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In Vitro Antimicrobial Activity of Aqueous, Ethanolic and Methanolic Leaves Extracts from *Salvia argentea*

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ABSTRACT
In view of the global rise of antimicrobial resistance, the discovery of new antimicrobial agents of plant origin has become essential. In this study, the therapeutic capacity of different extracts of *Salvia argentea* was evaluated *in vitro* by testing the antimicrobial activity on 16 bacterial strains and 4 yeast strains. Aqueous, ethanolic, and methanolic extracts of *Salvia argentea* were obtained, and their antimicrobial effects were evaluated using the method of diffusion in agar medium and microdilutions. Aqueous, ethanolic, and methanolic extracts were effective on a majority organism tested with inhibition zones of up to 23 ± 2.6 mm observed in bacteria, and 24 ± 1.5 mm in yeast. Significant MIC results were noted between 3.90 and 7.81 mg/mL for bacterial strains (*Klebsiella pneumonia*, *Pasteurella multocida*, Methicillin-resistant *Staphylococcus aureus*, *Citrobacter freundii*), and a MIC value of 3.90 mg/mL for the yeast strain *Saccharomyces cerevisiae*. The CMB/MIC ratio values recorded for the majority of bacterial strains attribute to *Salvia argentea* extracts a bactericidal action against multi-resistant bacterial strains. In addition, the CMF/MIC ratio values expressed for the different extracts with respect to yeast clearly show a fungicidal action on the three strains of *Candida albicans* whereas for *Saccharomyces cerevisiae* the extracts have a fungistatic action. The antimicrobial activities of *Salvia argentea* extracts was observed on all the strains tested.

Keywords: Silver Sage, antibacterial, antifungal

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INTRODUCTION

Infections caused by resistant bacteria often lead to a prolongation of the disease state, and increased mortality due to a loss of efficacy of antibiotic therapy leading to a therapeutic impasse[1]. In view of the spread of resistance and the limited number of antibiotics under development, the discovery of new antimicrobial agents has become essential [2]. There are many avenues of research, but the exploration of natural resources appears to be the most promising, as they constitute, due to their biodiversity, the largest reserve of active substances [1]. In recent years, a growing number of physicians and pharmacologists have been interested in the therapeutic value and safety of plants [3]. Clinical agents are being sought to cure a wide variety of diseases caused by tumors, viruses, and central nervous system dysfunction [4]. The genus *Salvia*, of the Labiatae or Lamiaceae family, belongs to the wide range of spontaneous medicinal and aromatic plants that characterize the Algerian flora. The genus alone has more than 900 species [5,6]; twenty-three species have been described in Algeria [7]. Studies on the bioactive secondary substances of different species of the genus *Salvia* have revealed their biological properties, which find their application in various fields including, medicine, pharmaceutics, cosmetology, and agriculture [8,9,10]. However, knowledge about the species *Salvia argentea,* as a plant with medicinal properties, is limited. Following our previous evaluation of this plant [11], in this study, we sought to determine the antibacterial and antifungal properties of three different extracts of *Salvia argentea* against 16 bacterial strains and 4 yeast strains.

MATERIAL AND METHODS

Plant material

The *Salvia argentea* leaves used in this study were harvested in the wilaya of Saida, precisely in the region of Hammam Rabi (34°56'22.83 "N, 0°12'29.67 "E). The harvest was carried out at the full flowering stage. Plant identification was carried out by a botanist from the Biology Department of the University of Saida (Professor O. Hasnaoui). A specimen of *Salvia argentea* was deposited in the herbarium of the Biology Department of the University. The leaves were washed and subsequently dried in the shade in a dry and ventilated location for one month prior to being ground to a fine powder.

Extraction process

To obtain the alcoholic extracts, ground plant material (10 g) was placed in a beaker containing 100 mL of 60° ethanol solvent and 60° methanol separately. The extraction was carried out under magnetic stirring at a temperature of 50°C for 2 h. The macerate obtained was subjected to a further extraction by adding the same volume of solvent (100 mL) during the same period (2h). After filtration on Wattman paper number 01, the filtrate was concentrated to dryness using a Heidolph rotary evaporator [12]. Two dry organic extracts were obtained: the ethanolic extract (E. E. S) and the methanolic extract (M. E. S). The extract yields were calculated and dissolved in dimethyl sulfoxide (DMSO) in glass tubes and stored in the dark until further use.

To obtain the aqueous extract, 25g of vegetable powder was placed in a 1 L flask containing 500mL distilled water and heated for 20 min at 100°C reflux. After cooling, the extracts were filtered on Wattman paper number 01 as previously described[13]. The yields of the aqueous extract (A. E. S) obtained were calculated. The extracts were recovered in glass tubes and stored in the dark until further use.

Microbial strains

In vitro antimicrobial activities of different *Salvia argentea* leaf extracts were evaluated on 16 bacterial strains: *Enterococcus faecalis* ATCC 49452, *Staphylococcus aureus*

ATCC 25923, *Listeria monocytogenes* ATCC 19115, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Proteus mirabilis* ATCC 25933, *Pasteurella multocida* ATCC 43137, *Salmonella typhimurium* ATCC 13311, *Campylobacter fetus* ATCC 27374, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 700603, *Enterobacter cloacae* ATCC 13047, *Citrobacter freundii* ATCC 8090, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13312, and 4 yeast strains : *Candida albicans*ATCC 10231, *Candida albicans* ATCC 26790, *Candida albicans* IP 444 et *Saccharomyces cerevisiae* ATCC 204508.

Agar diffusion method: Inhibition test

The agar disc diffusion method (Kirby–Bauer method) was used to evaluate the antimicrobial activity of the different *Salvia argentea* leaf extracts [14]. After swabbing the surface of the Mueller Hinton medium with the inoculum of the microbial strains, the paper discs were impregnated with different concentrations (100, 50, 25, and 12.5mg/mL) of ethanolic, methanolic and aqueous extract, which had previously been filter sterilized using a 0.22 µm micro-filters. DMSO, antibiotic and antifungal controls were impregnated onto the discs as the control. Discs were subsequently placed on the surface of the inoculated agar. After pre-diffusion, the petri dishes were incubated for 18–24 h at 37°C for bacteria [15] and 48 h at 30°C for yeast [16]. Following this incubation, the observed appearance of a circular transparent zone around the discs corresponded to the absence of microbial growth. The larger the diameter of these zones, the greater the sensitivity of the strains to the test extracts [17]. The sensitivity of the strains was classified according to the diameter of the inhibition halos (Ø of the disc was included, 6 mm). The strain with a diameter D < 8mm, 9mm ≥ D ≤ 14mm, 15mm ≥ D ≤19mm, D> 20mm was considered as resistant, sensitive, very sensitive, extremely sensitive, respectively [18,19].

Micro dilution method: Determination of minimum inhibitory concentration (MIC)

The MIC was evaluated on the different extracts using the microdilution method previously described by Daoud *et al* [20]. The stock solution of each extract was prepared in DMSO at a concentration of 125mg/mL, which is known to have no antimicrobial effect [21]. The MIC was defined as the lowest concentration of extract at which no visible growth was observed compared to the control without extract.

Determination of minimum bactericide concentrations (MBC)

The MBC was defined as the lowest concentration of the antibacterial agent, which inhibited 99.9% of the final bacterial concentration. Following the determination of the MIC (after 24 h incubation at 37°C), the two extract concentrations before the MIC, as well as the MIC concentration were used to determine the MBC. For this purpose, a 10 µL sample from each well (not showing growth) was transferred to Petri dishes containing nutrient agar medium. The plates were incubated in an incubator at 37°C for 24 h. This technique verified whether the cells were viable and cultivable. The plate representing the MBC value contained less than three colonies [22].

Determination of minimum fungicide concentrations (MFC)

MFC is defined as the lowest concentration of the antifungal agent that kills 99.9% of the cell concentration. The minimum fungicidal concentration was calculated using the method described by Canton *et al* [23]. Following the determination of the MIC (after 24 h incubation at 35°C), the two antifungal extract concentrations before the MIC, as well as the MIC, were used to determine the MFC. Here, 10 µL from each well was transferred to Petri dishes containing agar-agar Sabouraud medium. The plates were incubated in an oven at 35°C for 48 h. This technique verified whether the cells were viable and cultivable. The plate representing the MFC value contained less than three colonies [24].

RESULTS

Extraction yields

The characteristics of the various extracts and their yields are summarized in Table 1. A maximum 32.5% yield was obtained for the aqueous leaf extract of *Salvia argentea*. Both the ethanolic and methanolic fractions obtained had a similar weight, and a 23% and 21.2% recovered yields, respectively, in relation to the starting dry matter.

Table 1. Aspects, colors and yields of *Salvia argentea* extracts

Extract	Ethanolic extract	Methanolic extract	Aqueous extract
Quantity of powder used (g)	10g	10g	25 g
Yield (%)	23%	21.2%	32,5%
Appearance and color of the dry matter	Sticky, pasty, dark green	Sticky, pasty, greenish brown	pasty, greenish brown

Sensitivity of bacteria and yeasts to plant extracts

The results demonstrated that when in contact with the different concentrations of plant extracts, the strains of bacteria and yeasts showed variable sensitivity as confirmed by presence of inhibition zones. Table 2 summarizes the effects of the different extracts tested on the appearance of inhibition zones of all microbes' strains evaluated. *Salvia argentea* extracts were noted to be active on all bacterial strains tested. The average diameter of the inhibition zones fluctuated between 7 and 23 mm.

Table 2. Sensitivity of bacterial strains to different extracts of *Salvia argentea* (Diameters of inhibition zones are expressed in mm)

Test organisms	Extract	Concentration of plant extracts (mg/mL)			
		12.5	25	50	100
Enterococcus faecalis ATCC 49452	A. E. S	08 ± 1.0	08 ± 1.1	10 ± 0.6	10 ± 0.6
	E. E. S	07 ± 0.6	07 ± 0.6	07 ± 0.0	12 ± 1.5
	M. E. S	07 ± 0.6	07 ± 1.7	10 ± 0.0	10 ± 2.3
Staphylococcus aureus ATCC 25923	A. E. S	08.5 ± 1.0	09 ± 1.5	09.5 ± 2.1	11 ± 0.6

	E. E. S	08.5 ± 1.0	09 ± 0.6	09.5 ± 0.6	11 ± 2.6
	M. E. S	07.5 ± 0.6	09 ± 0.0	09.5 ± 0.6	10 ± 0.6
Listeria monocytogenes ATCC 19115	A. E. S	07 ± 1.0	07 ± 0.0	08 ± 0.6	08 ± 1.5
	E. E. S	07 ± 1.0	07 ± 0.0	07 ± 1.1	07 ± 0.0
	M. E. S	07 ± 0.6	07 ± 0.0	07.5 ± 1.0	07.5 ± 0.6
MRSA ATCC 43300	A. E. S	09 ± 1.1	09.5 ± 1.1	10 ± 1.1	10 ± 2.6
	E. E. S	10 ± 0.0	10.5 ± 0.6	11.5 ± 1.0	12 ± 0.6
	M. E. S	11 ± 1.0	11 ± 1.1	12.5 ± 1.5	15 ± 2.3
Bacillus subtilis ATCC 6633	A. E. S	10 ± 0.6	10 ± 0.6	10 ± 0.0	10 ± 1.1
	E. E. S	12 ± 0.6	12 ± 1.5	15 ± 0.0	15 ± 0.0
	M. E. S	10 ± 0.6	10 ± 2.9	11 ± 1.1	11 ± 1.1
Bacillus cereus ATCC 11778	A. E. S	10 ± 1.5	10 ± 0.0	15 ± 1.5	15 ± 0.0
	E. E. S	12 ± 0.6	12 ± 0.0	14 ± 0.0	17 ± 1.1
	M. E. S	10 ± 1.0	10 ± 0.0	10 ± 2.1	10 ± 1.5
Proteus mirabilis ATCC 25933	A. E. S	07 ± 0.0	07 ± 0.0	07 ± 0.0	07 ± 1.1
	E. E. S	08 ± 1.0	07 ± 0.6	07 ± 0.6	07 ± 1.0
	M. E. S	08 ± 1.0	08 ± 1.5	10 ± 0.6	10 ± 1.1
Pasteurella multocida ATCC 43137	A. E. S	12 ± 2.1	12.5 ± 0.0	14.5 ± 0.6	23 ± 2.6
	E. E. S	10 ± 1.0	10.5 ± 0.0	11.5 ± 0.6	12 ± 1.7
	M. E. S	12 ± 1.1	12 ± 0.0	14 ± 0.0	19 ± 2.1
Salmonella typhimurium ATCC 13311	A. E. S	07 ± 0.6	08 ± 1.0	08 ± 1.5	12 ± 0.0
	E. E. S	07 ± 0.0	07 ± 0.6	07 ± 0.0	10 ± 0.6
	M. E. S	07 ± 0.6	08 ± 1.5	08 ± 0.6	12 ± 0.6
Campylobacter fetus ATCC 27374	A. E. S	08 ± 0.6	08 ± 0.0	10 ± 1.1	10 ± 0.6
	E. E. S	08 ± 0.0	08 ± 0.0	08 ± 1.5	08 ± 0.0
	M. E. S	08 ± 1.1	08 ± 1.1	10 ± 0.6	12 ± 2.3
Escherichia coli ATCC 25922	A. E. S	08 ± 0.0	09 ± 1.5	09 ± 0.0	11 ± 1.5
	E. E. S	07 ± 0.6	09 ± 0.6	09 ± 0.6	11 ± 0.6
	M. E. S	08 ± 1.5	08 ± 0.0	08.5 ± 2.1	10 ± 0.6
Klebsiella pneumonia ATCC 700603	A. E. S	10 ± 2.3	10 ± 1.1	12 ± 1.5	16 ± 0.6
	E. E. S	09 ± 0.0	09 ± 1.5	09.5 ± 0.0	12 ± 1.7
	M. E. S	10 ± 1.1	10 ± 1.5	14 ± 1.5	20 ± 2.1
Enterobacter cloacae ATCC 13047	A. E. S	08 ± 0.0	08 ± 0.6	10 ± 0.0	10 ± 1.1
	E. E. S	07 ± 0.0	08 ± 0.0	08.5 ± 0.0	10 ± 1.1
	M. E. S	08 ± 1.1	08 ± 0.0	10 ± 1.1	10 ± 0.6
Citrobacter freundii ATCC 8090	A. E. S	08 ± 0.6	08.5 ± 1.7	10 ± 0.6	10 ± 1.5
	E. E. S	07 ± 0.0	08.5 ± 1.0	09 ± 1.0	10 ± 0.0
	M. E. S	08.5 ± 1.7	09.5 ± 0.6	10 ± 1.0	10.5 ± 0.6
Pseudomonas aeruginosa ATCC 27853	A. E. S	08 ± 0.6	08 ± 0.0	14 ± 1.5	23 ± 1.1
	E. E. S	07 ± 1.0	07 ± 0.6	10 ± 1.5	12 ± 1.5
	M. E. S	07 ± 0.0	07 ± 1.0	15 ± 0.6	20 ± 2.1
Salmonella enterica ATCC 13312	A. E. S	08 ± 1.5	10 ± 1.5	10 ± 0.0	11 ± 0.6
	E. E. S	07.5 ± 0.0	08 ± 0.6	09 ± 0.0	10 ± 0.6
	M. E. S	07 ± 0.6	08 ± 0.0	08 ± 1.1	09 ± 1.0
Candida albicans ATCC 26790	A. E. S	08 ± 1.0	10 ± 0.0	12 ± 1.1	14 ± 1.1
	E. E. S	07 ± 0.6	08 ± 0.0	08 ± 0.6	09 ± 1.0
	M. E. S	10 ± 0.0	10 ± 0.1	12 ± 0.6	17 ± 1.7
Candida albicans IP 444	A. E. S	10 ± 1.1	12 ± 0.6	15 ± 1.5	19 ± 0.6
	E. E. S	10 ± 0.0	10 ± 1.5	14 ± 0.6	18 ± 0.0
	M. E. S	10 ± 0.6	10 ± 0.6	15 ± 0.0	18 ± 0.6
Candida albicans ATCC 10231	A. E. S	10 ± 0.0	12 ± 2.1	14 ± 0.6	15 ± 1.5
	E. E. S	08 ± 0.6	10 ± 0.0	12 ± 0.0	14 ± 2.1
	M. E. S	10 ± 0.0	12 ± 0.6	14 ± 2.1	16 ± 0.0
Saccharomyces cerevisiae ATCC 204508	A. E. S	10 ± 1.5	12 ± 1.5	12 ± 1.5	24 ± 1.5
	E. E. S	08 ± 1.0	10 ± 1.1	13.5 ± 0.0	16 ± 0.0
	M. E. S	10 ± 0.6	12 ± 0.0	14 ± 1.1	16 ± 0.6

Values are expressed as mean ± standard error of the mean (SEM).

The data presented in Table 2 clearly shows that at the lowest plant extract concentration (12.5 mg / mL), strains of *Klebsiella pneumonia*, MRSA, *Bacillus subtilis*, *Bacillus cereus*, *Pasteurella multocida*exhibited the highest sensitivity to *Salvia argentea* extracts with inhibition zone diameters of 10, 11, 12, 12, and 12 mm, respectively. At the 50 mg/mL plant extract concentration, *Klebsiella pneumonia*, *Pasteurella multocida*, *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa* strains showed very high average inhibition zone diameters of 14, 14.5, 15, 15, and 15 mm, respectively. Extracts of *Salvia argentea* proved very active at the highest concentration tested (100 mg/mL). *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumonia*, *Pasteurella multocida,* and *Pseudomonas*

aeruginosa were considered as the most sensitive strains with mean inhibition zone diameters of 15, 17, 20, 23, and 23 mm, respectively. These findings showed that these latter strains exhibited a higher susceptibility to *Salvia argentea* extracts compared to the others tested.

All the yeast strains examined showed sensitivity to the different plant extracts, with inhibition zone diameters varying between 8 and 24 mm, depending on the different concentrations. Moreover, the extracts were more effective against the yeast strains than the bacteria, especially at the higher extract concentrations. Inhibition zones of approximately 17mm were observed by the *Candida albicans* ATCC 26790 strain when in contact with the methanolic extract. At the highest extract concentrations, the inhibition zones for *Candida albicans* IP 444 were 18, 18, and 19 mm for the ethanolic, methanolic, and aqueous extracts, respectively. For *Candida albicans* ATCC 10231, at extract concentration of 100 mg/mL, the inhibition zone diameter reached a maximum of 16 mm; similar inhibition zone diameters, with 1mm differences, were observed among the different extracts tested. High sensitivity against *Saccharomyces cerevisiae*, as observed by the markedly high inhibition zone values between 16 and 24 mm, was noted at the high plant extract concentrations.

Determination of antibacterial parameters: MIC and MBC

The MIC and MBC values for each of the microbes tested were evaluated. Data presented in Table 3 shows the lowest MIC values, 7.81 mg/mL, of the aqueous extract against the test organisms *Pasteurella multocida* and *Klebsiella pneumonia.*

Table 3. Minimum inhibitory concentrations and minimum bactericidal (fungicidal) concentrations (mg/mL) of aqueous extracts

Test organisms	MIC[a]	MBC[b] (MFC[c])	MBC[b](MFC[c])/MIC[a]
Enterococcus faecalis ATCC 49452	31.24	31.24	01
Staphylococcus aureus ATCC 25923	15.62	15.62	01
Listeria monocytogenes ATCC 19115	15.62	62.48	04
MRSAATCC 43300	15.62	15.62	01
Bacillus subtilis ATCC 6633	31.24	62.48	02
Bacillus cereus ATCC 11778	15.62	15.62	01
Proteus mirabilis ATCC 25933	15.62	62.48	02
Pasteurella multocida ATCC 43137	07.81	07.81	01
Salmonella typhimurium ATCC 13311	15.62	31.24	02
Campylobacter fetus ATCC 27374	31.24	31.24	01
Escherichia coli ATCC 25922	15.62	31.24	02
Klebsiella pneumonia ATCC 700603	07.81	07.81	01
Enterobacter cloacae ATCC 13047	15.62	31.24	02
Citrobacter freundii ATCC 8090	15.62	15.62	01
Pseudomonas aeruginosa ATCC 27853	15.62	31.24	02
Salmonella enterica ATCC 13312	15.62	15.62	01
Candida albicans ATCC 26790	15.62	15.62	01
Candida albicans IP 444	07.81	15.62	02
Candida albicans ATCC 10231	15.62	15.62	01
Saccharomyces cerevisiae ATCC 204508	03.90	15.62	04

[a]MIC: Minimum Inhibitory Concentrations.
[b]MBC: Minimum Bactericidal Concentrations.
[c]MFC: Minimum Fungicidal Concentrations.

Three strains showed MIC values of 31.24 mg/mL: *Enterococcus faecalis*, *Bacillus subtilis,* and *Campylobacter fetus*. These values were the highest concentrations recorded for the aqueous extract. An intermediate MIC value of 15.62 mg/mL was obtained for the other strains investigated. MBC values, close or similar to the MIC values, were noted for 15 bacterial strains. These results lead to the deduction that the aqueous extract of *Salvia argentea* exerts a bactericidal action against all strains, with the exception of *Listeria monocytogenes*, for which the extract was found to be bacteriostatic.

With respect to the yeasts tested, the results showed that the lowest MIC value for the aqueous extract was obtained with *Saccharomyces cerevisiae*, at 3.90 mg/mL. Two strains showed MIC values of 15.62 mg/mL: *Candida albicans* ATCC 26790 and *Candida albicans* ATCC 10231. These values were the highest concentrations recorded for the aqueous extract against the yeast strains. An intermediate MIC value of 7.81 mg/mL was observed for

Candida albicans IP 444. MBC values close or similar to the MIC values for all three strains of *Candida albicans* were noted. These results suggested that the aqueous extract of *Salvia argentea* exerted fungicidal actions against all strains, with the exception of *Saccharomyces cerevisiae*, for which the extract was found to be fungistatic.

For the ethanolic extract, data presented in Table 4 shows that the lowest MIC values were obtained with *Pasteurella multocida* and *Klebsiella pneumonia*, at approximately 7.81 mg/mL.

Table 4. Minimum inhibitory concentrations and minimum bactericidal (fungicidal) concentrations (mg/mL) of ethanolic extract.

Test organisms	MIC	MBC[b] (MFC[c])	MBC[b](CMF[c])/MIC[a]
Enterococcus faecalis ATCC 49452	15.62	31.24	02
Staphylococcus aureus ATCC 25923	15.62	15.62	01
Listeria monocytogenes ATCC 19115	31.24	31.24	01
MRSAATCC 43300	15.62	15.62	01
Bacillus subtilis ATCC 6633	31.24	62.48	02
Bacillus cereus ATCC 11778	15.62	31.24	02
Proteus mirabilis ATCC 25933	15.62	62.48	04
Pasteurella multocida ATCC 43137	07.81	15.62	02
Salmonella typhimurium ATCC 13311	15.62	31.24	02
Campylobacter fetus ATCC 27374	31.24	31.24	01
Escherichia coli ATCC 25922	15.62	31.24	02
Klebsiella pneumonia ATCC 700603	07.81	07.81	01
Enterobacter cloacae ATCC 13047	15.62	15.62	01
Citrobacter freundii ATCC 8090	15.62	15.62	01
Pseudomonas aeruginosa ATCC 27853	15.62	31.24	02
Salmonella enterica ATCC 13312	15.62	15.62	01
Candida albicans ATCC 26790	15.62	15.62	01
Candida albicans IP 444	15.62	15.62	01
Candida albicans ATCC 10231	15.62	31.24	02
Saccharomyces cerevisiae ATCC 204508	03.90	15.62	04

[a]MIC: Minimum Inhibitory Concentrations.
[b]MBC: Minimum Bactericidal Concentrations.
[c]MFC: Minimum Fungicidal Concentrations.

Three strains showed MIC values of 31.24 mg/mL: *Listeria monocytogenes*, *Bacillus subtilis,* and *Campylobacter fetus.* These values were the highest concentrations recorded for the ethanolic extract. An intermediate MIC value of 15.62 mg/mL was obtained for the remaining strains examined. MBC values close or similar to MIC values were observed for 15 bacterial strains. These results indicated that the ethanolic extract of *Salvia argentea* showed bactericidal actions against all strains except for *Proteus mirabilis*, for which the extract was found to be bacteriostatic.

Results relating to the antimicrobial parameters showed that the most basic MIC value for the ethanolic extract was obtained with *Saccharomyces cerevisiae,* at 3.90 mg/mL. *Candida albicans* showed a similar sensitivity with an MIC value of 15.62 mg/mL for all three strains. These values were the highest concentrations recorded for the ethanolic extract against the yeast organisms tested. MFC values close or similar to MIC values for all three strains of *Candida albicans* were recorded. These results demonstrated that the ethanolic extract of *Salvia argentea* exerted a fungicidal action against all strains except for *Saccharomyces cerevisiae*, for which the extract was found to be fungistatic.

Data obtained on the methanolic extracted showed that the most basic MIC value was obtained exclusively with MRSA, at 3.90 mg/mL (Table 5). This finding proved very interesting as this strain is known for its high resistance. Eleven strains showed intermediate MIC values between 15.62 and 31.24 mg/mL. A high MIC value of 62.48 mg/mL was obtained for the *Proteus mirabilis* strain. MBC values close or similar to the MIC values were recorded for 15 bacterial strains. These results suggested that the methanolic extract of *Salvia argentea* exerted a bactericidal action for all strains, except for *Bacillus subtilis,* for which the extract was bacteriostatic.

Table 5. Minimum inhibitory concentrations and minimum bactericidal (fungicidal) concentrations (mg/mL) of methanolic extract.

Test organisms	MIC	MBC[b] (MFC[c])	MBC[b](MFC[c])/MIC[a]
Enterococcus faecalis ATCC 49452	15.62	31.24	02
Staphylococcus aureus ATCC 25923	15.62	31.24	02
Listeria monocytogenes ATCC 19115	31.24	31.24	01
MRSAATCC 43300	03.90	07.81	02
Bacillus subtilis ATCC 6633	15.62	62.48	04
Bacillus cereus ATCC 11778	15.62	31.24	02
Proteus mirabilis ATCC 25933	62.48	62.48	01
Pasteurella multocida ATCC 43137	07.81	15.62	02
Salmonella typhimurium ATCC 13311	15.62	15.62	01
Campylobacter fetus ATCC 27374	15.62	31.24	02
Escherichia coli ATCC 25922	15.62	31.24	02
Klebsiella pneumonia ATCC 700603	07.81	07.81	01
Enterobacter cloacae ATCC 13047	15.62	15.62	01
Citrobacter freundii ATCC 8090	07.81	15.62	02
Pseudomonas aeruginosa ATCC 27853	15.62	15.62	01
Salmonella enterica ATCC 13312	15.62	31.24	02
Candida albicans ATCC 26790	15.62	15.62	01
Candida albicans IP 444	07.81	15.62	02
Candida albicans ATCC 10231	15.62	15.62	01
Saccharomyces cerevisiae ATCC 204508	03.90	15.62	04

[a]MIC: Minimum Inhibitory Concentrations.
[b]MBC: Minimum Bactericidal Concentrations.
[c]MFC: Minimum Fungicidal Concentrations.

Based on the antimicrobial parameters, the most basic MIC value for the methanolic extract was recorded with *Saccharomyces cerevisiae* at 3.90 mg/mL. All three *Candida albicans* strains showed MIC values between 07.81–15.62 mg/mL for the methanolic extract. MFC values close or similar to MIC values were recorded for all three strains of *Candida albicans*. These results demonstrated that the methanolic extracts of *Salvia argentea* exerted a fungicidal action, for all strains except for *Saccharomyces cerevisiae*, for which the extract proved to be fungistatic.

DISCUSSION

There is a growing interest in the use of natural plant extracts as a potential source of bioactive molecules for alternative medicine, especially for the treatment of infectious diseases. Therapeutic strategies against infections of bacterial and fungal origin are mainly based on the use of antibiotics and antifungal. However, inappropriate treatment prescriptions can lead to the selection of multi-resistant microbial strains. Therefore, there is an urgent need to direct research towards medicinal plants as a source of new molecules with different biological activities, in order to limit the alarming development multi-resistance organisms.

In this study, we aimed to test the efficacy of three extracts (aqueous, ethanolic, and methanolic) obtained from of *Salvia argentea* against 20 strains of microorganisms known for their intrinsic resistance to several antibacterial and antifungal agents, as well as their increasing capacity to acquire resistance during antibiotic therapy. The extraction techniques used in this study gave yields of 21.2% and 23% for the ethanolic and methanolic extracts, respectively. The aqueous extract gave a higher yield of approximately 32.5%. Mora *et al.*[12] reported extraction yields of 16.75% after 2 h maceration of the aerial parts of *Salvia elegans* with ethanol (60%). Whereas Alimpić *et al.* [25] showed very low yields after maceration of the aerial parts of *Salvia ringens* in ethanol and methanol, of 7.70% and 6.94%, respectively. These differences in yields may be due to the loss of some relatively volatile substances during the extraction processes, or to the drying time of the biomass [26].

Using the method of diffusion in agar medium, the evaluation of the antimicrobial activity of the *Salvia argentea* extracts showed relatively variable sensitivities, which was dependent on the microorganism tested, as well as on the different extract types and their test concentrations (12.5, 25, 50, 100 mg/mL). This method aimed to test the efficacy of the extracts with respect to the bacterial strains. The inhibition zones data clearly showed that all strains tested were sensitive to the ethanolic, methanolic, and aqueous extracts of *Salvia argentea* at varying concentrations.

Based on the data on the evaluation of the sensitivity of the bacteria to the different extracts, *Klebsiella pneumonia* and

Pasteurella multocida were found to be sensitive to all concentrations, with zones of inhibition greater than those observed by the other strains (19 and 20 mm) for *Klebsiella pneumonia,* and (20 and 23 mm) for *Pasteurella multocida*. Interestingly, these values corresponded to an MIC of 7.81 mg/mL. These findings do not concur with Petrović *et al*. [27] who tested the inhibitory effect of the essential oil of *Salvia amplexicaulis* against Gram-positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus,* and Gram-negative bacteria: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and a strain of *Candida albicans*, and showed the best inhibition effect against *Micrococcus luteus* followed by *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans*, concluding that Gram-negative bacteria are more resistant than Gram-positive bacteria.

Klebsiella pneumonia and *Pasteurella multocida* are strains that cause lung infections such as, nosocomial pneumonia, pneumonia and abscessed pneumonia [28,29]. In our study, we were particularly interested in the high sensitivity of these two strains to the different extracts tested. Our data supports the data of the ethnopharmacological survey on the exclusive use of *Salvia argentea* as a traditional remedy against respiratory diseases [11].

The sensitivity of MRSA to ethanolic and methanolic extracts of *Salvia argentea*is reported by Abouzeed *et al*. [30], who, by testing the inhibitory effect of various Libyan medicinal plants, including plants of the genus *Salvia*, revealed that the most active antimicrobial plants were *Salvia officinalis*, *Pistacia atlantica*, *Arbutus pavarii* and *myrtus communis*. Moreover, they concluded that methanolic extracts of these plants can be used as a source of antimicrobial molecules against the most resistant strains namely, MRSA.

The yeast sensitivity tests with *Salvia argentea* extracts show a high sensitivity for the different concentrations tested. However, *Saccharomyces cerevisiae* proved to be very sensitive to the 100 mg/mL concentration, with inhibition zones 24 mm. Therefore, *Saccharomyces cerevisiae*was shown to be more sensitive to these extracts than strains of *Candida albicans*. This corroborated with studies by Farcasanu and Oprea [31], which reported that yeast cells (*Saccharomyces cerevisiae*) were sensitive to ethanol extracts from the leaves of *Salvia officinalis*. Whereas Lee and Kim [32] confirmed the antifungal activity of *Salvia miltiorrhiza* extracts against *Candida albicans and* indicated that it was associated with changes in membrane permeability. Ünver *et al* [33] tested the *in vitro* inhibitory effects of methanolic extracts of sage (*Salvia officinalis*), laurel (*Laurus nobilis*), and thyme (*Thymbra spicata*) on clinical isolates of eleven yeast strains, indicating their effective capacity to control fungal growth.

In the present study, The MIC values expressed for the different extracts of *Salvia argentea* on the bacteria tested revealed interesting results. *Pasteurella multocida* and *Klebsiella pneumonia* showed an MIC value of around 7.81 mg/mL. The other strains recorded MIC values between 15.62 and 62.48 mg/mL. The methanolic extract exclusively had the lowest MIC value for the MRSA strain, at 3.90 mg/mL. These finding are confirmed by Kivrak *et al* [5], who tested the antioxidant, anticholinesterase, and antimicrobial effect on several microorganisms of the ethanolic extract of *Salvia potentillifolia* and demonstrated that this extract had strong activities against *Bacillus subtilis* and *Bacillus cereus*. Moreover, they noted the remarkable anticandidal activity against *Candida albicans* and *Candida. tropicalis* with MIC values of 18.5 and 15.5 mg/mL, respectively.

The MBC recorded for the different extracts against the bacterial strains clearly showed a bactericidal action by these extracts on the majority of the strains tested. However, the aqueous, ethanolic, and methanolic extracts of *Salvia argentea* exerted a bacteriostatic action on *Listeria monocytogenes*, *Proteus mirabilis*and *Bacillus subtilis*, respectively.

Toty *et al* [34], confirmed a bactericidal power of the aqueous extract on all strains tested: *Pseudomonas aeruginosa*, *Staphylococus aureus*, *Escherichia coli*, and *Proteus mirabilis*, with the exception of a single strain of *Salmonella typhimurium* against which the extract was bacteriostatic. This bactericidal power was previously reported on aqueous and methanolic plant extracts [35].

The MFC expressed for the different extracts, with respect to the yeast strains studied, demonstrated a fungicidal action on the three *Candida albicans* strains; whereas, for *Saccharomyces cerevisiae,* the extracts had a fungistatic action. Kivrak *et al*.[5], also noted that the ethanolic extract of *Salvia potentillifolia* showed a remarkable activity against *Candida albicans* and *Candida tropicalis* with MIC values of 18.5 and 15.5 mg/mL, respectively.

Taken together, methods based on the use of plant extracts against infectious diseases, can have an important role in the development novel therapies to substitute antibiotics, thereby helping to limit complex problems of microbial multi-resistance.

CONCLUSIONS

The antimicrobial activities of *Salvia argentea* extracts was observed on all the strains tested, with a majority showing bactericidal and fungicidal actions. However, some strains proved to be very sensitive among which: *Klebsiella pneumonia* and *Pasteurella multocida* for bacteria, and *Saccharomyces cerevisiae* for yeasts, revealed interesting inhibition zones diameters and MIC, MBC and MFC values. Collectively, this data confirmed the effectiveness of this plant against bacterial strains which are considered highly resistant, and which can affect the respiratory system.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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