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Phytochemical screening and the antioxidant, antibacterial and antifungal activities of aqueous extracts from the leaves of Salvia officinalis planted in Morocco

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Abstract

The aqueous extract of the leaves of Salvia officinalis planted in Morocco is analyzed using different methods in order to study the different phytochemical components (phytochemical screening) and to test in parallel the antioxidant, antibacterial and antifungal activities. The main phytochemical components of salvia officinalis are: phenolics complex 13.87±1.96 mg E.AG/g, flavonoids 7.38±1.91mg E.Q/g and tannins 0.18±0.03 µg E.Cat/g, as well as different concentrations of saponins, alkaloids and mucilage. The antioxidant activity is achieved by four different methods: the DPPH test (IC-50 = 8.2 µg/ml higher than BHT (1.2 µg/ml)); the ß-carotene decolorization test (the inhibition of oxidation of the aqueous extract (64.2%) was greater than the negative control (13. 87%)); the reducing power test (FRAP) (detection of a low reducing power of iron for the aqueous extract compared to quercetin) and the total antioxidant capacity (CAT), this test revealed an antioxidant capacity of 103.7±5.8 mg/g. The aqueous extract of salvia officinalis leaves showed antibacterial activity against four pathogenic bacteria: E. coli 57, K. pneumoniae, P. aeruginosa, S. aureus, noting MIC values of 0.25mg/ml; 0.125mg/ml; 0. The extract showed antifungal activity against two yeasts (S. sereveseae (0.25 mg/mlet C. albicans 0.25 mg/ml), using the agar diffusion method and the direct contact method.

Keywords: *Salvia officinalis; phytochemical; screening; antifungal activity; antimicrobial activity; aromatic and medicinal plant.*

1. Introduction

Since antiquity, human beings have used various plants found in their environment, in order to treat and cure all kinds of diseases, these plants represent a huge reservoir of potential compounds of secondary metabolites (phenols, flavonoids, tannin, mucilage, essential oil,) which have the advantage of being of a great diversity of chemical structure with a very wide range of biological activities [1]. Currently the World Health Organization (WHO) estimates that about 80% of the world's population uses traditional herbal medicines as primary health care [2,3].Among these plants widely consumed by the population in general and especially the Moroccan population, we note *Salvia officinalis* [4]. Commonly called "SALMIA", *Salvia officinalis* is a perennial, annual plant and belongs to the *Lamiaceae* family (formerly *Labiatae*)[5]. It forms a small sub-shrub of 50 cm to 80 cm high with woody, brownish roots, and fibrous. The stem is woody at the base, forming a bush sometimes exceeding 60 cm, green twigs. whitish quadrangular, and hairy. these flowers are purplish-blue in loose terminal spikes, arranged by 3 to 6 in spaced whorls visible from May to August. They are large, grouped at the base of the upper leaves [6].Sage is a panacea always very appreciated; it remains used in the modern pharmacopoeia not only in medicine, in cooking but also in the perfume industry thanks to its various phytochemical compounds and also thanks to its miraculous effect and phytotherapeutic activity [7]. Therefore, the main aim of the present work is to evaluate the antioxidant, antibacterial and antifungal activity of sage, even after the realization of a phytochemical screnning.

2. Materials and methods

2.1 Plant material

Salvia officinalis was harvested fresh from local farmers in the region of Haj Kadour in Meknes (33° 53' 42", 5° 33' 17") in northwest Morocco during May-June 2018. The identification of the species was carried out by the professor of botany Mr. REHOU Abderrahim of the department of biology, Faculty of Sciences, Moulay Ismail University Meknes-Morocco. In the present study, the part of the leaves of the plant was used to prepare the preparation of the aqueous extract.

2.2 Preparation of aqueous extract

The cleaning of the plant material and the washing with water after the harvest is the initial process to guarantee the good conservation of the plant. the second step is drying in an oven at a temperature of 35°C and in the dark for a few days. The material is then crushed by an electric grinder and stored in boxes. The aqueous extract is prepared by decoction for 30 minutes, with three repetitions each time. After filtration on filter paper, the filtrate is concentrated in an oven at a temperature of 35°C.

2.3 Phytochemical screening

Phytochemical tests consist in detecting the different families of compounds existing in the studied part of the plant of (leaves of *Salvia officinalis*), by qualitative reactions. For this reason, we have carried out a phytochemical screening for the detection of the following metabolites: flavonoids, tannins, terpenes, coumarins, cyanogenic compounds, alkaloids, quinones and saponins [8].

2.3.1 Determination of total polyphenols

the Singleton method with the Folin-Ciocalteau reagent is the selected approach to quantify total phenolic compounds. first, 500 µl of diluted F-C reagent (10/100) is added to 100µl of methanolic extract from our

plant. a few minutes later, 400µl of a sodium carbonate solution (75 mg/ml) is added to this reaction medium. secondly, the mixture is incubated in the dark for 2 hours at room temperature, then the absorbance is measured at 765 nm. The results obtained are expressed as µg A.G.Eq/mg extract using the linear regression equation of the calibration curve plotted for gallic acid [9].

2.3.2 Dosage of condensed tannins

The determination of the quantification of tannins in our plant extracts is carried out according to the method of vanillin in acid medium [10] according to the following procedure:

The vanillin reagent was prepared by mixing at equal volume the following dilutions: 8% HCl (v/v), 37% methanol (v/v) and 4% vanillin in methanol (w/v). The mixture was incubated at 30°C a few minutes before the test. 200 µL of each extract tested was added to 1 ml of vanillin reagent and incubated in the dark at 30°C for 20 min. Absorbance was measured at 500 nm by a spectrophotometer against a blank consisting of an equal volume mixture of methanol (37%) and HCl (8%). The results are expressed in µg catechol equivalent/mg dry extract with reference to the catechol calibration curve [10].

2.3.3 Determination of flavonoids

The content of total flavonoids in the extract of *Salvia officinalis* was carried out according to the method described by Dehpeur [11]: 500 µl of the extract to be analyzed is added to 1500 µl 95 % methanol, 100 µl 10 % (w/v) AlCl3, 100 µl 1 M sodium acetate and 2.8 ml distilled water. The mixture is stirred and incubated in the dark and at room temperature for 30 minutes. The blank is obtained by replacing the extract with 95% methanol. The absorbance is measured at 415 nm using a spectrophotometer. The results are expressed in mg quercetin equivalent / g dry plant matter by reference to the quercetin calibration curve [11].

2.4 Anti-oxidant activity of the extracts studied

The antioxidant capacity of the *Salvia officinalis* extracts studied was evaluated in this work by a series of four tests aimed at determining 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, the reducing power test (FRAP), the total antioxidant capacity (CAT) and the ß-carotene discoloration test.

2.4.1 Free radical scavenging DPPH- test

The method for trapping the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical was first described by Blois (1958). In the present work, the method used for the evaluation of DPPH radical scavenging by the plant extracts studied is the one described by BektaS in 2005 [12,13]. After preparation of the methanolic solutions of the extracts, 100 µL of each is taken and put into an Eppendorf tube and 750 µL of a methanolic solution of DPPH (0.004%) is added. The reaction mixture is immediately stirred and then placed for 30 min in the dark at room temperature in the laboratory. The absorbance of the reaction medium was measured at 517 nm using a spectrophotometer against a negative control (containing methanol instead of extract).

Le pourcentage d'inhibition du radical de DPPH a été calculé suivant la formule :

*PI (%) = (A0-A/A0) *100*

PI : Pourcentage d'inhibition ; A0 : Absorbance de la solution de DPPH sans l'échantillon (contrôle négatif) ; A : Absorbance de la solution de DPPH en présence de l'échantillon.

2.4.2 Reductive power test

following the Moattar protocol in 2016 [9,14,15], 500 µl of a phosphate buffer solution (0.2 M - pH = 6.6) and 500 µl of potassium ferricyanide [K3Fe (CN)6] (1%) are mixed with 100 µl of different concentrations of the prepared samples in methanol. The mixtures are incubated for 20 minutes in a water bath at 50°C. Immediately afterwards 500 µl of a 10% TCA solution, 500 µl of distilled water and 100 µl of 0.1% FeCl3 are added to the reaction medium. Then the absorbance is determined at 700 nm against a blank containing all reagents in the absence of the test sample. The results are expressed as a 50% effective concentration (EC50) which reflects the concentration of antioxidant required to achieve an absorbance of 0.5 nm.

2.4.3 Total antioxidant capacity test

in an Eppendorf tube mix 25 µl of plant extracts with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). leave the reaction tubes incubated at a temperature of 95°C for 90 min. After the specified time has elapsed, the tubes are cooled to room temperature and the absorbance is measured with the spectrophotometer at 695 nm with a blank containing 25 µl methanol instead of the extract [16].The total antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g extract) from an ascorbic acid calibration curve.

2.4.4 ß-carotene discoloration test

In a vial, 1 ml of ß-carotene solution in chloroform (20 mg/100 ml), 0.01 ml linoleic acid and 100 ml Tween 80 are mixed. The chloroform is evaporated at 45 °C for 5 minutes in a vacuum rotary evaporator and then 25 ml hydrogen peroxide is added to the residue. Finally, water was added slowly to the mixture, which was vigorously stirred to form an emulsion [17,18]. To 2.5 ml of the previous mixture, 0.1 ml of each of the extracts diluted in methanol was added in tubes. After incubating the tubes in a water bath at 50°C for 120 minutes, the absorbance was measured at 470 nm. The percentage of antioxidant activity was calculated from the following equation: $AA\% = (AE/ABHT) * 100$

AA%: percentage of antioxidant activity; AE: absorbance after 120 min with negative control; ABHT: absorbance after 120 min of BHT.

A blank containing the stock solution without β-carotene was used to bring the spectrophotometer to zero. The percentage of antioxidant activity was corrected with the BHT standard which was used under the same conditions for the extracts.

2.5 Antimicrobial activity

2.5.1 Culture media

To discover antimicrobial activity in this work, the following agar media are used: Muller Hinton Agar (MHA) and Sabouraud (SB). And for the determination of the minimum concentration, the media used are Malt extract broth (EM) and Muller Hinton broth (MHB). The media were sterilized in an autoclave at 120°C for 20 minutes [19].

2.5.2 Biological material

The bacterial strains chosen for this study are potentially pathogenic bacteria, in particular gram-negative bacteria *Escherichia coli* (ATB:57) B6N (CHU, Fez), *Escherichia coli* (ATB : 97) GMB (CHU, Fez), *Klebsiella pneumoniae* (LM, FMP, Fez), and *Pseudomonas aeruginosa* (LM, FMP, Fez); and gram-positive

bacteria including *Staphylococcus aureus* (LM, FMP, Fez); For Yeasts we used *Candida albicans* and *Saccharomyces cerevesea.*

2.5.3 Products used

this work evaluated the antimicrobial activity of the aqueous extract of the leaves of *Salvia officinalis*. the positive controls used for the comparison of results were Antibiotics, including Ampicillin (AMP) and Streptomycin (STR), and the antifungal agent: Fluconazole (AF).

2.5.4 Preparation of the inoculum

The microbial inoculum was prepared by the direct suspension method from 2 to 3 colonies of a fresh culture which were taken aseptically, and were suspended in a solution of sterile physiological water (PES; with 0.85 to 0.9 NaCl), after vortex agitation, the turbidity was adjusted to 0.5 McFarland. The bacterial suspensions contain approximately 1x108 to 2x108 CFU/ml, while the yeast suspension contains approximately 1x106 to 5x106 CFU/ml. The McFarland standard was prepared with a mixture of 99.5 ml of a 0.36N sulphuric acid (H2SO4) solution with 0.5 ml of a 0.048 M dehydrated barium chloride (BaCl2*2H2O) solution. The absorbance of the solution was checked using a UV-Visible spectrophotometer at λ= 625nm which should be between 0.08 and 0.12. This solution was poured into 10 ml test tubes. After preparation of the inoculum, the plates containing the agar medium (MHB and SB) were inoculated with 1 ml of the bacterial and fungal suspension by the flooding technique and thus the inoculum was dispersed over the entire agar surface (circular and back and forth movement) with removal of the excess inoculum using the propette. Finally, the boxes were dried for 10 min [20].

2.5.5 Disk broadcast technique

in order to assess the sensitivity of the microorganisms to an antimicrobial agent, the disc diffusion technique is used. after preparation of the kneading boxes and leaving them to stand for 10 minutes, sterile discs with a diameter of 6 mm were placed on the agar surface of the previously inoculated boxes and impregnated with 10 µl of test material: AMP 1.67 mg/disc, STR 0.02 mg/disc, plant extract and the concentration of FA is 5 mg/disc. Subsequently, the individual plates are incubated for 24 hours at a temperature of 37°C for bacteria and 30°C for yeast. Finally, the antimicrobial power after incubation is evaluated by measuring the growth inhibition zones in mm [21].

2.5.6 Microdilution technique

Dilutions were made as follows: first the microbial inoculum was diluted to 1/1000 of 0.5 McFarland in the MH medium for bacteria and EM for yeasts. These dilutions were then diluted ½ after inoculation of the microplate. The stock solutions (prepared in sterile distilled water) of MPA (1.66 mg/ml) and STR (5 mg/ml), i.e., 5 mg/ml for FA, were then diluted 1:10 in the MH medium for MPA and STR and the EM medium for FA.

3. Results

3.1 Phytochemical screening

Results of phytochemical tests on the aqueous extract of the leaves of *Salvia officinalis* indicate the presence of several compounds like all aromatic and medicinal plants: tannins, flavonoids, sterol, alkaloids,

saponosides and mucilage. Screening was the first step in this work for research on bioactive molecules. The screening results are presented in Table 1.

Table 1: Phytochemical families presented in the studied extract

		Plant extract
Tanin		+++
catechin tanins		-
Gallic tanins		+++
Flavonoïdes		++ (flavonol+flavanol+genine)
Sterol		++
Alcaloides	**Dragondorf test**	++
	Mayer test	+
Saponosides		+++
Cardiac Glycosides		+++
Oses and holosides		++
Mucilage		+++

+++: Strong presence; ++: Medium presence; +: Weak presence; -: Absent

3.2 Determination of Phenolic compounds

According to the linear calibration curve performed by gallic acid at different concentrations under the same sampling conditions according to the equation: $Y= 0.0425X + 0.2218$ and $R^2 = 0.985$, the results of the quantification of the total polyphenol content of the aqueous extract of the leaves of *Salvia officinalis* are shown in Table 2 expressed as equivalent mg of gallic acid per 1 g of extract. In the same way, the concentration of flavonoid compounds is determined by following the linear calibration curve performed by quercetin at different under the same operating conditions according to the following equation $Y = 0.006X - 0.0048$ and $R^2 = 0.9863$. Results are expressed in mg of quercetin equivalent per 1 g of extract. The quantification of the tannin content of the extracts was carried out according to a linear calibration curve ($Y= 0.6042X + 0.065$ and $R^2 = 0.9942$) performed by catechol under the same sampling conditions. results are shown in table 2.

Table 2: Concentration of phytochemical compounds measured

	Total polyphenols (mg E.AG/g)	**Flavonoids (Mg E.Q/g)**	**Tanins (µg E.Cat/g)**
Concentration	13,87±1,96	7,38±1,91	0,18±0,03

The results show that the concentration of total phenolic compounds in the aqueous extract of the leaves of *Salvia officinalis* is of the order of 13.87±1.96 mg gallic acid equivalent in one gram of extract. Then, the concentration of flavonoids in the extract is of the order of 7.38±1.91 mg quercetin equivalent present in one gram of extract of the studied plant; while the concentration of tannins remains of the order of 0.18±0.03 mg E. Cat/g of extract. It should be noted that the method used is the decoction which remains the most effective method for extraction because it requires a temperature that facilitates the solubility and release of compounds and the extraction of phytochemical compounds exist in the material of plant origin. Thus, increasing the decoction temperature can decrease the total polyphenol content [8].

3.3 Anti-oxidant activity of the extracts

3.3.1 DPPH Test

The free radical DPPH allowed the estimation of the antioxidant activity of isolated and identified compounds. It is a purple-colored synthetic radical that turns yellow when captured by the antioxidant compounds tested [9]. The intensity of the yellow color reflects the antiradical capacity of the molecule tested, and depends on the nature, the Figure 1A illustrates the percentages of antiradical activity of the aqueous extract isolated from the leaves of *Salvia officinalis* with respect to the free radical DPPH. It is also important to know that the IC50 is the concentration necessary to eliminate 50% of the free radicals, it is the parameter used to measure the activity of the extract to trap the radical free, it is inversely related to the antioxidant capacity of a compound because a low value of IC50 indicates a high antioxidant effectiveness of the extract (figure 1B) [22].

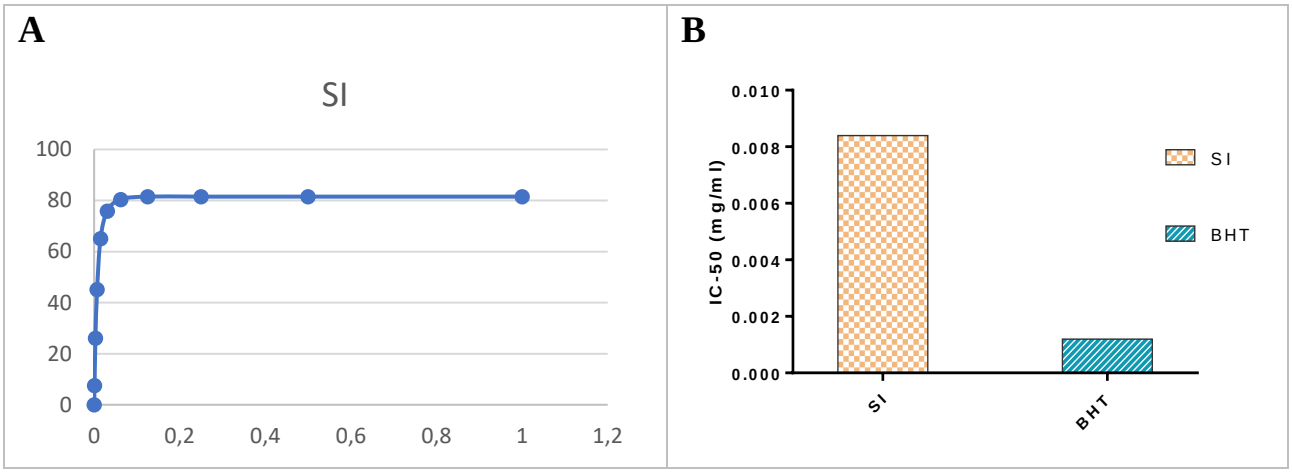

Figure 1: Antioxidant activity by the DPPH test for the aqueous extract OF *Salvia officinalis* (SI), A: percentage inhibition as a function of the extract concentration, B: the inhibitory concentration of half of the free radicals of DPPH.

From the results obtained, it was deduced that the leaf extract exerted a good activity towards the DPPH radical, exceeding 80% inhibition at a concentration of 0.01mg/ml of leaf extract, which was confirmed by the IC50 values, since more than 8.2 µg/ml for the extract from the leaves of *Salvia officinalis* which is higher than that of BHT record value of 0.12 µg/ml.

3.3.2 Decoloration of B-carotene

The degradation of fatty acids is one of the main causes of food spoilage. The inhibition of the oxidation of fatty acids through the use of natural preservatives is important in the food industry. In this test, the inhibition of oxidation of linoleic acid is measured in the presence of β-carotene, which is used as a marker.

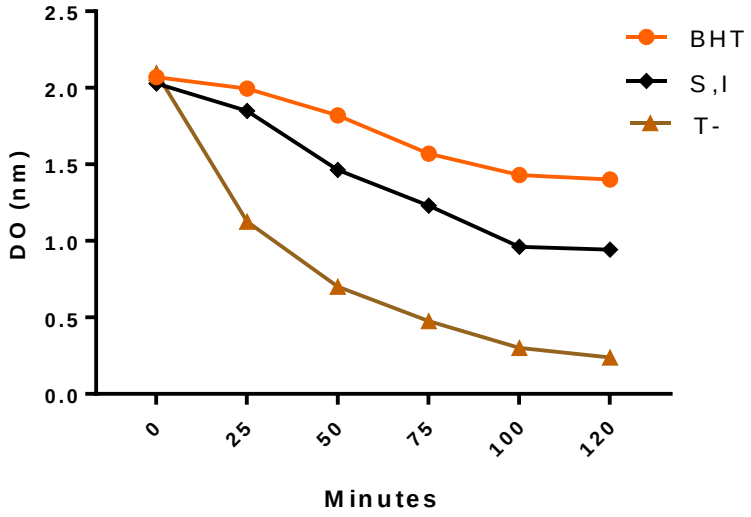

Figure 2: Evaluation of antioxidant activity by the B-carotene decoloration method.

Figure 2 shows the evaluation of the antioxidant activity by the B-carotene decolorization method. The results show that the absorbance of β-carotene in the presence of aqueous extract of the leaves of *Salvia officinalis*, BHT and the negative control decreases progressively. This decrease is very important at the beginning of the test, while after 100 min it becomes very low. The decrease of the (DO) of the negative control is the most important, followed by the aqueous extract of the leaves of our plant, and finally the BHT. This change in absorbance at different time intervals shows that no extract seems to be effective as an oxidation inhibitor of linoleic acid (figure 2).

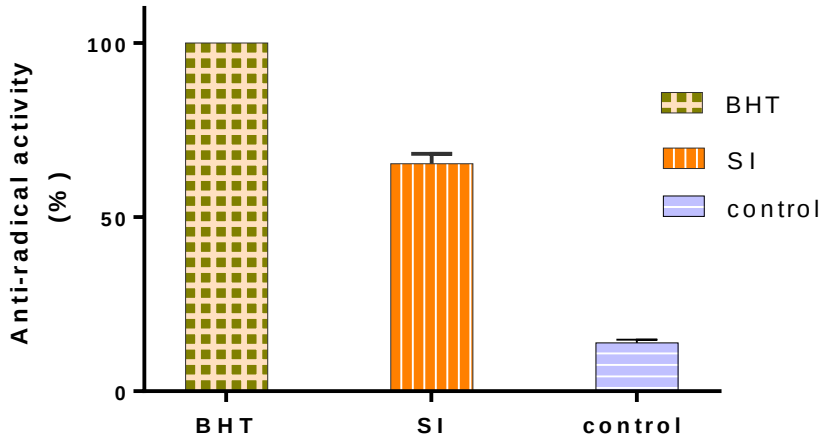

Figure 3: Anti-radical activity of extract, BHT and control in B-carotene discoloration.

The above-mentioned results are confirmed by the results of the antiradical activity mentioned in figure 3, it can be clearly seen that the oxidation inhibition was more important for the aqueous extract of the leaves of *Salvia officinalis* (66.17%) than for the negative control (13.87%), so that the antioxidant activity of both samples remains less important than the positive control used as reference (the antioxidant of BHT).

3.3.3 Reducing power

the third method used in the current work to study the antioxidant activity of the aqueous extract of the leaves of *Salvia officinalis* is the iron reduction test (FRAP). the principle of this method consists on the

observation of the change of the yellow color of potassium ferrocyanide to a blue-green color, the intensity of which depends on the reduction of the potency of each sample. The intensity of the latter depends essentially on the amount of reducing power present in the test medium. This results in an increase in absorbance, which is measured at 700 nm [23,24].

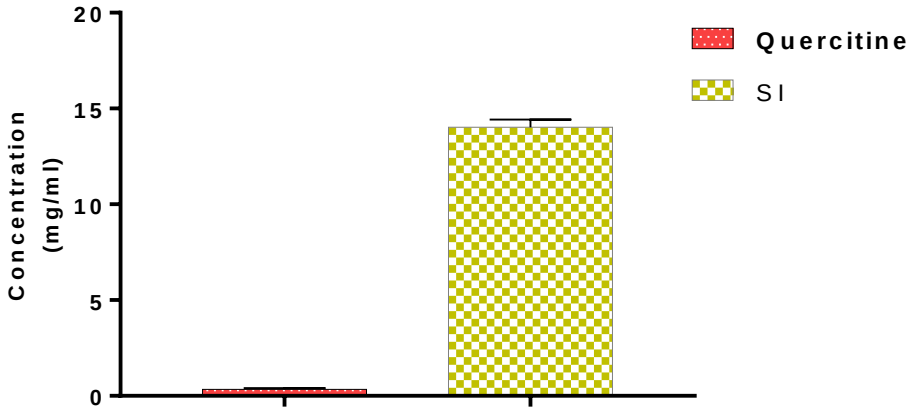

Figure 4: Antioxidant activity by the FRAP method

Figure 4 presents the results of the reduction of the ferric complex in the presence of the aqueous extract of the leaves of *Salvia officinalis* and compares them with the reduction in the presence of the positive control used as a reference (quercetin). The results show that the aqueous extract of our plant has a much lower antioxidant activity than the reference used (Quercitin). Metal ions are necessary for the functioning of biochemical and physiological cellular processes, but in some cases and when their mechanism of action is not well controlled, these same ions can cause lipid peroxidation, oxidative stress or tissue damage [23,24].

3.3.4 Total antioxidant capacity

the last method used in this work to quantify the antioxidant capacity of the aqueous extract of the leaves of *Salvia officinalis* is the total antioxidant capacity using ascorbic acid as standard at different concentrations expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g EXT). according to the calibration curve of ascorbic acid, the results show an antioxidant capacity of the order of 103.7±5.8 mg/g.

3.4 Antibacterial activity

The agar diffusion method is the method used to qualitatively evaluate the antibacterial activity of the aqueous extract of the leaves of *Salvia officinalis* (table 3) while the microdilution method is the method used to quantitatively measure the minimal inhibitory concentration of the extract of our plant (table 4). The results obtained are expressed by measuring the diameter of the inhibition zone. around the disk being used.
The results of the activity mentioned in Table 3 show that the aqueous extract has an antibacterial effect on all bacteria used whether Gram positive or negative with inhibition zones of the order of 1.8±0.2 cm, 1.3±0.1 cm and 1.6±0.2 cm for the Gram-negative bacteria *Escherichia coli 57, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively, and 0.8±0.1 cm for gram positive Staphylococcus aureus. The selected bacterial strains were resistant to antibiotics used as reference product, except for *S.aureus* which is sensitive to streptomycin (1.2±0.0 mm) (Table 3).

Table 3: Results of the diameter of the studied extract inhibition zone and antibiotics.

Compound	Gram-negative bacteria (cm)			Gram-positive bacteria (cm)
	Escherichia coli 57	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
SI	1.8±0.2	1.3±0.1	1.6±0.2	0.8±0.1
Streptomycine	-	-	-	1.2±0.0
Ampicilline	-	-	-	-

The minimum inhibitory concentration (MIC) of the aqueous extract of *Salvia officinalis* obtained is approximately the same in all the gram-negative and gram-positive bacterial strains used, which is of the order of 0.25 mg/ml, except for the *Klebsiella pneumoniae* bacterium which shows a lower MIC value than the other bacteria, of the order of 0.125 mg/ml. In general, however, the ICD remains accentuated compared with the reference antibiotic (table 4).

Table 4: The minimum inhibitory concentration obtained by the micro-dilution method.

Compound	Gram-negative bacteria (mg/ml)			Gram-positive bacteria (mg/ml)
	Escherichia coli 57	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
SI	0.25	0.125	0.25	0.25
Streptomycine	0.25	0.003	-	0.062
Ampicilline	-	-	-	-

3.5 Antifungal activity

Following the previous study, the anti-fungal effects of the aqueous extracts of the leaves of *Salvia officinalis* is evaluated against two types of yeast with varied metabolism*: Candida albicans (C.a), Saccharomyces cerevisiae (S.c)*. The methods used to evaluate the effects of the plant extracts are the direct contact technique and the MIC technique using a positive control reference: Fluconazole. The test results showed significant growth of mycelial fungal inhibition around the discs containing the aqueous extract on *C.albicans and S. cerevisiae (S.c)* with an inhibition zone of the order of 0.9±0.2 cm and 1.8±0.1 cm respectively. Comparing these values with the reference antifungal agent used (Fluconazole), it is noted that the inhibition zone is larger recorded on the extract, but in general remains significant (Table 5).

***Table 5: Extract inhibition zone results and Minimum concentration of water extract inhibitor* on fungal strains**

	Diametre de la zone d'inhibition (cm)		***Concentraton miminale inhibition (mg/ml)***	
Compound	*C. albicans*	*S. Sereveseae*	*C. albicans*	*S. Sereveseae*
SI	0.9±0.2	1.8±0.1	0.25	0.25
Fluconazole	2.3±0.2	2.6±0.1	0.03	0.03

The minimum inhibitory concentration of the aqueous extract of the leaves of *Salvia officinalis* is generally higher than that of the other reference antifungal agent. The results showed that the aqueous extract of the test plant is effective at a concentration of 0.25 mg/ml on *C. albicans* and *S. cerevisiae.* This value is greater than that of Fluconazole, which is of the order of 0.03 mg/ml (Table 5).

4. Discussion

To conclude, the present work used four different methods for testing the antioxidant activity of the aqueous extract of the leaves of *Salvia officinalis*, for reliable results given their complex composition, which requires the use of several protocols to assess antioxidant efficacy. firstly, according to some subsequent studies, there is a strong correlation between the content of the plant extract in total polyphenolic compounds and the antioxidant power, which allows researchers to direct their research towards the study of the molecular structure of plant phytochemical compounds in order to know the mechanism of action of certain phytochemical compounds, particularly phenolic compounds [5,25]. Other studies have reported a positive correlation between antioxidant activity and polyphenol structure. In general, polyphenols with a high number of hydroxyl groups have the highest antioxidant activity [22] due to their ability to give more atoms to stabilize free radicals [26], which explains the anti-free radical activity, which depends on the number, position and nature of substituents on the B and C rings (hydroxyl groups, metaxyl groups, glycosylated groups) and the degree of polymerization [5]. Thus, the antioxidant effect is not only dose-dependent but also depends on the structure [27]. On the other hand, numerous other publications have shown a link between antioxidant activities and the reducing power of phytochemical components in certain plant species [28]. The presence of hydroxyl groups in plant extracts gives them reducing power and can serve as electron donors. Indeed, antioxidants are considered to be reducing and inactivating oxidants [29]. The autoxidation mechanism of the plant extract depends on certain parameters such as temperature, concentration of metal ions and polyphenols, pH and the presence of complexing agents [30]. Some previous studies have also shown that the reducing power of a plant extract can serve as a significant indicator of its potential antioxidant activity. The study by [31] shows that isopropyl alcohol extract from *Salvia officinalis* had a high activity against *Corynebacterium pseudodiphtericum* (with a MIC of 10µg/ml), a lower activity against *Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus lugdunensis, Corynebacterium amycolatum* (with a MIC of 20µg/ml), a low activity against *Staphylococcus aureus, Enterococcusfaecalis, Morganellamorganii, Pseudomonas aeruginosa, Pseudomonas maltophilia, Micrococcus luteus* (with a MIC of 100µg/ml). On the other hand, this extract did not inhibit the growth of *Candida albicans, Candida krusei, E. coli, Klebsiellapneumoniae* and *Serratiamarcescens.* In the study conducted by [32], acetone and chloroform extracts of *Salvia sclarea L.* from Turkey (at 30µg/disc) did not inhibit the growth of *Escherichia coli* and *Listeria monocytogenes*. On the other hand, the efficacy was notable against *Bacillus megaterium, Bacillus brevis, Proteus vulgaris, Micrococcus luteus, Pseudomonas aeruginusa* and *Mycobacterium smegmatis* but did not reach that of the antibiotic. (*Streptomycin* at 20mg/disk). On another example, [33] found that the ethanolic extract from the leaves of *Salvia officinalis* studied was very effective against *P. gingivalis.* In addition, there is an abundance of research on the use of sage essential oils in different food models: against *Bacillus cereus, S. aureus* and *Salmonella typhimurium* found in poultry and beef pâté; against *B. cereus* and *S. aureus* found in boiled rice [34] and against *L. monocytogenes* found in soft cheese [35].

The study conducted by [36] showed a non-effectiveness antibacterial essential oils of *S. officinalis* from Tunisia vis-à-vis *P. aeruginosa ATCC 27853* and *S. aureus ATCC 25923*. Similarly, the essential oils of this plant grown in the south of the Brazil did not show antimicrobial efficacy against the different strains of *Pseudomonas* and *Staphylococcus* [37]. On the other hand, with respect *to E. coli ATCC 25922* the essential oil of sage was really effective [36,38].

5. Conclusion

To conclude, the present work analyzed the aqueous extract of *Salvia officinalis* leaves planted in Morocco by studying the antioxidant, antifungal and antimicrobial activities and by performing phytochemical screnning. The results of this study show that the plant studied is rich in phenols, flavonoids, alkaloids, saponoside, sterol and tannins, which explains the important antioxidant effect of the extract, and at the same time the antimicrobial and antifungal activity against bacteria and standard yeasts used.

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