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Phytochemical Study and Biological Activity of Sage (*Salvia officinalis* L.)

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***Abstract*—**This study presents an attempt to evaluate the antioxidant potential and antimicrobial activity of methanolic extract, and essential oils prepared from the leaves of sage (*Salvia officinalis* L.). The content of polyphenol in the methanolic extracts from the leaves of *Salvia officinalis* was determined spectrophotometrically, calculated as gallic acid and catechin equivalent. The essential oils and methanol extract were also subjected to screenings for the evaluation of their antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test. While the plant essential oils showed only weak antioxidant activities, its methanol extract was considerably active in DPPH (IC_{50} = 37.29 µg/ml) test. Appreciable total polyphenol content (31.25 mg/g) was also detected for the plant methanol extract as gallic acid equivalent in the Folin–Ciocalteu test. The plant was also screened for its antimicrobial activity and good to moderate inhibitions were recorded for its essential oils, and methanol extracts against most of the tested microorganisms.

The present investigation revealed that this plant had rich source of antioxidant properties. It is for this reason that sage has found increasing application in food formulations.

***Keywords*—**Antibacterial, Antioxidant, Flavonoid, Polyphenol, *Salvia officinalis*.

I. Introduction

MEDICINAL plants have served as rich sources of pharmacologically active substances. Herbs have been used in a diverse array of purposes, including medicine, nutrition, flavorings, beverages, dying, repellents, fragrances, cosmetics, charms, smoking and industrial uses. Today, herbs are still found in 40% of prescription drugs [1].

The family of *Lamiaceae* consists of about 230 genera and 7100 species worldwide. Many species from the *Lamiaceae* family are considered of high importance because of their uses in medicine, culinary and cosmetics, and production of essential oils. Some of the major genera belonging to *Lamiaceae* family are *Salvia*, *Menthe* and *Siderites* [2].

The genus's *Salvia* includes approximately 900 species that are cultivated throughout the world for use in folk medicine and for culinary purposes [3]. Essential oils and extracts from *Salvia* species have been shown to possess antimicrobial, antioxidant, anti-inflammatory, anti plasmodial, hypoglycemic and anti-carcinogenic properties [4], [5].

Salvia officinalis L. is a common herbal plant widely cultivated in various parts around the world, but it is native in the Mediterranean region. It is cultivated in several countries, mainly to obtain dried leaves to be used as raw material in medicine, perfumery, and food-industry [6]. Sage is rich in biologically active compounds, among which the most important are polyphenol compounds, plant secondary metabolites, which are divided into two basic groups: phenolics acids and flavonoids [7].

In the past few decades, sage has been the subject to intensive studies for its flavonoids and phenolics, which have been isolated from the plant. It is for this reason that sage has found increasing application in food formulations [8], [9].

Lima et al. [10] tested the antioxidant potential of the Salvia tea *in vivo* and showed that following 14 days of drinking Salvia tea the liver antioxidant status improved. The aqueous extract of *Salvia officinalis* possesses an antioxidant and antiviral effect, such as antimicrobial and fungicidal effects [11], [12].

The aim of this study is *in vitro* an assessment of antioxidant potential and antibacterial activities of the methanolic extracts in *Salvia officinalis* L.

II. Material and Methods

A. Plant Materiel

The plant material was collected in the university garden of Mostaganem during July 2012. The leaves of *Salvia officinalis* L. was dried immediately after harvesting in a shady and well-aired place in two weeks. Afterwards, they were packed in paper bags and kept in a dark. After that the leaves were crushed using a house blender (Mixy, Zepter International). The fresh aerial part of *Salvia officinalis* L. material was subjected to hydro-distillation during approximately 4 h in a *Clevenger-type* apparatus. The distilled essential oils were dried over anhydrous sodium sulphate and stored in tightly closed dark vials at 4–6°C before analysis. The oil's yield was 0.87% (w/w).

B. Phytochemical Screening

Chemical tests were carried out using standard procedures to identify the constituents as described by [13], [14].

Alkaloids: About 0.2 g of the extracts was warmed with 2% sulphuric acid for two minutes. It was filtered, and few drops of Dragendorff's reagent were added. Orange-red precipitate indicates the presence of alkaloids.

Saponins: About 0.2 g of the extracts was shaken with five ml of distilled water and then heated to boil. Frothing (appearance of creamy misses of small bubbles) shows the presence of saponins.

Terpenoids (Salkowski test): 0.2 g of the extracts was mixed with two ml of chloroform and three ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-

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brown coloration of the interface will form to indicate positive results from the presence of terpenoids.

Steroids (Liberman-Burchard's test): two ml of acetic anhydride acid. The color changes from violet to blue or green in samples indicate the presence of steroids.

Tannins: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark-green solution indicates the presence of tannins. One ml of extract was added to two ml of sodium chloride (2%), filtered and mixed with five ml of 1% gelatin solution. A precipitate indicates the presence of tannin.

Flavonoids: Extracts of about 0.2 g was dissolved in sodium hydroxide, and hydrochloric acid was added. A yellow solution that turns colorless indicates the presence of flavonoides.

C. Estimation of Total Phenol Content (TPC)

Total phenol content of the extracts was determined colorimetrically using Folin-Ciocalteau method [15]. This test is based on the oxidation of phenolics groups with phosphomolybdic and phosphotungstic acids. The aliquot (400µL) of each extract was mixed with 2 ml of Folin-Ciocalteau reagent and 1.6 ml of 4% sodium carbonate. The mixture was allowed to stand for 2 h with intermittent shaking for reaction. After oxidation, the green-blue complex formed was measured at 750 nm (Perkin Elmer, UV-Visible Spectrophotometer). Using gallic acid monohydrate, standard curve was prepared and linearity was obtained over the range of 10-50 µg/ml. Total phenolics was calculated using the standard curve, and the concentrations are expressed as gallic acid equivalent (GAE) in % w/w of the extracts. The calibration equation for gallic acid

$$y = 0{,}528x\text{-}0{,}109;\ (R^2=0{,}901).$$

D. Estimation of Flavonoid Content (FC)

Aluminum chloride colorimetric technique was used for total flavonoid estimation. Flavonoids are capable of forming complexes with metal ions and act as antioxidants. A known volume (1.0 ml) of the extract was mixed with 3ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. After incubation at room temperature for 30', the absorbance of the reaction mixture was measured at 415 nm with the help of Perkin Elmer UV Visible Spectrophotometer. The flavonoid content was expressed as quercetin equivalent in % w/w of the extracts. The calibration equation for quercetin was:

$$y = 0{,}075\ x - 0{,}016;\ (R^2 = 0{,}907).$$

All analyses were carried out aseptically in triplicate.

E. DPPH Radical Scavenging Assay

Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract Antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical, using a modified method [16].

The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from one to100 µg/ml. We prepared 0.002% DPPH in methanol. Then 1 ml of this solution was mixed with 1 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using a Cecil spectrophotometer against methanol. The blank was used as 1 ml of methanol with 1 ml of DPPH solution (0.002%). The optical density was recorded, and percent of inhibition was calculated using the formula:

$$\%\ \text{of inhibition of DPPH activity} = A\text{–}B/A \times 100$$

where: A is optical density of the blank; B is optical density of the sample.

F. Microbial Strains

The essential oils were obtained by hydro-distillation of the aerial part of *Salvia officinalis* L. The essential oil and extracts were individually tested against a panel of microorganisms, including *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10239. Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA). The microorganisms were obtained from the Laboratory of Pasteur Institute, Algiers. Cultures of these bacteria were done on Nutrient Muller-Hinton medium and were incubated at 37°C for 24h.

G. Antibacterial Assay

The antimicrobial activity was evaluated by paper disc diffusion [17] and dilution methods [18].

The pre-inoculum preparation was carried out by the cultivation of bacteria in LB medium, at 30°C for 24 hours. For the experiments in solid medium, Mueller and Hinton – MH (*Merck*) medium was poured into Petri dishes and cooled under sterile conditions. An aliquot of 200 µL the pre inoculums was then added to each Petri dish (approximately 108 and disks of filter paper (*Whatman* n° 3) with 7 mm UFC. mL diameters were placed on the solid medium: a disk containing 7.5 µL of chloramphenicol, corresponding to 30µg, an empty disk (without any compound), used as a negative indicator, and four disks with 5.0 µL of different essential oils. The Petri dishes were incubated at 36°C for 24 hours. The mean diameter of inhibition halo was measured for each disk using a caliper rule.

Minimum inhibitory concentrations (MIC) were determined by the evaluation of growth by optical density.

III. Results and Discussion

Phytochemical screening by simple chemical tests and showed a presence of flavonoid (caffeic acid, quercetin and luteolin), triterpenoids and steroids (β-sitosterol, β-amirin) and

cinnamic derivatives (chlorogenic acid) in *Salvia officinalis* (Table I).

TABLE I
PHYTOCHEMICAL SCREENING OF HYDRO ALCOHOLIC EXTRACTS OF *SALVIA OFFICINALIS* LEAVES (+) - PRESENCE, (-) – ABSENCE

Constituent	Tests	Hydro alcoholic extract
Tannins	Ferric chloride test	+
Flavonoids test	Sodium hydroxide test	+
Terpenoids and Steroids	Salkowski test	+
Alkaloids	Dragendorffs test	-
Saponins	Frothing test	-

A. Determination of Polyphenol

Based on the absorbance values of the various extract solutions, reacting with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, as described above, results of the colorimetric analysis of total polyphenol are given in Table II.

TABLE II
TOTAL PHENOLICS, FLAVONOIDS AND DPPH RADICAL SCAVENGING ACTIVITY EXTRACTS *SALVIA OFFICINALIS* LEAVES

Total phenolics (mg GAE/100 g DW)	Total flavonoids (mg GAE/100 g DW)	DPPH radical scavenging activity [IC_{50} (µg/ml)]
31,15±1,056	18,46±0,132	130,56±0,867

The total phenolics and flavonoids content of the methanolic extracts were found to be 31,15 ± 1,05 and 18,46 ± 0,13mg/g respectively compared with gallic acid, catechin equivalents. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities in this plant extract used for this study.

The total phenol content and total flavonoids content showed strong correlation with total antioxidant activity, with the correlation coefficient R^2 = 0,907 for *Salvia officinalis*. This indicates that the antioxidant activity of the extract from *Salvia officinalis* leave is due to its phenolics constituents. These results are in accordance with other reports in the literature, which showed positive strong correlation between antioxidant activities and total phenolics [19].

Phenolics compounds are known to have antioxidant activity, and it is likely that the activity of the extracts is due to these compounds [20]. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [21].

B. DPPH Radical Scavenging Activity*

The results on DPPH· radical scavenging activity of the aqueous tuber extracts along with the reference standards ascorbic acid (Vit C) and butylated hydroxyl toluene (BHT) are shown in Fig. 1.

Concentration of the sample necessary to decrease initial concentration of DPPH* by 50% (IC_{50}) under experimental condition was calculated. Therefore, lower value of IC_{50} indicates higher antioxidant activity of 130,56 µg/ml. In this, study indicates that the plant was potently active and these plant extract to contain polyphenol compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron which is responsible for radical's reactivity. Free radicals are chemical entities that can exist individually with one or more unpaired electrons [22].

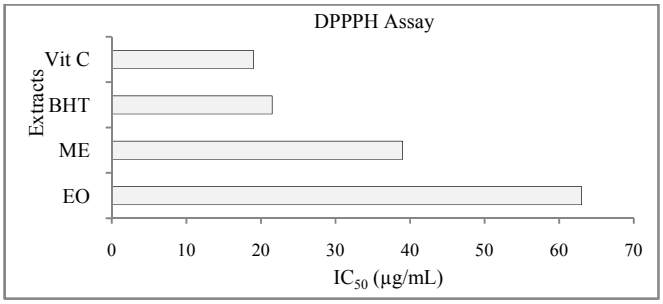


Fig. 1 Free radical scavenging capacities of the extracts measured in DPPH assay Vit C: Ascorbic Acid, BHT: Butylated Hydroxyl Toluene, ME: Methanolic Extract, EO: Essential Oils

The measurements of the absorbance of DPPH causes by the presence of different substances antioxidants (essential oils, methanolic extract) of *Salvia officinalis* L. for determining the inhibition percentage (% I) from each dilution (10 to 50 µg/ml). After calculating the inhibition percentage for different dilutions of the extracts, we found that the highest concentration used, which is 50 µg/ml, we obtained the % I = 86.73 % and 40.13 % of the crude extract and essential oil respectively.

The test results (DPPH) showed that the methanolic extracts of sage have been very high compared with essential oils antiradical activities. However, the activity of BHA is greater than that of vitamin C (Figs. 2-4).

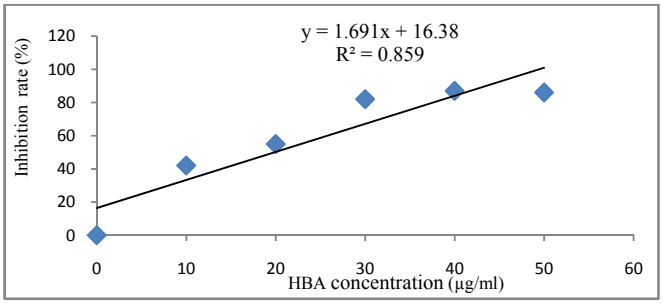


Fig. 2 Percent inhibition of free radical (DPPH) of BHA

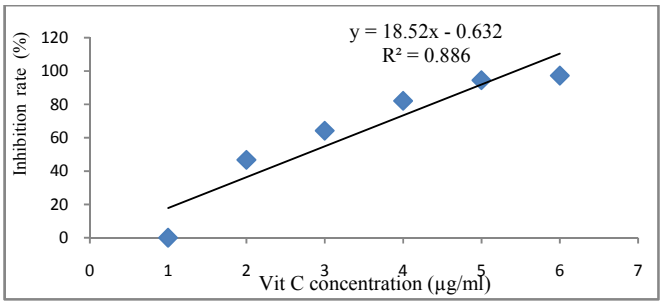


Fig. 3 Percent inhibition of free radical (DPPH) of Vit C

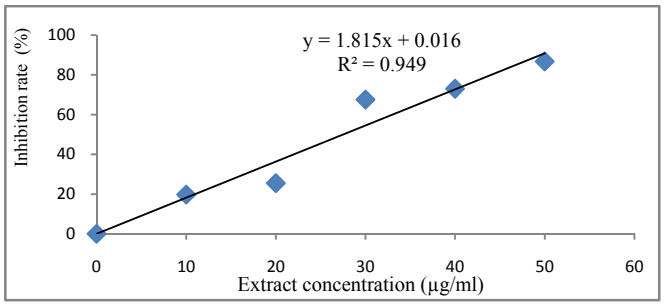


Fig. 4 Percent inhibition of free radical (DPPH) of extract

Antioxidants are directly related to quantitative and / or qualitative diversity of compounds present in the extracts [23]. In our case, a mixture of extracts was rich in phenolics compounds by supplying essential oils. Therefore, his action was the characteristic rather rapid antioxidant.

The results from our work showed antioxidant effects of *Salvia officinalis* extracts. Such properties have been previously reported for leaf extracts from the plant [24]. We found that also extracts of sage leaves exhibited strong antioxidant activity. It was observed that due to variety of antioxidant compounds presented in sage.

In general, antioxidant activity of various Salvia species extracts is described, but not so much is reported on their essential oils to this regard. The antioxidant activities of *S. officinalis* L. has been widely demonstrated [25]-[27].

C. Determination of IC_{50}

Results may be expressed as percentage of the antiradical activity of DPPH or remaining percentage, or may also be expressed using the parameter IC_{50}, which is defined as the concentration on the substrate causes a loss of 50% of the DPPH activity [28].

The results obtained showed that the methanolic extract of *Salvia officinalis* has been very similar to that of BHA and Vit C activity (IC_{50} = 2.70 and 14.84) respectively (Table III), this activity is as well higher than that found in essential oils. These results can be explained by the low solubilization of these constituents in water [29].

TABLE III
IC_{50} VALUES (MG/ML) OF METHANOLIC EXTRACT AND ESSENTIAL OILS OF *SALVIA OFFICINALIS* L.

	Es.Oil	Extract	Polyph	Flavo.	BHA	Vit C
IC_{50} (µg/ml)	62.65	27.53	33,77	18,14	14.84	2.73

D. Antibacterial Activity

The essential oil and leaves extracts of *Salvia officinalis* has been documented to have a wide range of antimicrobial effects [30]. The antimicrobial actions are suggested due to its particular chemical constituents.

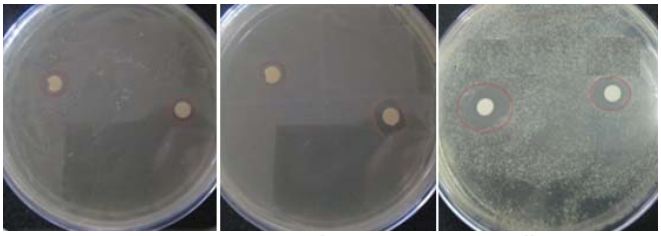
Results obtained from disc diffusion method, followed by measurements of MIC, indicate that *Bacillus cereus* 14579 is the most sensitive microorganism tested, with the lowest MIC values (16µg/ml) in the presence of the oil isolated from *Salvia officinalis* (Table IV).

Escherichia coli ATCC 25922 and *Candida albicans* were other sensitive ones against the oil with an MIC value at 75µg/ml, respectively. No activity was observed against two microorganisms (*Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923).

The antibacterial activity of *Salvia officinalis* essential oil was tested against several microorganisms (Table IV, Fig. 5). The essential oil exhibited the best antibacterial activity against, *Escherichia coli* ATCC 25922, *Bacillus cereus* 14579 and *Candida albicans* with 18, 12 mm and 22 mm, inhibition zone diameters, respectively.

TABLE IV
INHIBITION ZONE AND MINIMAL INHIBITORY CONCENTRATION OF THE ESSENTIAL OILS EXTRACT OF *SALVIA OFFICINALIS* L.

Microorganism	Inhibition zone *(mm)	MIC (µg/mL)
Escherichia coli ATCC 25922	18	75
Pseudomonas aeruginosa ATCC 27853	-	-
Staphylococcus aureus ATCC 25923	-	-
Bacillus cereus 14579	12	16
Candida albicans	22	20



B. cereus *E. coli* *C. albicans*

Fig. 5 Inhibition zones of methanolic extract from *Salvia officinalis* for bacterial strains tested

The antimicrobial activity from the major compounds of the oil studied here was previously well defined by several researchers [19], [31]-[34].

IV. CONCLUSION

In conclusion, the present study shows that polyphenol content in the leave's extracts of *Salvia officinalis* L. is high, and these extracts exhibit strong antioxidant activities compared to that of the standard compounds such as Ascorbic acid (Vit C) and Butylated hydroxyl Toluene (BHT). Hence, this investigation suggested that the plant naturally having rich source of antioxidants could be used in the prevention of free radical diseases and general health tonic.

Salvia officinalis L. is a plant which has been used in a variety of food preparations. In this work, we showed the significant antibacterial activity of the essential oils of *Salvia officinalis* L. Some earlier studies have demonstrated sage antibacterial activity against food borne bacteria [35].

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