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Article

Acute toxicity assessment of bioactive constituents from *Salvia algeriensis* (Desf.) extracts: a promising natural agent against clinical bacterial isolates and pathogenic fungi

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ABSTRACT

Salvia species are emerging as promising therapeutic agents due to their diverse bioactivity against various pathologies. This study was conducted to investigate the phytochemical profile, antimicrobial activity, and acute Toxicity of hydromethanolic extracts from *Salvia algeriensis* (Desf.) leaves, flowers, and roots. Chemical reaction tests and chromatographic analysis were employed to determine the chemical composition, while microdilution was used to determine the tested microorganisms' minimum inhibitory concentration (MIC). The acute Toxicity of the leaf extract was carried out following the rules and guidelines of OECD 425. Toxicity parameters in Swiss albino mice were evaluated after a single dose of 500 mg/kg and 2000 mg/kg. According to the preliminary phytochemical screening results, terpenoids and polyphenols (flavonoids and tannins) were found in all plant parts, but coumarins were only found in the root extract. The HPLC-DAD analysis revealed the presence of 16 phenolic compounds in varying amounts across the three extracts, of which rosmarinic acid, quercetin, caffeic acid, and 3-hydroxybenzoic acid were the most abundant. Selective antimicrobial activity was noticed, with the root extract demonstrating the most substantial effect against the two fungal strains tested. MIC values ranged from 0.3 to 10 mg/mL, and Gram-positive bacteria generally showed greater susceptibility compared to Gram-negative bacteria. The LD_{50} was found to be greater than 2000 mg/kg. There were no overt clinical symptoms of Toxicity. Body weights, organ weights, and temperatures were not significantly altered, and hematological analysis showed no significant differences. *Salvia algeriensis* (Desf.) extracts emerge as potential candidates for natural, non-toxic antimicrobial agents.

Keywords: Acute Toxicity; antimicrobial; HPLC-DAD; polyphenols; Salvia

INTRODUCTION

The use of natural remedies is widespread and has increased recently; approximately 80% of the Arab population currently uses medicinal plants to treat diseases and meet their health needs[1]. Traditional herbal medicine has been used by Algerian society since ancient times. In particular, Algeria's unique geographical location and diverse climate contribute to its variety of flora, which serves as a vast resource for developing and applying herbal remedies[2]. Algeria is host to 23 different species of *Salvia*, one of the medicinal plants[3]. *Salvia,* a genus with approximately 1,000 species, is the most taxonomically diverse member of the *Lamiaceae* family. These remarkable plants have found a home across the globe, particularly in temperate and tropical climates, with a strong presence in the Mediterranean, Southeast Africa, and Central and South America[4,5]. *Salvia* species have long been used in folk medicine in multiple locations across the world[6]. In China, *salvia* species have been used as analgesics and for hepatitis[7], while in Anatolia, they were used for wound healing, tuberculosis, rheumatism, diabetes, and skin disorders[8]; in South Africa, people used *salvia* species to treat digestive disorders, and infections[9], In Europe, sage has also been used to alleviate inflammation[10]. *Salvia* has many bioactivities, including antiviral[11], anti-inflammatory[12], antioxidant[13], antidiabetic[5], antihypertensive[14], and cytotoxic properties on tumor cell lines[15]. These diverse bioactivities are further reflected in the chemical composition of *Salvia* species.

Although they all have some commonalities, such as the presence of volatile monoterpenoids, flavonoids, and triterpenoids in their aerial parts, the composition of their roots varies, with diterpenoids being the most common component[16]. *Salvia algeriensis* (Desf.) is a medicinal plant native to the northwestern regions of Algeria and northeastern Morocco[3]. Thriving at elevations up to 600 meters, *Salvia algeriensis* (Desf.) is an annual sage that typically reaches a height of around 1 meter when cultivated. Researchers have found a clear difference in the chemical composition of the essential oils from *Salvia algeriensis* (Desf.) flowers and leaves, with the leaf oil having a more significant antifungal effect. While flower oil mainly included globulol and viridiflorol, leaf oil was rich in benzaldehyde, eugenol, and phenylethyl alcohol[17].

No prior research has explored the phytochemical characterization, antimicrobial potential, or Toxicity of extracts from *Salvia algeriensis* (Desf.) root, flower, and leaf. This study aims to break new ground by assessing the plant's chemical profile using standard methods and HPLC-DAD analysis, evaluating the extract's antimicrobial activity via the MIC determination method, and testing the acute Toxicity of orally administered extracts in Swiss albino mice.

MATERIAL AND METHODS

Plant sampling and preparation

Salvia algeriensis (Desf.) plants were collected from Ouled Fares province in Chlef, Algeria. The leaves, flowers, and roots were carefully washed with tap water to remove any impurities and then dried in the shade for two weeks at a controlled temperature and humidity. Finally, the dried plant parts were ground into a fine powder and stored in sealed containers at 4°C until further analysis.

Preparation of hydromethanolic extracts

Hydromethanolic extracts were prepared from leaves, flowers, and roots of *Salvia algeriensis* (Desf.) following a maceration method[18]. Briefly, 20 g of each finely powdered plant part was macerated with 200 mL of methanol/water (80/20, v/v) for 24 hours at room temperature with continuous stirring. The extracts were then filtered (Whatman filter paper), and the residues were re-extracted with the same solvent volume. Combined filtrates were evaporated to dryness under reduced pressure using a rotary evaporator at 45°C. The extracts were stored at 4°C for further analysis.

Qualitative Phytochemical screening

The plant extracts were screened for the presence of various phytochemical constituents, including Alkaloids, Terpenoid, Sterols, Triterpenes, Reduced sugar, Saponins, Anthraquinones, Coumarins, Tannins, Flavonoids, and Mucilage, using standard methods described by Iqbal et al[19].

HPLC-DAD analysis

The phenolic compounds in the extracts were analyzed using HPLC-DAD according to Caponio et al. [20]. Ten microliters (µL) of each prepared sample solution (in methanol, 20mg/mL) were injected into a 250×4.6 mm C18 Ultra sphere-ODS column, homogenized and filtered, the eluates were detected at (254; 278; 287; 330 nm). The mobile phase comprises 2% acetic acid (A) in water and methanol (B). The system was run with the following elution gradient: 95% A/5% B for 3 min, 80% A/20% B in 15 min and isocratic for 2 min, 60% A/40% B in 10 min, 50% A/50% B in 10 min, 100% B in 10 min until the end of the run. The standards used are summarized in Table 1.

Standard	Retention time (min)
3-HydroxyBenzoic acid	22.545
4-HydroxyBenzoic acid	17.647
Benzoic Acid	47.629
Catechin Hydrate	11.499
Chlorogenic Acid	16.239
Caffeic Acid	21.476
Epicatechin	20.169
Gallic Acid	5.912
Hesperidin	65.989
P-Coumaric Acid	33.597
Quercetin	76.313
Rosmarinic Acid	70.655
Sinnapic Acid	37.264
Syringic Acid	22.628
t-Cinnamic Acid	75.207
t-Ferrulic Acid	37.202

Table 1. Phenolic compound standards are used for identification in extracts, with their corresponding retention times.

Microorganisms

Clinical bacterial strains were isolated from patients at Trás-os-Montes and Alto Douro Hospital Center (Vila Real, Portugal). These strains included both Gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Morganella morganii*) and Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes*, and *Methicillin-resistant Staphylococcus aureus* (*MRSA*)). *Aspergillus fumigatus* (ATCC 204305), and *Aspergillus brasiliensis* (ATCC 16404) obtained from Frilabo, Porto, Portugal were also used.

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and fungicidal concentration (MFC)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using a colorimetric assay[21]. Samples were dissolved in a 5% (v/v) Dimethyl sulfoxide (DMSO) and 95% autoclaved distilled water solution to a final 20 mg/mL concentration. Serial dilutions were performed in a 96-well microplate containing Tryptic Soy Broth (TSB) to obtain a concentration range of 10 to 0.03125 mg/mL. Standardized inoculum (1.5 x 10^6 CFU/mL, 10 µL) was added to all wells for a final inoculum of 1.5 x 10^5 CFU/well. The microplates were then incubated at 37°C for 24 hours. MIC was determined following incubation with 40 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) for 30 minutes at 37°C. The lowest concentration inhibiting visible bacterial growth was considered the MIC. For MBC determination, liquid (10 µL) from wells without color change was plated on Blood Agar (7% sheep blood) and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration, showing no bacterial growth. Fungal cultures were maintained on malt agar at 4°C. Before antifungal activity testing[22], cultures were transferred to fresh malt agar and incubated at 25°C for 72 hours. For the assay, fungal spores were harvested from the agar surface using a sterile 0.85% saline solution containing 0.1% Tween 80 (v/v). The spore suspension was then adjusted with sterile saline to a final concentration of approximately 1.0 x 10^5 spores/mL in a 100 µL volume per well. Similar to the bacterial assay, samples were first dissolved in a solution of 5% DMSO and 95% autoclaved distilled water to reach a final concentration of 20 mg/mL. This solution (100 µL) was then added in duplicate to the first wells of a 96-well microplate containing 90 µL of Malt Extract Broth (MEB). The remaining wells of the 96-well microplate were filled with 90 µL of fresh Malt Extract Broth (MEB). Serial dilutions of the samples were then performed within the plate to create a concentration range of 10 to 0.03125 mg/mL. The Minimum Inhibitory Concentration (MIC) was determined using this dilution technique. The MIC was defined as the lowest concentration of the sample that prevented visible fungal growth, as observed under a binocular microscope. To determine the Minimum Fungicidal Concentration (MFC), 2 µL aliquots of the serially diluted samples were transferred to new microplates containing 100 µL of MEB per well. These plates were then incubated for 72 hours at 26°C. The MFC was defined as the lowest concentration that showed no visible fungal growth after this incubation period, indicating a 99.5% kill rate of the initial fungal inoculum.

Acute toxicity assessment

The experiment involved 35 male mice, aged 4 to 5 weeks old, obtained from the Pasteur Institute of Algiers, Algeria. These mice were bred and housed following the rules and guidelines for the care of laboratory animals at the animal house of the Hassiba Benbouali University of Chlef. Following best practices (OECD 425)[23], five animals per group were housed in plastic cages with a 12-hour light/dark cycle. Temperatures were maintained between 25 and 30°C, and humidity levels were between 50 and 60%.

Before the experiments, the animals were acclimated for seven days. They had ad libitum access to a standard rodent diet (ONAB) and tap water. The extracts were administered by oral gavage as a single dose (500 and

2000 mg/kg) to one mouse per group. Following administration, each mouse was observed individually for signs of Toxicity, including modifications to the skin, eyes, mucous membranes, respiratory and nervous systems, somatomotor activity, and behavior. Observations were conducted during the first four hours, then again at 24 hours, and then once daily for the entire experiment duration (14 days). Mice were fasted for 2 hours before each gavage. The negative control group received distilled water.

Body and organ weight analysis

Each animal in each group had its body weight measured at various points throughout the experiment, and the relative weight of its heart, lungs, spleen, liver, and kidneys was calculated using the body weight of each animal on the day of sacrifice.

Body temperature analysis

Rectal temperature was measured using a thermometer inserted rectally 2mm deep. Measurements were taken before gavage and then again at 2, 4 hours, day 7, and day 14. All procedures were carried out at room temperature.

Blood sample analysis

A total of 1.5 ml of blood was obtained through cadaveric puncture and transferred in EDTA tubes. Hematological parameters were measured using an automated hematology analyzer.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was performed using a one-way ANOVA test followed by Tukey's post-hoc test in IBM SPSS Statistics 21 software. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Qualitative Phytochemical screening

The results of the preliminary phytochemical screening of the hydromethanolic extract of *Salvia algeriensis* (Desf.) are presented in Table 2. Terpenoids (triterpenes), polyphenols (flavonoids and tannins), reduced sugars, and mucilage appear to be abundant in the aerial parts (leaves and flowers) .As for the roots contain terpenoids (triterpenes), polyphenols (flavonoids, tannins, and coumarins), sterols, and saponins. Anthraquinones have been detected at deficient levels, whereas alkaloids, reduced sugar, and mucilage were not detected.

Phytoconstituent	Leaves	Flowers	Roots
Alkaloid	-	-	-
Terpenoid	+	+	+
Sterols and Triterpenes	+	-	+
Reduced sugar	+	+	-
Saponins	-	-	+
Anthraquinones	-	-	+/-

Coumarins	-	-	+
Tannins	+	+	+
Flavonoids	+	+	+
Mucilage	+	+	-

Key: positive test (+); negative test (-); presence as a trace (+/-)

Table 2. Phytochemical screening of hydromethanolic extracts of *S.algeriensis* (Desf.).

HPLC-DAD *analysis*

A total of sixteen standards were used to identify the phenolic compounds in the flowers, leaves, and root extracts. Results presented in Table 3 revealed the presence of several phenolic compounds with varying amounts depending on the plant part. All three extracts contained sinapic acid, syringic acid, t-ferulic acid, t-cinnamic acid, quercetin, hesperidin, caffeic acid, 4-hydroxy benzoic acid, and 3-hydroxy benzoic acid, while rosmarinic acid predominated at concentrations of 936.3, 674.9, and 316.8 µg/g for the plant's flowers, leaves, and roots, respectively. Flowers have quercetin (319.4 µg/g) as their most abundant phenolic compound after rosmarinic acid. Caffeic acid (11,498 µg/g) plays the same role in roots, while leaves have 3-hydroxybenzoic acid (18,741 µg/g) as their second highest. Chlorogenic and gallic acids were exclusively detected in the aerial parts of the plant, while p-coumaric acid was explicitly found in the roots. The flower extract exhibited the presence of catechin hydrate.

Phenolic Compounds	Leaves (µg/g)	Flowers (µg/g)	Roots (µg/g)
3-HydroxyBenzoic acid	18,741	7,461	6,712
4-HydroxyBenzoic acid	2,612	2,259	0,553
Benzoic Acid	3,257	ND.	1,745
Catechin Hydrate	ND.	6,777	ND.
Chlorogenic Acid	1,037	1,394	ND.
Caffeic Acid	7,363	8,998	11,498
Epicatechin	N.D.	11,929	7,166
Gallic Acid	0,186	0,581	N.D.
Hesperidin	3,899	13,977	10,695
P-Coumaric Acid	N.D.	N.D.	0,524
Quercetin	10,117	319,4	9,007
Rosmarinic Acid	674,9	936,3	316,8
Sinnapic Acid	3,146	5,609	0,861
Syringic Acid	2,521	3,747	2,820
t-Cinnamic Acid	2,08	0,327	4,814
t-Ferrulic Acid	2,191	4,035	0,479

ND not detected.

Table 3. Phenolic composition of *Salvia algeriensis* (Desf.) hydromethanolic extracts.

Antimicrobial activity

The current study investigated the antimicrobial susceptibility of various extracts against clinically isolated bacteria and fungi. The microdilution technique was used to determine the Minimal Inhibitory Concentration

(MIC) of the tested extracts against a panel of microorganisms. The panel included five Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) and three Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes*, *MRSA*), along with two fungal species (*Aspergillus brasiliensis*, *Aspergillus fumigatus*). Table 4 summarizes the Minimum Inhibitory Concentration (MIC) as well as the Minimum Bactericidal Concentration (MBC), and fungicidal concentration (MFC) values obtained. These values ranged from 0.3 mg/mL to 10 mg/mL, indicating a spectrum of inhibitory activity. Interestingly, Gram-positive bacteria generally demonstrated greater susceptibility to the extracts compared to Gram-negative bacteria. However, *Listeria monocytogenes* emerged as an exception, exhibiting either a 10 mg/mL MIC or complete resistance (MIC > 10 mg/mL, MBC > 10 mg/mL) to all extracts tested. The leaf extract exhibited promising antimicrobial activity against *Klebsiella pneumoniae*, demonstrating the lowest Minimum Inhibitory Concentration (MIC) of 1.25 µg/mL among all tested extracts. Furthermore, both leaf and root extracts displayed satisfactory inhibitory potential against *Morganella morganii*, with an MIC of 2.5 µg/mL. The root extract emerged as particularly effective against *Proteus mirabilis*, *Enterococcus faecalis*, and *MRSA*. Notably, it displayed the lowest MIC (ranging from 0.3 µg/mL to 2.5 µg/mL) among all extracts against these pathogens. None of the tested extracts demonstrated inhibitory activity against *Pseudomonas aeruginosa*, as evidenced by MIC values exceeding 10 µg/mL. *Aspergillus brasiliensis* was most susceptible to the root extract, exhibiting the lowest MIC of 2.5 µg/mL. *Aspergillus fumigatus* remained susceptible to both flower and root extracts, with similar MIC values around 5 µg/mL.

Microorganisms	**Leaves**		**Flowers**		**Roots**	
	MIC	MBC/F	MIC	MBC/F	MIC	MBC/F
Gram-positive bacteria						
Escherichia coli	10	>10	10	>10	>10	>10
Klebsiella pneumoniae	1.25	>10	5	>10	5	>10
Morganella morganii	2.5	>10	5	>10	2.5	>10
Proteus mirabilis	5	>10	10	>10	2.5	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10
Gram-negative bacteria						
Enterococcus faecalis	2.5	>10	2.5	>10	0.3	>10
Listeria monocytogenes	10	>10	>10	>10	10	>10
MRSA	2.5	>10	2.5	>10	0.3	>10
Fungal strains						
Aspergillus brasiliensis	5	>10	10	>10	2.5	>10
Aspergillus fumigatus	10	>10	5	>10	5	>10

Table 4. Minimum inhibitory, bactericidal, and fungicidal concentration (MIC/MBC/MFC) of the hydromethanolic extract from *Salvia algeriensis* leaves, flowers, and roots on the growth of different microorganisms.

Acute Toxicity of extracts

This study investigated the acute oral Toxicity of hydromethanolic extracts from *Salvia algeriensis* (Desf.) leaves, flowers, and roots in male Swiss albino mice. Two doses were evaluated: 500 mg/kg and 2000 mg/kg. No mortality was observed within 24 hours following a single oral administration at either dose level. However, one out of five mice in the 2000 mg/kg group (Leaves extract) died within eight days after treatment.

This particular animal exhibited diarrhea before death. No other treatment groups displayed clinical signs of Toxicity, physiological changes, or behavioral alterations compared to the control group. Food and water intake remained normal across all treated groups. Since no mortality was observed at the initial dose of 500 mg/kg and following the OECD 425 (2008) guidelines, the dose was increased to 2000 mg/kg. Based on these results, the estimated median lethal dose (LD_{50}) of the *Salvia algeriensis* (Desf.) extracts for all plant parts (leaves, flowers, and roots) is considered to be greater than 2000 mg/kg.

Body and organ weight analysis

Throughout the study period, all groups exhibited a gradual increase in body weight, with no instances of body weight loss recorded. Figures 1 and 2 show that the body weight changes followed a similar pattern for treated and control groups. Statistical analysis revealed no significant differences in weight gain between treated and control groups ($p>0.05$). These findings indicate that the *Salvia algeriensis* (Desf.) extracts did not affect body weight at the tested doses.

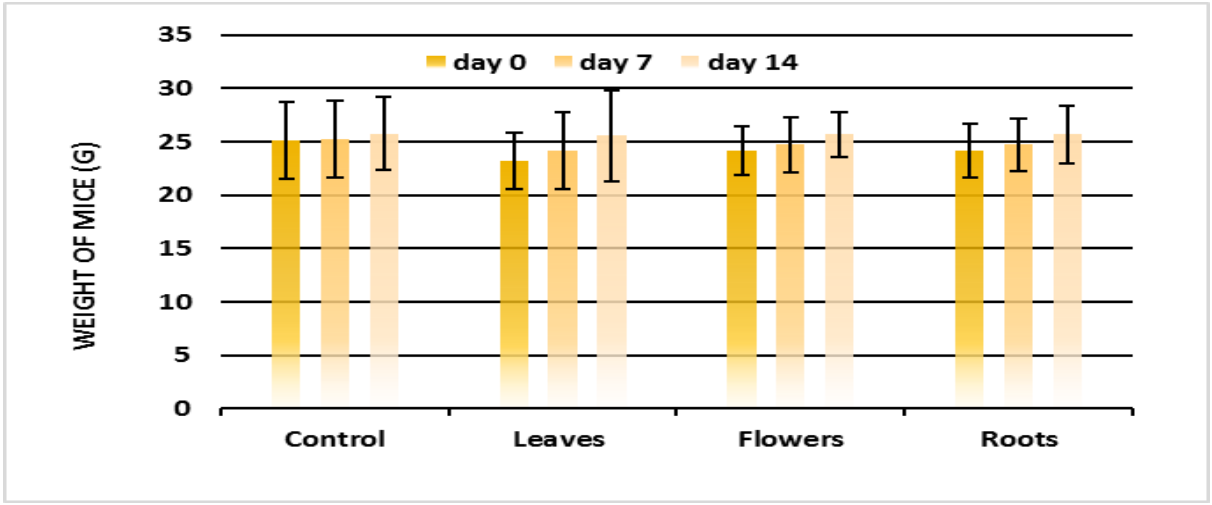

Figure 1. Body weight of mice treated orally with 500 mg/kg of Plant Extracts. Values expressed as mean ± SD, (n= 5) per each group.

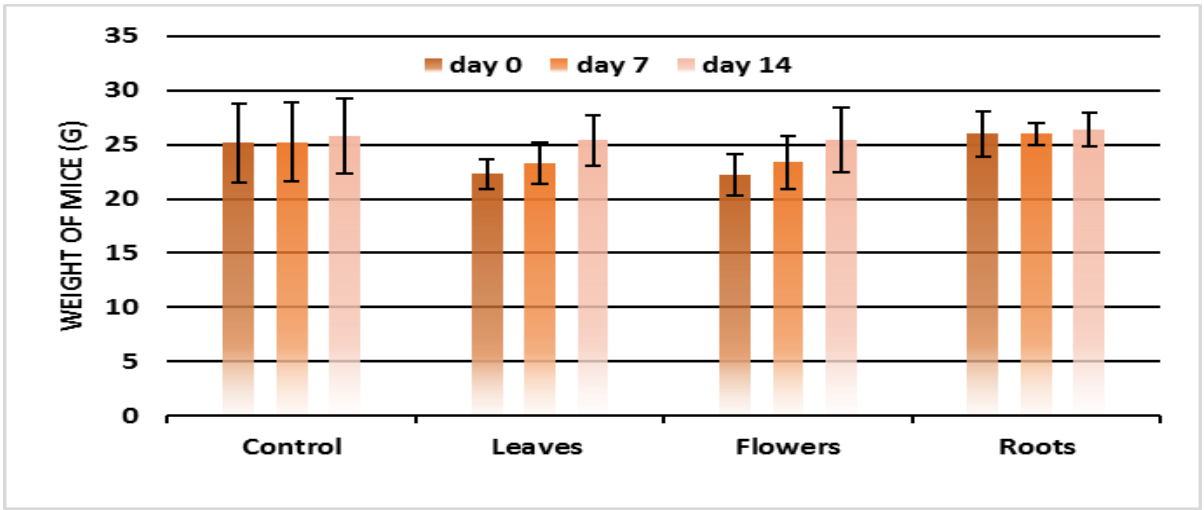

Figure 2. Body weight of mice treated orally with 2000 mg/kg of Plant Extracts. Values expressed as mean ± SD, (n= 5) per each group.

There were no statistically significant differences observed in the relative weight of major organs (heart, lung, spleen, liver, left kidney, and right kidney) between the extract-treated groups and the control group ($p>0.05$) (Table 5). This suggests that the tested doses did not cause any major changes in organ size or weight. Macroscopic evaluation of the tissues did not reveal any pathological changes.

Organ	Control	Leaves (mg/kg)		Flowers (mg/kg)		Roots (mg/kg)	
		500	2000	500	2000	500	2000
Heart	0.534 ± 0.115	0.456 ± 0.042	0.508 ± 0.059	0.525 ± 0.102	0.589 ± 0.064	0.609 ± 0.069	0.587 ± 0.039
Lung	0.876 ± 0.303	0.721 ± 0.056	0.806 ± 0.064	0.706 ± 0.115	0.809 ± 0.158	0.850 ± 0.084	0.852 ± 0.120
Spleen	0.570 ± 0.167	0.502 ± 0.076	0.642 ± 0.243	0.455 ± 0.124	0.464 ± 0.092	0.399 ± 0.075	0.459 ± 0.073
Liver	4.732 ± 0.632	4.806 ± 0.449	4.462 ± 0.748	4.299 ± 1.006	4.246 ± 0.721	5.392 ± 0.976	5.110 ± 0.353
Left Kidney	0.729 ± 0.079	0.656 ± 0.118	0.664 ± 0.091	0.684 ± 0.156	0.752 ± 0.099	0.800 ± 0.183	0.767 ± 0.120
Right Kidney	0.729 ± 0.079	0.619 ± 0.116	0.626 ± 0.087	0.753 ± 0.169	0.720 ± 0.116	0.749 ± 0.220	0.730 ± 0.146

No statistically significant differences were seen following the administration of plant extracts compared to the control group.

Table 5. relative organ weight of mice treated orally with Plant Extracts. Values expressed as mean ± SD, (n= 5) per each group.

Body temperature

Rectal temperatures were measured at baseline (0 h), at 2 and 4 hours post-administration, and on days 7 and 14 (summarized in Table 6). No significant differences in body temperature changes were observed within the treated groups compared to the control group ($p>0.05$). This suggests that the hydromethanolic extracts did not significantly affect this physiological parameter.

Time	Control	Leaves (mg/kg)		Flowers (mg/kg)		Roots (mg/kg)	
		500	2000	500	2000	500	2000
0 h	36.66 ± 0.53	36.30 ± 1.12	36.04 ± 1.03	35.96 ± 0.27	35.88 ± 0.64	36.06 ± 0.77	35.72 ± 0.71
2 h	36.34 ± 0.78	35.72 ± 0.75	36.46 ± 0.69	36.76 ± 1.26	35.98 ± 0.43	36.50 ± 0.70	36.66 ± 0.82
4 h	36.76 ± 0.58	35.76 ± 0.89	36.60 ± 0.65	37.00 ± 0.37	36.66 ± 0.58	36.12 ± 0.31	36.18 ± 0.67
7 d	36.18 ± 0.56	36.14 ± 0.66	36.28 ± 0.54	36.06 ± 0.63	35.90 ± 0.52	36.98 ± 1.00	36.84 ± 0.65
14 d	36.56 ± 1.30	36.02 ± 1.24	35.98 ± 0.78	36.38 ± 1.38	35.96 ± 0.42	36.50 ± 1.15	37.18 ± 1.16

No statistically significant differences were seen following the administration of plant extracts at the time intervals examined compared to the control group and time 0 h.

Table 6: Body temperature of mice treated orally with plant extracts. Values expressed as mean ± SD, (n= 5) per each group.

Hematological parameters

Hematological parameters, including red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT) (Table 7), showed no significant changes in the treated groups compared to the control group on day 14 ($p>0.05$).

Parameters	Control	Leaves (mg/kg)		Flowers (mg/kg)		Roots (mg/kg)	
		500	2000	500	2000	500	2000
RBC[1]	8.92 ± 0.65	9.08 ± 0.80	8.59± 0.29	7.83 ± 1.21	7.51 ± 1.89	8.08 ± 1.06	9.27 ± 1.23
HGB[2]	14.50 ± 0.74	14.74 ± 0.60	13.66 ± 0.89	12.76 ± 1.77	12.12 ± 3.09	13.23 ± 1.64	15.22 ± 1.80
HCT[3]	45.18 ± 3.85	45.55 ± 2.58	42.95 ± 3.01	40.80 ± 5.34	37.18 ± 10.14	41.15 ± 5.79	48.18 ± 5.44
MCV[4]	51.48 ± 2.13	50.36 ± 1.55	49.89 ± 1.90	50.22 ± 3.11	49.24 ± 2.34	50.90 ± 1.15	51.88 ± 1.56
MCH[5]	16.80 ± 1.06	16.32 ± 0.73	15.89 ± 0.51	16.70 ± 1.38	16.10 ± 1.03	16.43 ± 0.28	16.32 ± 0.67
MCHC[6]	32.62 ± 1.58	32.37 ± 0.58	31.84 ± 0.42	32.64 ± 1.71	32.70 ± 1.29	32.27 ± 0.81	31.44 ± 1.60
WBC[7]	8.48 ± 1.87	8.48 ± 1.29	7.32 ± 2.51	7.89 ± 1.14	7.21 ± 3.05	8.89 ± 1.92	7.87 ± 0.54
PLT[8]	0.750 ± 0.344	0.867 ± 0.202	1.052 ± 0.153	0.990 ± 0.50	0.857± 0.456	0.918± 0.164	0.673 ± 0.236

No statistically significant differences were seen following the administration of plant extracts in comparison to the control group.

[1] (×106 mm^{-3}), [2] (g/dl), [3] (%), [4] (fl), [5] (pg), [6] (g/dl), [7] (×103 mm^{-3}), [8] (×103 mm^{-3})

Table 7: hematological parameters of mice treated orally with plant extracts. Values expressed as mean ± SD, (n= 5) per each group.

DISCUSSION

Plants are an abundant source of several distinct secondary metabolites, holding immense potential for development into pharmaceuticals, food additives, and industrial applications. Phytochemical screening remains a crucial tool for researchers, enabling the identification of these valuable compounds[24]. Our investigation successfully identified a broad spectrum of secondary metabolites in various parts of the plant we studied. These findings align with those of Salimikia et al. [25], who demonstrated the abundance of tannins and flavonoids in the aerial parts of *S. multicaulis*, devoid of anthraquinones and saponins. Results presented by Umer et al. [26] revealed that *S. schimperi* leaf extracts contain polyphenols (tannins and flavonoids) and terpenoids, but no alkaloids were detected. While the occurrence of anthraquinone and coumarins is somewhat limited in Salvia species[27, 28], the abundance of phenolics and terpenoids in the studied extracts calls for further exploration of these valuable compounds to comprehend the medicinal potential of the plant extracts completely.

Our HPLC-DAD analysis revealed a rich profile of phenolic compounds in the *Salvia* sp. extracts, with rosmarinic acid, a caffeic acid derivative, being the most abundant. This finding aligns with prior research on *Salvia* species, where rosmarinic acid was also identified as a major component. Furthermore, studies have shown high levels of rosmarinic acid in the leaves of 27 Iranian *Salvia* species[29]. Rowshan and Najafian[30] noted that the methanolic extract of *S. multicaulis*'s aerial parts contained the highest amount of rosmarinic acid (7.358 mg/g) compared to other identified phenolics like chlorogenic acid (0.53 mg/g) and p-coumaric acid (0.015 mg/g). Different phenolic compounds were also observed in *Salvia officinalis* leaf extracts, including syringic acid, caffeic acid, p-coumaric acid, catechin, epicatechin, and quercetin[31].

The investigated plant extracts displayed a spectrum of antimicrobial activity with varying selectivity based on the target microbe and the plant part, possibly due to the variation in the phytochemical composition within tested extracts. A diverse array of secondary metabolites, encompassing flavonoids, phenolic acids, diterpenes, and triterpenes, is a promising source of antimicrobial agents. These compounds exert their inhibitory effects on various microbes by targeting different components of microbial cells through many different mechanisms[4]. Previous research had demonstrated similar activity in other *Salvia* species. Firuzi et al. [32] reports

promising antibacterial activity in four *Salvia* species (*S. eremophila, S. limbata, S. santolinifolia, and S. sclarea*) with MICs ranging from 0.31 to 5 mg/mL, showing particular effectiveness against *E. coli, K. pneumoniae*, *S. typhi*, and *Staphylococcus* strains. Aqueous extracts from *S. africana*, *S. officinalis*' Icterina', and *S. mexicana* displayed MICs ranging from 0.63 to 10 mg/mL against various microbes, indicating broad-spectrum activity within the Salvia genus[33].

For new plant-based medicines (phytomedicines), assessing acute Toxicity is a crucial first step. This in vivo test battery is crucial for determining the medicine's safety before human use. It involves monitoring weight changes, clinical signs, blood cell counts (hematology), organ function (serum biochemistry), and tissue changes (histopathology)[34]. This study evaluated the acute Toxicity of *Salvia algeriensis* extract in mice at doses of 500 and 2000 mg/kg body weight, employing a reduced sample size to minimize animal suffering following ethical guidelines[23]. No signs of Toxicity (body weight loss and changes in body temperature), behavioral changes, or mortality were observed at either dose. Hematological parameters, including red blood cell count, white blood cell count, hemoglobin, hematocrit, and platelet count, showed no significant differences between the control and treated groups, indicating that *S. algeriensis* extract did not influence blood cell production or circulation. The unchanged relative organ weights compared to the control group suggest that *Salvia algeriensis*, at the administered doses, did not induce organ swelling, atrophy, or hypertrophy. These findings suggest a low acute toxicity profile for the extracts on short-term oral administration, even at the high oral dose of 2000 mg/kg recommended by OECD guidelines-423[23], supporting its potential for further safety and efficacy evaluation. Our study aligns with previous findings by Ramírez et al[35] who reported no toxicity in mice administered the aqueous extract of *S. scutellarioides* at both tested doses of 1 and 2 g/kg and Semaoui et al[36] who observed no toxicity signs within 4 hours and no mortality within 14 days after single-dose oral administration of *S. chudaei* aerial part infusion, suggesting an LD_{50} exceeding 5000 mg/kg. Several reports, including a study by Guaouguaou et al. (2018)[37] on *S. verbena*, support the low toxicity profile of various *Salvia* species. This study demonstrated no toxicity in male and female mice following single-dose oral administration of the extract at 2000 mg/kg, and no toxicity in male and female rats following single-dose dermal administration at the same dose. Additionally, a 28-day dermal sub-chronic toxicity assessment of the same extracts in male and female rats revealed no significant adverse effects on hematological, biochemical, or renal parameters. Histopathological examination of treated rats' liver, kidney, and skin tissues showed normal architecture, indicating no treatment-related morphological alterations.

While our preliminary findings are promising, several limitations must be considered. The single-dose administration and short observation period preclude definitive conclusions regarding long-term Toxicity. Additionally, histopathological analysis of the kidneys and liver is necessary to confirm the absence of nephrotoxicity and hepatotoxicity. Future research should expand on our findings by employing a more comprehensive dose range, more extended observation periods, larger sample sizes, and including both sexes to comprehensively assess the toxicity profile. Sub-acute, chronic, mutagenicity, and carcinogenicity studies are necessary to fully evaluate the safety of *S. algeriensis* extract for therapeutic applications.

CONCLUSIONS

This study is the first to evaluate the biological activity, phytochemical profile, and acute Toxicity of *Salvia algeriensis (Desf.)*. The hydromethanolic extracts from various plant parts (leaves, flowers, roots) exhibited broad-spectrum antimicrobial activity against a range of gram-negative and gram-positive bacteria and fungi.

This means the extracts were effective against a wide variety of microbes. Importantly, these extracts were found to be safe at both tested oral doses. These findings suggest that *S. algeriensis (Desf.)* could be a promising source of natural antimicrobial agents, which could be useful in the fight against infectious diseases. The presence of beneficial phenolic compounds, including caffeic acid derivatives and flavonoids, potentially contributes to the observed activity. Further investigations are necessary to elucidate the specific mechanisms by which these phytochemicals inhibit microbial growth.

Author Contributions: Abdelkader Saadi conceived the study and designed the research goals. Fatima Zohra Bouarsa developed the research methodology. The software used in the analysis was developed/implemented by Djafri Karima. Yavuz Selim Çakmak performed validation of the results; he secured the necessary resources. Abdallah Noui conducted the formal analysis and data collection. At the same time, Djafri karima, and Fatima Zohra Bouarsa managed the research data (data curation), and the first draft of the manuscript was written by Fatima Zohra Bouarsa, and Abdallah Noui conducted the review and editing. Djafri Karima created the figures and tables (visualization). Abdelkader Saadi provided overall research guidance (supervision). All authors have read, critically revised, and agreed to the published version of the manuscript.

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