.10
4	β-Pinène	4.409	0.37	14	Bornéol	13.560	2.94
5	β-Myrcène	4.859	0.25	15	α-terpenéol	14.166	0.25
6	D-Limonène	5.667	0.20	16	Caryophyllène	20.106	1.54
7	Eucalyptol	6.253	3.27	17	α-caryophyllène	21.617	1.34
8	Cinéole	6.345	22.97	18	α-longipinène	22.638	0.23
9	α-Thujone	10.525	36.74	19	β-Humulène	29.112	3.15
10	β-Thujone	10.950	8.81				

A: Area, RT: Retention time

The extraction of flavonoïds gave a yield of 0.41 g for 10 g of plant drugs, which corresponds to a rate of 4.1 %. The TLC analysis of flavonoïds and the observation of chromatograms under a UV lamp enabled us to note the presence of active components at 254 nm and fluorescence at 366 nm. The tasks and fluorescences seen in different colors inform us after chromatogram revelation with Aluminum Chloride ($AlCl_3$) on the presence of flavonoïds in the leaves of our plant species.
Three different colors were detected in the presence of Aluminum Chloride reactive: green yellow clear yellow task and ocher task. This may possibly mean that the leaves of *Salvia*

officinalis L. contains three different compounds or groups of compounds with frontal reports 0.128, 0.642 and 0.928 respectively.

From the antibacterial activity evaluated through *in vitro* tests on Mueller-Hinton medium, it is clear that there is a great variability in the obtained results. The selected germs reacted more or less well depending on the nature and the concentration of plant extracts isolated from the leaves of *Salvia officinalis* L. (Tab.3).

In general, the lower of concentrations of flavonoïds and essential oils caused a greater inhibitory effect of the majority of strains.

Flavonoïds:

The three *Escherichia coli* strains (*Escherichia coli* 12, *Escherichia coli* 1554 and *Escherichia coli* 1429) appear to be more sensitive to flavonoïds diluted to ¼. For this, diameters of inhibition zones ranging between 22.70 and 39.40 mm were recorded. *Proteus mirabilis* and *Klebsiella pneumonia* also reacted similarly with 31.66 and 34.42 mm of diameter of inhibition respectively towards flavonoïds diluted to ¼.

Concerning *Pseudomonas aeruginosa* 7244, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* species, they appear to be more resistant to flavonoïds diluted to ¼ because of a less widespread inhibition zones varying between 12.70 and 17.40 mm of diameter were recorded. The *Pseudomonas aeruginosa* 7244, although strongly resistant to the antibiotic (*Rif 5µg*) was more affected by the different concentrations of the used flavonoïds, with a more pronounced effect in the presence of flavonoïds diluted to ½ (14 mm of diameter).

Essential oils:

Essential oils appear to be slightly more effective than flavonoïds except for minimal concentrations (EO ¼). The best inhibition zones were obtained with the solution of EO diluted to ½ and exceeded those recorded with flavonoïds diluted to ½.

For the four strains of *Escherichia coli*, the values were ranging between 12.65 and 34.48 mm in diameter whose best result was observed for *Escherichia coli* 1554 strain with 34.48 mm of diameter against 24.16 mm towards flavonoïds diluted to ½. This inhibition was far greater than that noted with the antibiotic. The *Staphylococcus aureus* germ did not show any kind of resistance to the used antibiotic (40 mm of diameter). However, it seems slightly sensitive to different dilutions of essential oils with 22.65 mm of inhibited surface for EO diluted to ½ and 21.45 mm for EO diluted to ¼. The *Pseudomonas aeruginosa* 7244 strain which was completely resistant to the used antibiotic is not able to develop next to these essential oils.

Among all the tested strains, that of *Pseudomonas aeruginosa* ATCC 27853 has attracted more our attention for its great performance against the wide variety of antibiotics, it proved here under the action of essential oils of *Salvia officinalis* L. less powerful (30.54 mm for EO diluted to ¼) and (31.22 mm for EO diluted to ½).

On the whole, essential oils seem to be more effective against the majority of the studied strains, especially with the dilution ½. Better results than those obtained in the presence of the antibiotic rifampin were recorded; even multiresistant strains such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* were somewhat affected face to EO diluted to ½, on the other hand the dilutions to ¼ were less efficient ¼. However, the diameters of the halos formed around the discs are not negligible as is the case for example of *Staphylococcus aureus* (21.45 mm) and *Pseudomonas aeruginosa* 7244 (15.50 mm) against (0 mm) for the antibiotic.

Table 3: Inhibition zones diameters (mm) of the bacteria growth against the tested products.

	Flavonoïds			Essential oils			Antibiotic (*Rif 5µg*)
	pure Flav	Flav ½	Flav ¼	pure EO	EO ½	EO ¼	
Escherichia coli 12	22.65	21.40	22.70	27.40	27.50	19.35	00.00
Escherichia coli 1554	17.40	24.16	34.50	23.32	34.48	28.04	27.72
Escherichia coli 1429	11.32	23.34	39.40	31.56	33.62	29.64	30.50
Escherichia coli ATCC 25922	10.00	12.00	14.70	13.40	12.65	29.35	00.00
Proteus mirabilis	25.12	29.44	31.66	24.32	26.74	29.96	28.32
Klebsiella pneumonia	06.00	25.72	34.42	27.56	27.28	24.14	21.68
Pseudomonas aeruginosa 7244	11.50	14.00	12.70	05.40	10.00	15.50	00.00
Pseudomonas aeruginosa ATCC 27853	06.70	30.20	45.92	37.08	31.22	30.54	27.42
Staphylococcus aureus	15.40	15.50	17.40	24.00	22.65	21.45	40.00

3.2 Discussion

In light of the obtained results from the preliminary tests carried out on the leaves of *Salvia officinalis* L., the extraction, analysis and identification of essential oils and flavonoïds, it seems obvious that the studied species is of aromatic nature with a Chemotype of Thujone (α and β - Thujone) and is characterized by the presence of various compounds or groups of biochemical compounds (tannins, saponins, cardinolides ...).

More or less similar results were announced by [7] on the same plant species whose yields of obtained EO are ranging between 1 and 2.5 % and with about 60 % of thujone (mixture of α- and β-Thujone). However, in another work realized by [13] on Spain officinal Sage, the author reported the presence of a Chemotype to different terpene compounds with a predominance of camphor (11 to 36 %), followed by cineol (11 to 25 %) and very minor amount of thujone (< 0.5 %). A divergence was also observed between essential oils of the studied officinal Sage and those of Tunisian officinal Sage characterized by a Chemotype with 44 terpene compounds which the most abundant one is the β-Thujone (17.76 %) against only 7.71 % for α- Thujone [6]. In the same context and according to [19], the extraction of essential oils from the leaves of a Syrian officinal Sage was able to give a yield of about 0.14 to 0.52 % and a rate varying between 55 and 62 % of the major compound (1,8 Cineole) against only 2.1 to 3.3 % of the thujone which was the most abundant compound in the biochemical composition of the essential oils of the studied officinal Sage.

On the other hand, and from a study carried out on officinal Sage of Batna (East - Algeria) a great divergence was observed. In other words, the used officinal Sage is characterized by the predominance of Thujone in the biochemical composition of its essential oils (about 45 %) whereas it is less frequent (only 31 %) despite its predominance in the biochemical composition of essential oils of officinal Sage of the region of Batna [21]. This divergence in results explains the potential role of environmental and climatic factors on the biochemical composition and the quality of the extract of the plant species [33, 22].

From the evaluated tests the antibacterial activity, these EO seem to be more effective against the majority of the tested bacterial strains. Good results were recorded; even against the most resistant strains such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This antibacterial activity of EO has been announced in many studies especially those realized by [10] on the essential oils of *Salvia officinalis* L. in the region of southern Brazil, which caused antibacterial properties on *Escherichia coli* and *Staphylococcus aureus* .

Pseudomonas aeruginosa ATCC 27853 strain has attracted more our attention for its great performance to antibiotics, it proved here sensitive to the action of EO. This positive effect of EO against this strain was also demonstrated by [3] with EO extracted from Rosemary and Oregano.

The same thing was mentioned by [11] on the same strain subjected to flavonoïds of *Sambucus nigra* L. but cultivated on Chapman medium in which it was noted an average sensitivity of 24 mm of inhibited surface. We can mention here the importance of the culture medium for the antimicrobial activity.

Concerning flavonoïds, they are mainly efficient with the concentration ¼ and more particularly for *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* 1429, *Escherichia coli* 1554 and *Klebsiella pneumonia*.

The strong antimicrobial activity of flavonoïds was evoked by [11] in a study carried out on the effect of diluted flavonoïds of *Sambusus nigra* L. put in contact with *Staphylococcus aureus* cultivated on Mueller-Hinton medium. Their work highlighted the resistance of this strain face to these substances. However, the same bacterial species cultivated on the same medium (MH medium) seems slightly sensitive to different dilutions of our EO, moreover our results were comparable with those of [27] concerning the EO of *Lippia multiflora* L. leaves where the average bactericidal power on wild germs of *Staphylococci* and *Enterococci* was demonstrated.

In the same context and according to [19], a similarity is observed because the EO of a Syrian officinal Sage could have a good inhibitory activity against *Staphylococcus aureus* strain which was also the subject of our study. However, a divergence in the results is recorded when talking about the effect of these EO of the Syrian region face to various colonies of *Pseudomonas aeruginosa* ATCC 27853 strain. in other words, these EO could cause only a bacteriostatic effect against *Pseudomonas aeruginosa* ATCC 27853 whereas it is strongly inhibited under the action of the EO of our officinal Sage.

Furthermore, *Staphylococcus aureus* and *Klebsiella pneumonia* that were sensitive to the action of EO extracted from *Salvia sahendica* of the Iranian region [32], could not develop when they are put in contact with EO of our officinal Sage; which allow us to judge the strong inhibitory power of its EO.

On the other hand, an investigation conducted by [21] on EO of *Salvia officinalis* L. in the region of Batna (Eastern Algeria) announced an inhibitory effect on *Escherichia coli* such as the inhibition of the development of *Escherichia coli* ATCC 25922 which was very sensitive to different concentrations of EO isolated from our plant species of northeastern Algeria.

We even include the work of [12] on the nosocomial germs of hospital infections whose author evoked the effectiveness of thirty essential oils renowned for their antimicrobial activity against bacterial strains such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* . The bactericidal efficacy was also sought by [24] on a Lamiaceae *Ocimum graticimum* whose extracted essential oils involved a strong inhibiting capacity on the growth of the standard strain *Escherichia coli* ATCC 25922 which was also the subject of our study. In our case, the volatile extracts of *Salvia officinalis* L. showed a lesser degree of bactericidal power.

The biological activity of the active principles like that of essential oils is related to their Chemotype it means the biologically active molecules and present predominantly, their composition or the functional groups of the major compounds (alcohol, phenols, terpene and ketone compounds) and for their synergistic effects because an essential oil often contains 50 to 100 different biochemical molecules.

Generally, the action of essential oils is treated as a bacteriostatic effect. However, some of their chemical constituents seem to have bactericidal properties. This bactericidal activity of

natural substances is explained by the lysis of the bacterial membranes. Essential oils, flavonoïds, alkaloids and even tannins could induce an escape of potassium ions in the membrane and thus formation of an irreversible lesions on the level of this membrane. This permeability to potassium is a precursor effect of their death.
The power of the antibacterial activity of natural substances (flavonoïds, EO, tannins ...) shows increasing their effectiveness against multiresistant bacteria. Furthermore, many bacteria have recently developed a resistance to the majority of antibiotics. Therefore, we think that the active principles isolated from various medicinal plants could present an interesting alternative to the use of antibiotics. Indeed, many studies have shown that EO could - well be a particularly credible candidature.

4. Conclusion

At the end of this work, it is interesting to present the principal results:
The preliminary biochemical tests revealed the presence of five compounds of secondary metabolism (gallic tannins, saponins, flavonoïds, Cardinolids, terpenes and sterols) and the absence of anthocyanins, leuco-anthocyanins and alkaloids.
The extraction, analysis and identification by GC/MS and TLC detected on the hand, a Chemotype of nineteen terpene compounds with a predominance of α-Thujone for essential oils and on the other hand the presence of three compounds or group of compounds with three different colors: green yellow, clear yellow and ocher, and of frontal reports varying between 0.128 and 0.928 for the obtained flavonoïds.
From the antibacterial activity evaluated by *in vitro* tests, it appears that flavonoïds and essential oils have a important inhibitory effect on the multi-resistant germs, responsible for infectious diseases. The growth inhibition varies with the bacterial species, the nature and the concentration of the tested product. On the whole, essential oils seem more effective than flavonoïds at all used concentrations. Among all tested strains, five of them were very sensitive towards these natural substances: *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis*, *Escherichia coli* 1554, *Escherichia coli* 1429 and *Klebsiella pneumonia*. The recorded inhibition zones most often exceed those of the antibiotic (*Rif 5 µg*).
These results confirm that the extracts of *Salvia officinalis* L. species may well rival the synthetic chemicals, antibiotics used in nosocomial infectious treatments or even other respiratory infections, urinary and genital. These antibacterial tests provide a scientific validation of the massive traditional use of this species by the population.

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