In Vitro Antimalarial, Antimicrobial and Antioxidants Activities of *Salvia chudaei* Batt. & Trab. (Lamiaceae) Extracts

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ABSTRACT

The present research focuses on the biological activities study of the essential oils and extracts preparations using organic solvents of different polarity from Salvia chudaei Batt. & Trab. (Lamiaceae) plant, wild growing in Algeria.

The essential oils and solvent extracts were tested in vitro against two reference clones of Plasmodium falciparum: the K1 chloroquine-resistant and the 3d7 chloroquine-sensitive strains. A significant inhibitory activity was observed with IC_{50} values between (IC_{50}=2.39 µg.mL^{-1} and 7.99 ± 1.06 µg.mL^{-1}).

The antimicrobial activity of the essential oil and the others organic extracts was evaluated against Staphylococcus aureus ATCC 27923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 25853 and Candida albicans using disc diffusion method. The essential oil showed an efficient antibacterial activity against Staphylococcus aureus with the strongest inhibition zone (26 mm). The minimal inhibitory activity (MIC) ranged from 0.019 to 37.92 µg.mL^{-1}.

The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) and 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. The essential oil of plant exhibited noticeable scavenging effects. In DPPH free radical scavenging, the IC_{50} value of the antioxidant capacity was between 4.22 ± 0.105 µg.mL^{-1} and in ABTS˙$^+$ scavenging assay; the IC_{50} was 12.74 ± 0.216 µg.mL^{-1}.

The composition of essential oil was analyzed by GC and GC-MS. Twenty constituents (94.72%) of the total oil were identified. The main compounds were (43.18%) dillapiole, (14.82%) myristicin and (7.82%) Alpha-pinene.

The in vitro antimalarial, antimicrobial and antioxidants activities support the historic and present use of Salvia species in traditional medicine in Algeria.

Keywords: *Salvia chudaei*, Antimalarial, Antimicrobial, Antioxidant, Activities

INTRODUCTION

Since ancient times the aromatic plants extracts have been in use for different purposes, such as food, drugs and perfumery [1]. The essential oils are considered among the most important antimicrobial agents present in these plants, and may also have antioxidant

and anti-inflammatory activities [2-4].

In the recent decades, antimicrobial plant products have gained special interest because of the resistance to antibiotics that some microorganisms have acquired [4], the increasing popular concern about the safety of food and the potential impact of synthetic additives on health [5].

Historically, many drugs effective against parasitic diseases stem from traditional medicine, such as quinine and artemisinin [6]. Malaria, a parasitic disease caused by *Plasmodium* sp. and transmitted by *Anopheles mosquitoes*, currently ranks highly among the most important infectious diseases around the world. The disease mainly affects Southern countries where malaria is a major public health problem [7].

The genus *Salvia*, the largest genus of the Lamiaceae family, includes nearly 900 species spread throughout the world. Many *Salvia* spp. are used as herbal tea and for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industry. *Salvia* species have been reported to have a wide range of biological activities including anticholinesterase, antibacterial, antimalarial, anticancer also been reported to have a potent antioxidative effect [8-13].

Salvia species indicates that 1,8-cineole (eucalyptol), linalyl acetate, camphor, borneol and *E*-caryophyllene are its main constituents. However, several authors have documented significant variations in the concentration of these compounds and/or presence of others in high concentrations in the species [3,9,14-16].

Moreover, the essential oil composition of *Salvia* species, as occurs with other medicinal and aromatic plants, is highly influenced by genetic and environmental factors [16-18].

The aim of the present work is to study *in vitro* antimalarial, antioxidant and antimicrobial activities of the extracts and the essential oils of *Salvia chudaei* Batt. & Trab. from Algeria, in addition to the chemical compositions of essential oils, in an attempt to validate the claims made by traditional medicine practitioners of the effectiveness of this plant.

MATERIALS AND METHODS

Plant material

The aerial parts of *Salvia chudaei* Batt. & Trab. were collected in January 2013 from Tamanrasset (23º81' 756″ N, 05º93' 888″ E), in south Algeria was dried at room temperature in the shade. The identification was performed according to the flora of Sahara [19] and the botanists of the National Forest Research Institute in Tamanrasset (Algeria). The voucher specimens have been deposited at the Herbarium (PM/03) of the Laboratory of Biogeochemistry of Desert Environments, Ouargla University, Algeria.

Preparation of solvent extracts

The extraction by maceration with an increasingly polar gradient was carried out to obtain the extracted enriched in molecules of interest.

Increasing polarity solvent systems used were: hexane, chloroform (low polarity used for extracting apolar compounds), ethyl acetate (medium polarity used for extracting aglycone flavonoid and monosides) and ethanol (high polarity used for extracting the flavonoid heteroside). The extraction process was third repeated [20]. The combined filtrates were then concentrated under reduced pressure at 40ºC using vacuum rotary evaporator. The yields of hexane (SH), chloroform (SC), ethyl acetate (SA) and ethanol (SE) extracts were 1.85%, 8.9%, 2.66% and 4.59% respectively. All extracts obtained were kept in the dark at +4ºC prior to use.

Preparation of essential oil

The essential oils of dried plant material were extracted by hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4ºC prior to analysis. Yield based on dry weight of the sample was calculated.

Essential oil analysis

The composition of the essential oils was investigated by GC and GC/MS. GC analysis was carried out on a HP-6890 gas chromatograph equipped with a FID and a HP5MS capillary column (30-0.25 mm ID, film thickness of 0.25 μm). The column temperature was programmed from 60-275ºC at 4ºC.min⁻¹. Other operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min; injector temperature, 250ºC; detector temperature, 250ºC; split ratio, 1:20. The GC/MS was performed in a Hewlett-Packard 5973 MSD System. An HP-5 MS capillary column (60 m to 0.25 mm ID, film thickness of 0.25 μm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 0.7 mL.min⁻¹. Oven temperature was programmed (60ºC for 8 min then programmed at rate of 2ºC.min⁻¹ to 280ºC and held isothermal for 10 min. Injector port: 250ºC, detector: 250ºC. Volume injected: 0.2 μL by split method 1/20; mass spectrometer: HP5973 recording at 70 eV; scan time: 2.83 s; mass range (m/z): 27-550 Th. Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the essential oil constituents was based on a comparison of their retention indices relative to (C_6-C_{28}) *n*-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their MS data compared to the NIST mass spectral library, and published mass spectra [21,22].

Antiplasmodial activity

The essential oils and solvent extracts of *Salvia chudaei* were tested *in vitro* against two reference clones of *Plasmodium falciparum*: the K1 chIoroquine-resistant and the 3d7 chloroquine-sensitive strains. The antiplasmodial assay was the same one described by Sanon et al. [6] and applied in the National Centre for Research and Training on Malaria (Burkina Faso).

Antimalarial effects were quantified as inhibition of parasite growth, as measured by the production of *Plasmodium* lactate dehydrogenase (pLDH). Inhibition testing was performed in three steps in duplicate in 96-well flat bottom plates (TPP, Switzerland).

Malaria parasites were cultured with different concentrations of each extract and parasite growth was assessed by the production of pLDH. Each extract or reference control (chloroquine diphosphate salt (Sigma Aldrich)) was applied in a series of seven duplicate dilutions (final concentrations ranging from 0.78 $\mu g.mL^{-1}$ to 50 $\mu g.mL^{-1}$ of the extracts and 1.56-100 nM for reference control (CQ)) on two rows. 100 μL of asynchronous parasitised erythrocytes at a hematocrit of 2% with parasitemia between 1.5% and 2% were prepared in 100 μL of each extract. The plates were placed at 37°C in a modular incubator chamber with a humidified atmosphere for 72 hrs. Infected and uninfected erythrocytes O^+ were used as positive and negative controls, respectively. Parasite growth was determined by measuring the content of parasite lactate deshydrogenase using Malstat, NTB/PES reagents. The microplates were read with a spectrophotometer (Biotek EL x 808) at 650 nm. Absorbance data were entered into Excel to calculate the inhibition percent relative to positive control from the mean of raw data for each concentration. MatLab version 2016 was used to plot inhibition curves and calculate the inhibition concentration of extract that reduced the level of parasitaemia to 50% (IC_{50}).

Antimicrobial activities

Microbial strains

The essential oil was tested against four microorganisms. Reference strains were: *Staphylococcus aureus* ATCC 27923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 25853; clinically isolated strain was obtained from the Microbiology laboratory, Med Boudiaf Hospital Ouargla Algeria: *Candida albicans*.

Antimicrobial screening

The agar disc diffusion method was employed for the determination of antimicrobial activity of the extracts. Briefly; a suspension of the test microorganism (2.108 $CFU.mL^{-1}$) was spread on the solid media plates. Filter paper discs (5 mm in diameter) were individually impregnated with 10 μL of the oil or the solvent extracts (1 $mg.mL^{-1}$), then placed on the inoculated plates, were incubated at 37ºC for 24 hrs in Mueller-Hinton broth for the bacteria and at 25ºC for 48 hrs in Sabouraud broth for the yeasts. The diameters of the inhibition zones were measured in millimeters. A broth micro dilution method was used to determine the Minimum Inhibitory Concentration (MIC) [23]. Different dilutions (100-0.005 $\mu g.mL^{-1}$) were prepared. The first dilution was made in Dimethyl Sulphoxide (DMSO) and further dilutions in the culture medium. The first column of the plate was reserved for negative control wells (without inoculants) and the last column, for the positive growth control wells (without antimicrobial agents). The MIC was considered as the lowest concentration of the essential oil inhibiting the total growth of microorganisms. MIC was detected by lack of visual turbidity (corresponding the negative growth control). Levofloxacin served as positive control. Each test was repeated three times.

Antioxidant activities

Free radical scavenging activity: the diphenyl-picrylhydrazyl (DPPH) test

The free radical-scavenging activity was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH˙) assay described by Blois [24]. Briefly, 6×10^{-6} $mol.L^{-1}$ solution of DPPH˙ in methanol was prepared and 3 mL of this solution was added to 100 μL of sample solution of essential oil. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

% inhibition=$((A_{blank} - A_{sample})/A_{blank}) \times 100$.

Antioxidant activity of standard (Trolox and ascorbic acid) or samples was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. All measurements were performed in triplicate.

Free radical scavenging ability by the use of a stable (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) ABTS radical cation

The free radical-scavenging activity of the essential oil was also determined by (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) ABTS radical cation (ABTS˙) decolorization assay as described by Cano et al. [25] with some modifications. In brief, ABTS˙ was produced by mixing 8 mM ABTS with 3 mM potassium persulfate in 25 mL of distilled water. The solution was incubated in the dark at room temperature for 16 h before use. The ABTS˙ solution was diluted with 95% ethanol to obtain an absorbance of 0.8 to 0.9 at 734 nm.

For each sample, diluted solution of the essential oil (20 μL) was allowed to react with fresh ABTS solution (980 μL), and then the absorbance was measured 10 min after initial mixing. Ascorbic acid was used as a standard. The capacity of free radical scavenging was expressed by IC_{50} ($\mu g.mL^{-1}$) values which represents the concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

<center>RESULTS AND DISCUSSION</center>

Chemical composition of the essential oil

The essential oil was obtained by hydrodistillation from the aerial parts of *Salvia chudaei* Batt. & Trab. plant with a yield of 0.4% on a dry weight basis. 58 constituents, representing 94.72% of the oil, were identified. Qualitative and quantitative analytical results are shown in Table 1.

<center>**Table 1: Chemical composition of *Salvia chudaei* essential oil**</center>

Components	R.I.[a]	% Composition
Alpha-Thujene	662	0.54
Alpha-Pinene	666	7.82
Camphene	672	0.04
Sabinen	686	0.53
Beta-Pinene	688	2.04
Beta-Myrcene	696	0.32
Alpha-Phellandrene	1006	4.13
Delta.3-Carene	1011	2.74
O-Cymene	1025	1.37
dl-Limonene	1030	5.28
Cis-Ocimene	1041	5.36
Alpha-Terpinolene	1086	0.33
Alloocimene	1127	0.15
Terpinene-4-ol	1173	0.15
Trans-Caryophyllene	1400	0.24
Germacrene-D	1475	1.95
Myristicin	1530	14.82
Dillapiole	1651	43.18
Beta-Eudesmol	1663	3.32
Butylidene phthalide	1679	0.41
Amount of identified compounds		94.72

[a]Retention indices relative to C_6-C_{28} n-alkanes on the HP-5 MS column; The MS-tentatively identified on the basis of computer matching of the mass spectra of peaks with the NIST/NBS and Wiley libraries

Dillapiole (43.18%) was the main compound of the essential oil, followed by Myristicin (14.82%) and *Alpha* -Pinene (7.82%). Analyzed essential oils mainly consisted of oxygenated sesquiterpenes (61.73%) followed by monoterpenes hydrocarbon (30.65%). The major oxygenated sesquiterpenes were: Myristicin, Dillapiole and *Beta* Eudesmol. While, *Alpha* Pinene, *Alpha* Phellandrene, Limonene and *Cis*-Ocimene were the main monoterpenes hydrocarbon, whereas, *Trans* Caryophyllen and Germacrene D were the important sesquiterpene hydrocarbon.

The compounds were found in the essential oil of other spaces of the genre *Salvia*, but with different percentages depending on season, geographic origin, environmental factors, extraction methods, plant organ, phonological stage, sampling techniques and genetic differences [16-18].

Chemical composition of *Salvia sclarea* L. essential oil from Bulgaria shows that oxygenated monoterpenes were the major group representing 84.57% of the total oil content, followed by sesquiterpenes 12.34%, monoterpenes 1.47%, oxygenated sesquiterpenes 0.61%. The major components detected were as follows: linalyl acetate 56.88%, linalool 20.75%, germacrene D 5.08% and *Beta*-caryophyllene 3.41% [26].

The major class of substances in the essential oil of *Salvia pratensis* was the sesquiterpene hydrocarbons group (53.7%), followed by aliphatic compounds (15.7%). On contrary, oxygenated monoterpenes were found to be the major class of substances (35.1%) in the essential oil of *Salvia bertolonii*, followed by sesquiterpene hydrocarbons (21.9%) and aliphatic compounds (10.8%).The main compound in essential oil was *E*-caryophyllene (26.4%), Epi-bicyclosesquiphellandrene (5.6%), *Z-Beta*-farnesene (6.0%) and *Beta*-cubebene (5.6%). Caryophyllene oxide (35.1%), *Z*-caryophyllene (11.4%), *Alpha*-humulene (3.3%) were the majors components in the essential oil of *Salvia bertolonii* [15].

In *Salvia hydrangea*, Camphor (54.2%), *Alpha* -humulene (4.0%), *Cis*-sesquisabinene hydrate (2.8%), myrtenol (2.6%), *Beta*-bisabolol (2.2%) and 1,8-cineole (2.1%) were found to be predominant components. The oil was also characterized by relatively high amount of oxygenated monoterpenes (69.6%) [11].

Camphor (19.1%), 1,8-cineole (16.4%), borneol (11.9%), viridiflorol (11.5%) and bornyl acetate (2.4%) were found to be the major constituents in the oil of *Salvia cryptantha*. The major constituents in the oil of *Salvia heldreichiana* were linalool (9.4%), *Alpha*-pinene (5.6%), 1,8-cineole (5.6%), borneol (5.6%), cryptone (5.3%), linalyl acetate (4.9%), *Alpha*-terpineol (4.4%), camphor (3.9%), terpinen-4-ol (3.3%) and *Trans*-linalool oxide (2.9%) [27].

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In The essential oil of *Salvia officinalis*, the oxygenated monoterpenes being the dominating constituents (66.7%) with the main compounds *Alpha*-thujone and *Beta*-thujone (24.9%, 15.6%) respectively. Monoterpenes hydrocarbons (17.2%) were also represented in this oil among them myrcene (5.0%) and *Alpha*-pinene (3.2%) as major compounds [28].

1.8-cineol (60%), *Beta*-pinene (9%), myrcene (3.7%), *Alpha*-pinene (3.4%) and germacrene-D (2.9%) were found to be the major constituents in the oil of *Salvia aramiensis* oil. In *Salvia cyanescens*, spathulenol (32.5%), myrtenal (7.8%), *Alpha*-pinene and *Beta*-pinene (6.9%) and *para*-cymene (5.7%) were the main compounds [29].

Antiplasmodial activity

The *in vitro* inhibitory effects of the *Salvia* extracts against the chIoroquine-resistant *P. falciparum* (K1) and the chloroquine-sensitive (3d7) are shown in Table 2.

Table 2: *In vitro* **antimalarial activity of the extracts (IC$_{50}$ values) against** *Plasmodium falciparum* **(K1and 3d7)**

	IC$_{50}$ (3d7) (μg.mL^{-1})	**IC$_{50}$ (k1) (μg.mL^{-1})**
Extract SH	4.91 ± 2.91	5.69 ± 0.3
Extract SC	6.41 ± 0.31	5.69 ± 2.71
Extract SA	NT	NT
Extract SE	7.99 ± 1.06	8.43 ± 0.38
Essential oil	2.39 ± 0.24	2.40 ± 0.77
Reference control (CQ)	4.81 ± 1.17	23.36 ± 1.17

NT: Not Tested

The antimalarial activity of the extracts (IC$_{50}$ values) ranged from 2.39 ± 0.24 μg.mL^{-1} to 8.43 ± 0.38 μg.mL^{-1}, with the essential oil being the most active. So, the antimalarial activity varied greatly between volatile (essential oil) and non-volatile (solvent extracts) components.

The solvent extracts exhibited moderate antimalarial activity compared with essential oils (5 μg.mL^{-1}≤ IC$_{50}$<10 μg.mL^{-1}). In except, the hexane extract (SH) as good antimalarial activity with IC$_{50}$ value: 4.91 ± 2.91 μg.mL^{-1} against the chloroquine-sensitive *P. falciparum* (3d7) strain.

All the extracts were more potent against the chloroquine-sensitive (3d7) than the antimalarial reference (CQ) (IC$_{50}$ value: 23.36 ± 1.17 μg.mL^{-1}).

The solvent extracts from *Salvia chudaei* exhibited antimalarial properties against the chloroquine-resistant *P. falciparum* K1 and the chloroquine-sensitive (3d7) strains, which decreasing antiplasmodial activity with an increasing solvent polarity (hexane, chloroform than ethanol extract) was observed.

Other *in vitro* studies demonstrated that *Salvia* species displayed antiplasmodial activity. Eleven essential oils and 17 solvent extracts of indigenous *Salvia* species were tested to inhibit the *in vitro* growth of *Plasmodium falciparum* FCR-3 strain using the [3H]-hypoxanthine radiometric method. The outcome showed that both the essential oils and the solvent extracts displayed antiplasmodial activity. The IC$_{50}$ values of the essential oils ranged from 1.20 to 13.50 μg.mL^{-1} and displayed promising activity compared to the solvent extracts (IC$_{50}$ values ranging from 3.91-26.01 μg.mL^{-1} [2]. Clarkson et al. [30] demonstrated that *S. repens* displayed antimalarial activity against the chloroquine-sensitive D10 strain of *P. falciparum* (IC$_{50}$ value: 10.8 μg.mL^{-1}) using the pLDH assay.

Lamien-Meda et al. [31] have also demonstrated decreasing antiplasmodial activity with an increasing solvent polarity: K1 stain IC$_{50}$s 4.5>5>20 μg.mL^{-1} for dichloromethane, ethanol and methanol extracts successively from *Cochlospermum planchonii* roots from Ivory Coast. The non-polar solvent hexane has been identified as the best extracting solvent (compared to dichloromethane, ethanol and water) of antiplasmodial compounds from *C. planchonii* roots from Burkina Faso against FcB1 strain with IC$_{50}$ value ranging from 0.4 to 22 μg.mL^{-1}.

In this study, the essential oils showed good antimalarial activity with IC$_{50}$<5 μg.mL^{-1}.

The antimalarial activity of essential oils may be attributed to their high sesquiterpene content [2]. Mono and sesquiterpenoids are credited with numerous biological properties, including antiplasmodial activity. Pinene and linalyl acetate were identified in the majority of essential oils of indigenous *Salvia* species. These compounds may therefore be responsible for the antiplasmodial activity of the oils [32].

The essential oil composition of indigenous *Salvia* species is highly variable qualitatively and quantitatively [30,32,33].

These differences may be related to many parameters, including the local environment and the collection periods, the laboratory techniques used as extraction methods because the ability of extracting a solvent of chemical groups is related to its polarity. Concerning biological assessment, the results are often based on the sensitivity of techniques used [2,6,31].

Finally, plants could be effectively more active against *Plasmodium falciparum* in man than *in vitro*, as is the case for plants containing prodrugs. Prodrugs are non-active by themselves and need a metabolization step in order to become active drugs; they cannot therefore be detected in an *in vitro* test model. This could also explain why non polar extracts are generally more active *in vitro* than aqueous extracts; these extracts are even closer to the remedies produced by the traditional preparation method, but

contain polar compounds such as heterosides. Like prodrugs, it is possible that these heterosides are inactive *in vitro* but active *in vivo* after metabolization, and that non polar extract contains the corresponding aglycone, which may be capable of being active *in vitro* without the need of a metabolization step [7].

Very little antimalarial research has been done with Algerian plants described for their antimalarial use. Our study appears to be the first to demonstrate the antimalarial effects of *Salvia chudaei* plant. This study confirms the pharmacological properties of the plant. It is recommended that future studies should consider this plant as a potential source of antiplasmodial molecules.

Antimicrobial activity

The antimicrobial activity of *Salvia chudaei* Batt. & Trab. extracts was tested against *Staphylococcus aureus* ATCC 27923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Candida albicans*, using disc diffusion and broth micro-dilution methods. The disc diameters of zone of inhibition and minimum inhibitory concentrations (MIC) of different extracts for the microorganisms tested are shown in Table 3.

Table 3: Antimicrobial activity (inhibition zones and MIC) of *Levofloxacin* and the extracts of *Salvia chudaei* Batt. & Trab. plant

	Essential oil		SH		SC		SA		SE		Levofloxacin	
	DD	MIC[b]	DD	MIC[b]	DD	MIC[b]	DD	MIC[b]	DD	MIC[b]	DD[a]	MIC[b]
Staphylococcus aureus ATCC 27923	26	37.5	20	67.5	8.5	15	12	12	22	12	34	0.3
Escherichia coli ATCC 25922	19	0.56	8	15	11	15	18	15	14	12	34	0.3
Pseudomonas aeruginosa ATCC 25853	16	37.92	11	26.25	25.5	12	NT	NT	11	30	20	0.15
Candida albicans	15	0.019	11.5	15	18	30	8	12	18	12	NT	NT

DD, diameter of zone of inhibition (mm) including disc diameter of 5 mm. NT: Not Tested; [a]Tested at a concentration of 5 µg/disc. [b]Values given as µg.Ml^{-1}

The result of the antimicrobial activities of the essential oil indicated that *Staphylococcus aureus* ATCC 27923 was the most sensitive microorganism tested, with the strongest inhibition zone (26 mm), followed by *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with strong inhibition zones (19-16 mm) respectively. *Candida albicans* exhibited a weak inhibition zone (15 mm). But, the inhibition zones were lower than those of antibiotics, which showed wide inhibition zones at very low concentrations.

The results of the MIC determination indicated the oil inhibited all microorganisms tested. *Candida albicans* and *Escherichia coli* ATCC 25922 had the lowest MIC (0.19-5.69 µg.mL^{-1}). *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 27923 had the highest MIC (37.925 and 37.5 µg.mL^{-1}) respectively.

Although this is the first report on the antimicrobial activity of *Salvia chudaei* Batt. & Trab. from Tamanrasset (Algeria), some other members of the *Salvia* genus have been subjected to antimicrobial activity evaluation and the results similar to us are obtained in most cases [9,11,14,26,28,34,35].

Generally such activity is attributed to the presence of 8-cineole, thujone, limonene, *Trans* caryophyllen and camphor [12,35-39] in reality, even if antimicrobial activity of an essential oil is often attributed mainly to its major components, today it is known that the synergistic or antagonistic effect of one compound in minor percentage of mixture has to be considered [40].

The solvents extracts (hexane, chloroform, ethyl acetate and ethanol) of *Salvia chudaie* were tested against a number of microorganisms and the results clearly show the antimicrobial effect of all of these strains, but with different inhibition diameters. The results reveal that the chloroform extract has a considerable antibacterial effect on *Pseudomonas aeruginosa* ATCC 25853 and *Candida albicans* with zones of inhibition of 25.5 mm and 18 mm respectively. This effect is low compared to that of levofloxacin, used as a reference antibiotic. The ethyl acetate extract, however, has no effect vis-à-vis *Pseudomonas aeruginosa* ATCC 2585, but he has a strong bactericidal effect on *Escherichia coli* ATCC 25922 (18 mm). The inhibitory actions of the ethanol extract were remarkable on *Staphylococcus aureus* ATCC 27923 and *Candida albicans* with the values 22 mm and 18 mm respectively. The Hexane extract was more active against *S. aureus* ATCC 27923 (20 mm) compared to other strains.

According to Gali-muhtasib et al. [41] and Stagos et al. [42], the genus *Salvia* has a strong inhibitory effect against bacterial growth.

Antioxidant capacity

Two methods have been used to measure the antioxidant activities of the essential oil and the solvent extracts from *Salvia chudaei* Batt. & Trab. plant: DPPH and ABTS`$^+$ radical scavenging assays. The results are shown in Table 4.

In DPPH assay, the IC$_{50}$ value of the antioxidant capacity of the essential oil was 4.22 ± 0.105 µg.mL^{-1}. But in ABTS`$^+$ scavenging assay, the IC$_{50}$ value of the antioxidant capacity was 12.74 ± 0.216 µg.mL^{-1}. The essential oil was more effects to the standards used. The antioxidant activity of the other *Salvia* plant species are attainable [9,10,35,38].

Table 4: Antioxidant activity of the extracts of *Salvia chudaei* Batt. & Trab. plant

	IC$_{50}$ DPPH (µg.mL^{-1})	IC$_{50}$ ABTS (µg.mL^{-1})
Essential oil	4.22 ± 0.105	12.74 ± 0.216
Extract SH	380 ± 0.20	240 ± 0.34
Extract SC	570 ± 0.20	120 ± 0.25
Extract SA	280 ± 0.20	20 ± 0.17
Extract SE	330 ± 0.41	660 ± 0.19
Trolox	26.75 ± 0.01	25 ± 0.001
Ascorbic acid	42.95 ± 0.001	40.90 ± 0.01

Against by the antioxidant activity of the organic extract of *Salvia chudaei*, the ethyl acetate extract was the most potent with IC$_{50}$ values for both ABTS and DPPH test 280 ± 0.20 and 20 ± 0.17 mg.mL^{-1} respectively. The previous studies and ours confirm the presence of good to moderate antioxidant potentials in the plants of *Salvia*.

From the results obtained in these tests, it is clear that the interaction of an antioxidant with the DPPH or ABTS depends on its chemical structure. Some compounds react very rapidly with the DPPH or ABTS. Athamena et al. [43] also found that this activity differs depending on the test used. Antioxidant activity not only depends on the concentration, but also on the structure and nature of antioxidants [44].

CONCLUSION

Natural products, especially those produced by medicinal plant species, are currently under special interest as food additive due to their safety, usefulness and accessibility. This study contributes to the knowledge of the *in vitro* antimalarial, antioxidant and antimicrobial activity of *Salvia chudaei* Batt. & Trab. extracts. This findings candidate the plant as a good case for more in-depth studies and we wish our future research lead to the identification and structure elucidation of biologically active molecules present in its extract.

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