Phytochemical characterization and evaluation of the antibacterial potential of *Solenostemma oleifolium* (Nectoux) Bullock & E.A. Bruce ex Maire essential oil

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Key words: GC-MS, NMR, *Solenostemma oleifolium*, Linalool, antibiotic.

Contributions: DKB was the main investigator performing or participating in all the manipulations and wrote the draft of the manuscript. BC supervised the bacteriological work, provided the strains, and critically corrected the manuscript. BD provides critical scientific feedback. HA and SB participated in the interpretation of chemical data. DCGAP opened the facilities to the use of GC-MS and NMR equipment and performed the chemical characterization of the EOSO by both techniques. SZ performed the statistics. MHB was the mentor of this study.

Conflict of interest: the authors declare that they have no conflicts of interest.

Funding: no specific funding for this work which is part of the global work of the Animal Health and Production Research Laboratory of the Higher National Veterinary School of Algiers.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Acknowledgments: the authors thank the University of Aveiro and the FCT/MCT for the financial support for the LAQV-REQUIMTE (UIDB/ 50006/2020 and UIDP/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. We thank Dr Kris Vernelen of Sciensano the Belgian Health Institute for providing the bacterial strains used in this study.

Received: 30 January 2024.
Accepted: 22 April 2024.
Early view: 5 June 2024.

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Licensee PAGEPress, Italy
Journal of Biological Research 2024; 97:12321
doi:10.4081/jbr.2024.12321

Abstract

Solenostemma oleifolium is a tropical plant growing in the Algerian desert that is traditionally used to treat several illnesses, including infection. We investigate Essential Oil components from leaves of *S. oleifolium* (EOSO) and its antibacterial activity. Using Gas Chromatography and Mass Spectrometry (GC-MS), twenty compounds were identified in EOSO, including linalool (57.10%), terpineol (12.95%), *trans*-geraniol (12.65%), and nerol (4.67%). Nuclear magnetic resonance (NMR) analysis allowed us to confirm linalool as the main component of EOSO. Antibacterial activity was tested by agar diffusion and microdilution methods for minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). For EOSO, the inhibition diameters ranged from 16.79 to 39.84 mm, the MIC ranged from 1.066 to 8.54 mg mL^{-1} and the MBC ranged from 20 to 100 mg mL^{-1}. For linanool, the inhibition diameters ranged from 11.1 to 31.87 mm, the MIC ranged from 2.68 to 14.3 mg mL^{-1} and, the MBC ranged from 40 to 100 mg mL^{-1}. EOSO and linalool exhibited significant antibacterial activity against all the tested bacteria. This study confirmed the antibacterial activity of the *S. oleifolium* essential oil and that linalool is the principal constituent of the EOSO.

Introduction

In recent years, the increased incidence of multiple resistance in animal and human pathogenic bacteria has been widely observed, largely due to the bad use of antibiotics extensively used in the treatment of infectious diseases in animals and humans.[1] The excessive use of antibiotics and the consequent selection pressure is the most important factor in the appearance of resistant and multiple resistant microbes.[2] The emergence of infections due to antibiotic-resistant bacteria led to high death rates (approximately 700,000 deaths per year worldwide) and are estimated to exceed 10 million deaths per year in 2050.[3] Strategies to control bacterial infections, the current lack of effective drugs and the limited number of new antibiotics in

the clinical arsenal will require the development of new alternative therapeutic options.[4]

Essential oils are complex mixtures of natural, volatile, and aromatic compounds synthesized by aromatic plants that have been often used in traditional medicine.[5] Currently, they are used to treat different ailments, including stress, pain, and infectious diseases.[6] Various essential oils and their major components from medicinal and aromatic plants, including *Achillea clavennae*, *Artemisia absinthium*, *Cinnamomum zeylanicum*, *Coriandrum sativum*, *Mentha pulegium*, *Origanum vulgare*, *Rosmarinus officinalis*, and *Thymus vulgaris*, have been reported to possess broad antibacterial potential.[7] Indeed, essential oils are endowed with an interesting property which is hydrophobicity, which allows them to distribute with the lipids present in the cell membrane of bacteria and mitochondria. It increases their permeabilities by disturbing their cellular structures leading to the death of the bacteria by the excessive leakage of molecules and ions from the bacterial cell.[8] In this regard, essential oils and their major compounds could be excellent candidates for developing new alternative products to overcome the problem of bacterial resistance.

Solenostemma oleifolium is a tropical species belonging to the Asclepiadaceae family widespread in the Saharan zones of certain countries including Algeria, Sudan, Egypt, and Libya.[9] *S. oleifolium* is also *called S. argel.*[9]

The aerial parts of the plant have been used in the traditional medicine by the local population of Algerian Sahara for the treatment of various infectious illnesses, such as urinary tract infection,[10] bronchitis, and influenza states.[11] Several studies have evaluated the antibacterial activity of aqueous and hydroalcoholic extracts of *S. oleifolium*.[12-16] However, only one study has demonstrated the antibacterial effect of *S. oleifolium* essential oil by the agar diffusion method against four bacterial strains without determination of minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs).[17] Moreover, three previous studies have allowed the phytochemical analysis of the *S. oleifolium* essential oil (EOSO) essential oil by Gas Chromatography and Mass spectrometry (GC-MS), however, the compositions obtained are different from one study to another.[17-19]

The main objectives of the present study are: i) to determine the phytochemical characterization of EOSO by GC-MS and to confirm by nuclear magnetic resonance (NMR) its main component, ii) to evaluate the antibacterial activity (MICs, MBCs) of EOSO and its major compounds to determine its contribution in the activity recorded on both Gram positive and Gram negative pathogenic bacteria.

Materials and Methods

Chemicals and drugs

Dimethyl sulfoxide (DMSO), INT (*p*-iodonitrotetrazolium), Muller Hinton Broth, and the standard drugs gentamicin and linalool (99.9%) were purchased from Sigma Aldrich Corporation (St. Louis, MO, USA).

Plant

The aerial parts (leaves and stems) of *S. oleifolium* were collected between January and March 2022 at Abalessa, 120 km from Tamanrasset (22° 47′ 13″ North, 5° 31′ 38″ East) - Algeria. The aerial plant part was air-dried at room temperature in a dry and aerated place. The leaves were removed and used for the extraction. The studied plant was identified by Professor Benhouhou of the botanical department of "Ecole Nationale Supérieure d'Agronomie" (ENSA) of El-Harrach in Algiers, Algeria, and she delivered us a certificate of identification. Voucher specimens were deposited in the herbarium of "Santé et Productions Animales" Laboratory Research of the "Ecole Nationale Supérieure Vétérinaire of Algiers" (SPA.031).

Extraction

Extraction of EOSO was done by hydrodistillation using a Clevenger-type apparatus. One hundred and fifty grams of crushed leaves were put in a 1000 mL round flask and added to distilled water. The mixture was hydrodistilled for 4 h. The resulting oil was stored in an amber-sealed bottle and stored in the refrigerator at 4°C until use. The yield percentage of essential oil was calculated as volume (mL) of essential oil per 100 g of plant material (v/w).

Preparation of linalool

Linalool (99.9%) was obtained from Sigma Aldrich Corporation (St. Louis, MO, USA). A 57% linalool solution was prepared from the linalool (99.9%) using DMSO and then used for the evaluation of the antibacterial activity.

GC-MS analysis

The GC-MS analysis was conducted as described previously,[20] using an Agilent Technologies 7890A series gas chromatograph interfaced with an Agilent 5975 C Mass selective detector. Instrument and data acquisition were performed with Chem-station software (Agilent Technologies, Wilmington, DE, USA). The analytical capillary column was HP-5ms (Agilent, Santa Clara, CA, USA). The injector temperature was 250°C, injection volume was 0.2 µL and the split ratio was 1:50. The temperature program was 60°C for 8 min, 2°C/min to 250°C for 20 min. The temperature of the MS source and quadrupole source were respectively 230°C and 150°C, the impact of the ionization mode was 70 eV over the scan range of 29-550.

The identification of compounds was carried out based on the GC retention indices (RI) calculated from a series of alkanes injected under the same conditions with the sample, and by comparing the mass spectral fragmentation patterns and their RI with those stored in the database NIST Mass Spectral and Wiley Registry of Mass Spectral Data. The percentage of EOSO compounds was calculated from the GC peak areas.

NMR analysis

NMR spectra were performed as described previously,[20] and were recorded on a Bruker Avance 300 spectrometer (Bruker Daltonics, Bremen, Germany) (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Tetramethylsilane (TMS) was used as the internal standard. The chemical shifts (δ, ppm) described were obtained at room temperature in a solution of deuterated chloroform.

Cultures

Both Gram-positive and Gram-negative reference bacterial strains of medical interest from the American Type Culture Collection (ATCC) were selected. The microbial strains were obtained from the Belgian Institute for Health Sciensano, Brussels, Belgium, and were as follows: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538, *Streptococcus pyo-*

genes ATCC 19615, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922 and *Salmonella enterica choleraesuis* ATCC 14028.

Antibacterial assay by agar diffusion method

The test was performed according to the standard agar diffusion method to test the sensitivity of bacteria to the EOSO.[21] A bacterial suspension was prepared to obtain a turbidity equivalent to that of the 0.5 Mac Farland standard. The Petri dishes previously prepared with Mueller Hinton agar were seeded in uniform streaks over the entire surface of the agar. Four sterile discs 6 mm in diameter were deposited as follows: one disc soaked with 15 µL of pure EOSO, a second with 15 µL of 57% linalool solution, a third disc serving as a negative control with 15 µL of DMSO, and a 30 µg gentamicin disc as a positive control. The technique was performed in triplicate during three successive tests. The dishes were incubated at 35±2°C within 15 minutes of the application of the discs. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition with a Vernier caliper after 18 to 24 hours of incubation.

Microdilution on microplate for Minimum Inhibitory Concentration (MIC)

The test was performed using the micro dilutions method on a microplate for the determination of MIC.[22] A bacterial suspension was prepared to obtain a turbidity equivalent to that of the 0.5 Mac Farland standard. Three stock solutions were prepared: a 20% EOSO solution, a 20% linalool solution, and a 40 mg/L gentamicin solution for all the bacteria tested except for *Escherichia coli* ATCC 35218 and *Escherichia coli* ATCC 25922 for which 10% solutions of EOSO and linalool were tested. Subsequently, a 96-well microplate was used. Mueller Hinton Broth (MHB; 100 µL) was placed in the 12 wells of the microplate. Wells 11 and 12 were used to control the growth and sterility of the medium. In the first well, 100 µL of substance to be tested was added. Subsequently, 100 µL of the mixture of MHB and substance were taken from the first well and placed in the well 2, passing them from the second well to the third and so on up to well 10 to obtain a range of successive dilutions from half to half. The 100 µL remaining at the end of the dilution were discarded. Finally, 100 µL of the previously prepared inoculum was added to all wells except for well 12. The microplate was sealed and placed in an oven at 35°C for 24 h. After the incubation period, a solution of *p*-iodonitrotetrazolium (INT) was prepared at the rate of 0.2 mg mL^{-1} in distilled water. The results were read after adding 40 µL of INT to each well. Subsequently, the microplate was incubated at 35°C for 30 to 120 min. The presence of live bacteria was detected by the pink stain of INT. Each test was performed three times during three successive experiments.

Determination of the Minimum Bactericidal Concentration (MBC) in a solid medium

The same concentration range, achieved by the microdilution technique, was used to determine the MBC of EOSO. Samples were taken from the well serving as growth control and from each of the wells devoid of bacterial growth and then deposited in streaks on Muller Hinton agar. The inoculated dishes were incubated for 24 hours at 37°C. The essential oil's MBC was deduced from the first bacteria-free box. Each experiment was replicated twice, during three successive experiments.[23]

Statistical analysis

The treatment of data was carried out on the IBM SPSS Statistics version 20 and the XLSTAT version 7.1 software. All values were expressed as means ± standard deviation. The means were compared using a one-way ANOVA followed by multiple comparisons (Tukey test). Results were considered as statistically significant when $p<0.05$ and as highly significant when $p<0.01$.

Results

GC-MS analysis

The yield of EOSO obtained by hydrodistillation per 100 g of the plant is 0.3% (v/w). The GC-MS technique was used to carry out the phytochemical characterization of EOSO. Twenty compounds were identified, representing 95.44% of the total EOSO components (Table 1). The compounds were identified using the GC-MS database, the calculated retention index, and pure standards injected in the same conditions. The analysis showed that the main compound was linalool (57%).

NMR analysis

The GC-MS indicated that linalool accounted for 57% of the EOSO (Figure 1A), and this was confirmed by the NMR analysis of the same essential oil. The EOSO proton ^{1}H NMR (Figure 1B) clearly shows the three methyl groups and the vinylic protons at the expected chemical shifts, and the integration confirms that these signals correspond to the major constituent. The vinylic system composed of carbons C-1 and C-2 can be identified by the two protons linked to carbon C-1, which are not equivalent and appear at δ 5.05 and 5.23 ppm with coupling constants typical of geminated protons (J=1.3 Hz) and a vicinal coupling constant typical of a *cis* (J=10.7 Hz) and *trans* (J=16.9 Hz) configuration. These last constants are the result of the coupling with proton H-2, which appears as a double doublet at δ 5.91 ppm and J=10.5 and 16.5 Hz, and allows the identification of the proton $H\text{-}1_{cis}$ at δ 5.05 ppm and $H\text{-}1_{trans}$ at δ 5.23 ppm, due to the coupling constants (Figure 1). Additionally, correlation spectroscopy (COSY) experiments confirm the correlation between these protons, and heteronuclear single quantum coherence (HSQC) confirmed that the mentioned protons H-1 were linked to the same carbon, C-1, at δ 111.7 ppm.

The other experiments, such as ^{13}C NMR spectrum, HSQC, and heteronuclear multiple-bond correlation spectroscopy (HMBC), allowed the confirmation of proton H-6 assignment at δ 5.09-5.15 ppm and the methyl groups identification (Figure 1B). Moreover, they let the assignments of carbons C-2, C-6, and C-3, just to mention the most significative ones, at δ 145.0, 124.3 and 73.5 ppm, respectively. Altogether these data confirm that linalool is the EOSO main compound, and generally, it is accepted that the major component determines the pharmaceutical activities of the essential oil.[23]

Antibacterial assay by agar diffusion method

The results obtained by the agar diffusion method revealed the significant antibacterial effect of EOSO against the Gram-positive and Gram-negative bacteria tested (Figure 2).

The EOSO showed antibacterial activity against *E. coli* ATCC 25922 ($p<0.001$) and *E. faecalis* ATCC 29212 ($P<0.028$) greater than that of the gentamicin standard and a comparable effect against *E. coli* ATCC 35218, *S. aureus* ATCC 6538 and *S. pyogenes* ATCC 19615. Furthermore, EOSO showed a better antibacterial effect than

that of 57% linalool against all the bacteria tested ($p<0.0001$) (Figure 2).

Among Gram-negative bacteria, *E. coli* ATCC 25922, and *E. coli* ATCC 35218 showed the highest zones of inhibition with diameters of 41 mm and 32.97 mm, respectively, while that of *S. choleraesuis* ATCC 14028 was 16.79 mm. In addition, the Gram-positive bacteria *E. faecalis* ATCC 29212 recorded the largest zone of inhibition with a diameter of 39.84 mm, while the bacteria *S. aureus* ATCC 6538 and *S. pyogenes* ATCC 19615 showed zones of similar inhibition (Figure 2).

Linalool, the main component of EOSO, represented 57% of its total composition. To demonstrate the relationship between the antibacterial effect of EOSO and the action of its major component linalool, a regression test was carried out after having proved by the Shapiro-Wilk test that the tested variables followed the normal distribution.

The graphical representation of the inhibition diameters of EOSO as a function of those of 57% linalool made it possible to obtain a very significant linear regression ($p<0.0001$) whose mathematical equation is: EOSO=1.106 Lin57% + 6.284 (Figure 3).

The obtained results show that the two variables follow the same trend with a correlation coefficient r=+0.87, which is between -1 and 1 ($-1 < r < 1$); therefore the correlation is positive. This value is close to 1 which means that the antibacterial effects of EOSO and linalool 57% are strongly correlated. Furthermore, the value of the coefficient of determination obtained is $R^2=0.754$, which means that the antibacterial effect of EOSO is responsible for 75% of the linalool effect.

Determination of MIC and MBC

The results of MIC and MBC showed that EOSO exhibited significant antibacterial activity on all the bacteria tested (Table 2).

The recorded MIC values ranged from 1.066 to 4.269 mg mL^{-1} for Gram-negative bacteria and from 1.279 to 8.541 mg mL^{-1} for Gram-positive bacteria. Furthermore, linalool exhibited MIC values greater than those of EOSO for all bacteria tested. These results corroborate those obtained by the agar diffusion method. The gentamicin standard recorded MIC values lower than those of EOSO, ranging from 0.078 to 5 mg L^{-1}. In a commercial antibacterial drug, the active molecule is pure but in essential oil there is a mixture of compounds.[24]

Discussion

The composition of the EOSO was examined using the GC-MS method. Considering the general guidelines[23] indicating that a component is considered major if it represents 20 to 70% of the components present in the mixture, linalool only can be considered as a major component representing more than 57% of the essential oil components of *S. oleifolium*. Nevertheless, other components such as terpineol (12.95%) and trans-geraniol (12.66%) represent more than 10% of the mixture and can participate in the recorded biological effects.

Our results agree with a previous study indicating that the essential oil of *S. oleifolium* was principally characterized by oxygenated monoterpenes (94.3%) represented by linalool (59.0%), α-terpineol (14.5%) and geraniol (12.4%), followed by small amounts of nerol

Table 1. Identification of GC-MS data from the essential oil of *S. oleifolium*. Calculated retention index (C-RI), compounds identification, theoretical retention index (T-RI) and area percentage (% GC-MS).

N°	C-RI[a]	Compounds	T-RI[b]	% GC-MS[c]	Identification
1	983	*cis*-2,6-Dimethyl-2,6-octadiene		0.126	RI, MS
2	1000	*trans*-2-(2-Pentenyl)furan	1007	0.326	RI, MS
3	1024	Limonene	1025	0.903	RI, MS
4	1035	*Z*-β-Ocimene	1038	0.552	RI, MS
5	1045	*E*-β-Ocimene	1048	0.949	RI, MS
6	1083	α-Terpinolene	1085	0.885	RI, MS
7	**1106**	**Linalool**	**1103**	**57.103**	**RI, MS**
8	**1107**	**3,7-Dimethyl-1,5,7-octatrien-3-ol**	**1104**	**1.047**	**RI, MS**
9	1173	Terpinene-4-ol	1174	0.245	RI, MS
10	**1190**	**Terpineol**	**1189**	**12.954**	**RI, MS**
11	1212	*p*-Menth-1-en-9-al	1232	0.430	RI, MS
12	**1228**	**Nerol (*cis*-Geraniol)**	**1226**	**4.678**	**RI, MS**
13	**1257**	***trans*-Geraniol**	**1258**	**12.658**	**RI, MS**
14	1286	Dihydroedulan I,	1289	0.679	RI, MS
15	1379	β-Damascenone	1382	0.487	RI, MS
16	1449	Geranyl acetone	1452	0.124	RI, MS
17	1480	β-Ionene	1483	0.184	RI, MS
18	1713	Pentadecanal	1713	0.441	RI, MS
19	1844	6,10,14-Trimethyl-2-pentadecanone	1843	0.21	RI, MS
20	1970	Hexadecanoic acid	1970	0.462	RI, MS
% Identification	**95.443**				

[a]Retention index with respect to C5–C28 n-alkanes calculated on non-polar HP5-MS capillary column; [b]Retention index given in literature (NIST or Wiley on non-polar HP-MS or DB5-MS capillary column); [c]Percentage calculated from the peaks areas of GC chromatogram on non-polar HP5-MS capillary column.
The major compounds are in bold.

Table 2. MIC and MBC values of *S. oleifolium* essential oil and 57% linalool.

	Gentamicin (mg mL^{-1})	EOSO(mg mL^{-1})			57% Linalool (mg mL^{-1})		
	MIC	MIC	MBC*	MBC/MIC	MIC	MBC*	MBC/MIC
E. coli ATCC 35218	0.104±0.04	1.07±0.3	20	18.76	10.725±0	160	14.92
E. coli ATCC 25922	0.156±0	1.28±0	20	15.64	2.676±0	40	14.96
S. choleraesuis ATCC 14028	1.67±0.59	4.27±1.21	50	11.71	14.3±5.06	100	6.99
E. faecalis ATCC 29212	5±0	8.54±2.42	100	11.71	10.725±0	100	9.32
S. aureus ATCC 653	0.078±0	1.28±0	20	15.64	2.676±0	40	14.95
S. pyogenes ATCC 19615	0.156±0	1.28±0	20	15.64	2.676±0	40	14.95

*, the three repetitions gave the same results (SD=0).

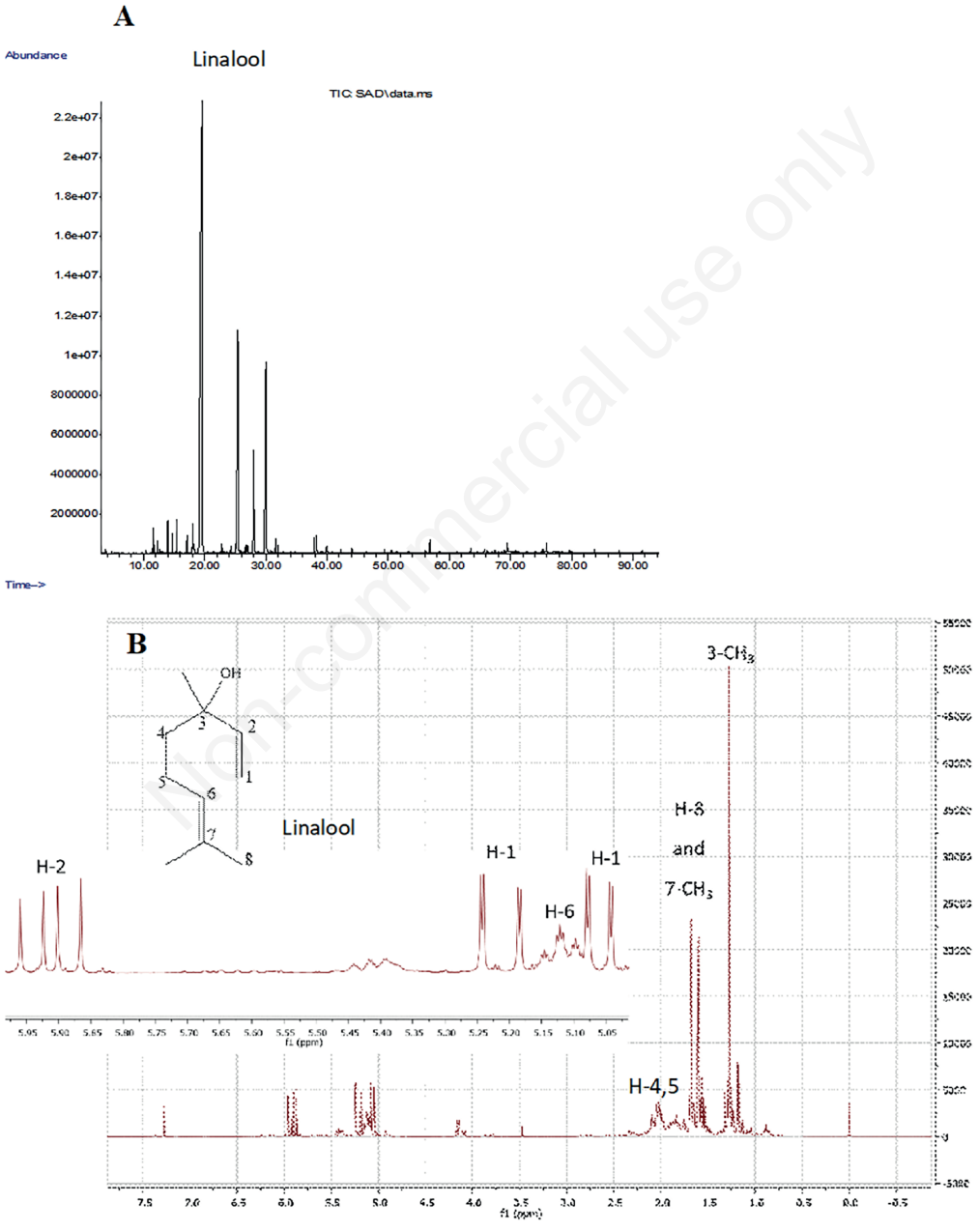

Figure 1. A) GC-MS chromatogram of *S. oleifolium* essential oil. Abundance in function of time. The highest peak is the linalool. B) ^{1}H NMR spectrum of *S. oleifolium* essential oil, showing that linalool is the main compound. The number on the peaks are related to the number on the molecule picture.

(3.7%) and piperitone (3.6%).[19] Contrary to the previous study and our study, another investigation indicated that the composition of *S. oleifolium* can be slightly different with thujone as the major compound (43.73%).[17] Nevertheless, it is not clear if the essential oil came from the leaves or the fruit and the site of collection of the plant were different regions of Algeria (Northwest in this case[17] and far South in our case). The composition of the essential oil can be influenced by the ecological growth conditions.

The antibacterial activity of several *S. oleifolium* extracts (petroleum ether, chloroform, ethyl acetate, ethanol, and distilled water) has been demonstrated.[26] However, only one previous study with the essential oil of *S. oleifolium* isolated from leaves or fruit showed antibacterial activity against *Streptococcus pneumoniae*, *E. coli*, *Salmonella typhimurium*, and *Bacillus cereus* and did not include the MIC and MBC analysis.[17]

Some research has proven that the antibacterial effect of essential oils may be greater, less than, or equal to the action of their main compounds.[27] The antibacterial mechanism of active substances present in essential oils may be due to a global effect induced by different interactions between the compounds of essential oils.[27,28]

Generally, essential oils have a greater antibacterial effect than

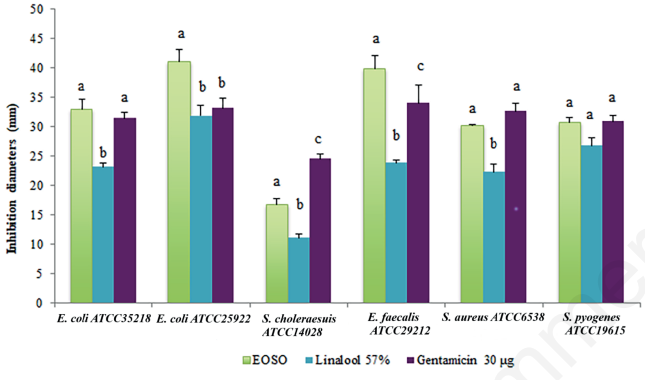

Figure 2. Diameters of inhibition (mm) for gentamicin, linalool, and EOSO against the tested bacteria. For a specific bacterium, the same letters on the bars indicate that the diameters were not significantly different ($p \geq 0.05$) and different letters indicate that the diameters were significantly different ($p < 0.05$).

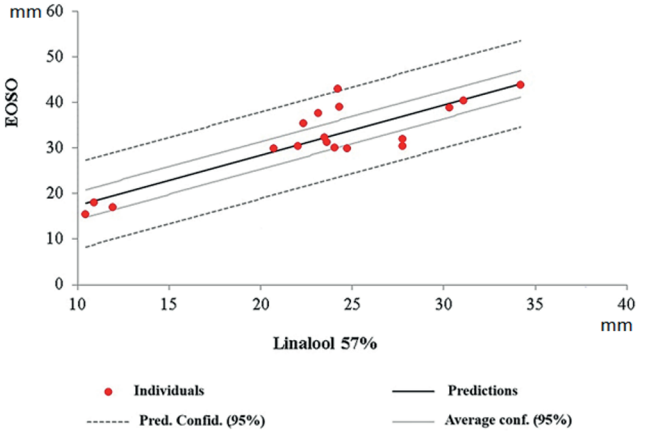

Figure 3. Linear regression between inhibition diameters (mm), EOSO *versus* 57% linalool.

their main compounds, suggesting possible interactions between their components.[27] In this study, EOSO showed an antibacterial effect against the tested bacteria, superior to that of its major component linalool. The obtained results showed that the antibacterial activity of EOSO is attributed for 75% to the action of linalool; the remaining 25% could be the result of the synergistic or additive action of other components, namely, geraniol, terpineol, terpinene-4-ol, and limonene.

Indeed, linalool has an antibacterial effect against several microorganisms such as *Bacillus subtilis, Pasteurella multocida, Escherichia coli,* and *Staphylococcus aureus*. This antibacterial property has been assigned to a functional change in the balance of the bacterial membrane and to an enhancement in the sensitivity of bacteria to common antibiotics.[29] Studies by different scientists have established that treatment with linalool exhibits antibacterial effects against *Candida albicans*, *Staphylococcus aureus,* and *Escherichia coli*.[30] In addition, it is possible to highlight the mechanism of action of linalool, which would lead to the destruction of the integrity of the membrane, an increase in membrane permeability, and leakage of nucleic acids, in addition to the depolarization of the cell membrane, irregularity of the cell metabolism activity and damage to the respiratory chain. These changes ultimately led to cell death.[31]

Geraniol, another component of EOSO, may also participate in its antibacterial activity thanks to its hydrophobic character. Indeed, the probable mechanism of action of the antibacterial effect of geraniol is described by its capacity to adhere to the lipid membrane of the microorganism, interacting with its constituents, making it more permeable, and binding to inside sites, to weaken their structures,[32] thus resulting in ions leak, a reduction in the electrical potential of the membranes, a loss of proton function and a decrease in ATP. These changes promote the cell death of bacteria.[33]

In addition, the terpineol and terpinene-4-ol present in the composition of EOSO have a characteristic antibacterial activity which could contribute to the recorded antibacterial effect. Indeed, the bacteriostatic mechanism of these two isomeric terpineols could be attributed to the damage to the integrity of the cell membrane and of the structure of the cell wall, thus changing its permeability; this results in the loss of intracellular substances such as nucleic acid and proteins, and the depolarization of cell membrane. These modifications lead to cell breakdown and death.[34]

Finally, the limonene present in EOSO could also contribute to the antibacterial effect observed insofar as it has demonstrated significant antibiotic effects against *S. aureus* and *S. pyogenes*.[35]

The mechanism of action of EOSO needs further investigation. Nevertheless, it is active on both Gram-positive and Gram-negative bacteria with different cell wall structures. Therefore, the mechanism of action is to be found in the common characteristics of the two types of bacteria. Our study confirms both the antibacterial and the bactericidal effect of the EOSO and linalool on the bacteria tested. The mechanism of action of EOSO must be investigated in the future. A favorable MBC would typically be the same as or no more than 1 or 2 dilutions greater than the MIC of compounds that are normally considered bactericidal.[36] Therefore, in our experimental conditions both the EOSO and the linalool can be considered bacteriostatic meaning that in low concentrations the growth of the bacteria is blocked and at higher concentrations the bacteria are killed.

Conclusions

This work indicates that *S. oleifolium* essential oil has an antibiotic activity both on Gram negative and Gram positive bacteria. Moreover, most of the antibiotic activity (75%) is due to its major

compound linalool. Nevertheless, the full antibiotic activity is probably due to the synergistic interaction of different compounds of the essential oil. Further investigations will focus on the identification of the mechanism of action of the EOSO using resistant mutants.

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