https://doi.org/10.31925/farmacia.2025.6.6 **ORIGINAL ARTICLE**

THERAPEUTIC POTENTIAL OF *SALVADORA PERSICA* AND *NIGELLA SATIVA* ESSENTIAL OILS: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES AGAINST MULTIDRUG-RESISTANT PATHOGENS

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Abstract

The rise of antibiotic-resistant bacteria poses a significant public health challenge. This study explores the potential of *Salvadora persica* and *Nigella sativa* essential oils, extracted *via* hydrodistillation, as natural alternatives. Hydrodistillation yielded 9% essential oil from *S. persica* and 1% from *N. sativa*. Chromatographic analysis revealed distinct chemical profiles: *S. persica* oil primarily contains oleic, palmitic and stearic acids, while *N. sativa* oil is rich in terpenes and fatty acids. Both oils exhibited significant antioxidant activity, with *N. sativa* showing stronger antiradical and iron reduction effects. Antibacterial efficacy was tested against multidrug-resistant strains, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. Both oils demonstrated antibacterial activity at 100 µg/disc, except against *E. coli*. However, *N. sativa* extract was effective against *E. coli* at higher concentrations (MIC: 1000 µg/mL, MBC: 2500 µg/mL), while *S. persica* showed the lowest MIC (1000 µg/mL) and MBC (2500 µg/mL) against *L. monocytogenes*. These findings highlight the potential of *S. persica* and *N. sativa* essential oils in combating multidrug-resistant bacteria and their applications in pharmaceuticals, cosmetics and aromatherapy.

Rezumat

Rezistența la antibiotice reprezintă o provocare pentru sănătatea publică. Acest studiu explorează potențialul efect antimicrobian al uleiurilor volatile de *Salvadora persica* și de *Nigella sativa*, extrase prin hidrodistilare. Hidrodistilarea a produs 9% ulei esențial din *S. persica* și 1% din *N. sativa*. Analiza cromatografică a relevat profiluri chimice distincte: uleiul *S. persica* conține în principal acizii oleic, palmitic și stearic, în timp ce uleiul *N. sativa* este bogat în terpeni și acizi grași. Ambele uleiuri au prezentat o activitate antioxidantă semnificativă, *N. sativa* având efecte antiradicalice și de reducere a fierului mai puternice. Eficacitatea antibacteriană a fost testată împotriva unor tulpini multirezistente, inclusiv *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes* și *Staphylococcus aureus*. Ambele uleiuri au demonstrat activitate antibacteriană la 100 µg/disc. Aceste rezultate evidențiază potențialul uleiurilor volatile de *S. persica* și *N. sativa* în combaterea bacteriilor multirezistente și aplicațiile lor în produse farmaceutice, cosmetice și aromaterapie.

Keywords: antibacterial activity, antioxidant activity, *Salvadora persica* L., *Nigella sativa* L.

Introduction

Since ancient times, aromatic and medicinal plants have been used to cure a wide range of illnesses. Today, their application in a variety of industries, including food preservation, cosmetics and perfumery, has grown [1, 2]. It is believed that medicinal plants are the primary source, either directly or indirectly, of at least 25% of all current medications [3]. Although, there have been attempts since the early 1900s to characterise these attributes in the laboratory, the antibacterial effects of aromatic and medicinal herbs and their extracts have been known since antiquity [4]. In fact, medicinal plants are being used more often in industrialised nations to counteract the negative effects of standard medications. This is especially true in light of the rise in antibiotic-resistant germs

and the introduction of rare ailments that make it difficult to treat existing medications [5].

Furthermore, in underdeveloped nations, medicinal plants continue to be the cornerstone of the treatment of many ailments. In fact, 60% to 80% of people on the planet still receive their common ailments from traditional healers [6].

Antioxidants, which combat free radicals, can also be utilised to cure a number of illnesses, including as diabetes, cancer and neurological disorders, according to new studies. However, the usage of synthetic antioxidants has been linked to a number of disorders, including carcinogenicity [7].

The usage of synthetic antibiotics has a concerning effect on human health today [8]. Due to the emergence of a significant number of bacteria resistant to broad-spectrum antibiotics, the development of new antibacterial drugs and research on their mechanisms of action are becoming increasingly crucial [9], like plant extracts and essential oils [10]. The phenomenon of antibiotic resistance has existed since the discovery of antibiotics by Alexander Fleming in 1928, which prevented the chaos caused by the misuse of antibiotics. Because antibiotics are an important element both in patient care and in the treatment of diseases and infections, bacterial resistance to antibiotics is a major health problem because it makes the elimination of these bacterial agents by drug treatment more difficult [11, 12].

Essential oils have a high potential to combat antimicrobial resistant organisms and reduce the uncontrolled use of antimicrobials. These molecules are biologically active chemicals with antibacterial and anti-inflammatory properties, as well as analgesic activity. The antimicrobial properties of essential oils are essential to combat the resistance of microorganisms to drugs, which is rapidly increasing [13]. In 2016, around 6 million people died from upper respiratory tract infections, tuberculosis or diarrheal diseases worldwide. According to the WHO report on drug resistance, *Klebsiella pneumoniae* is resistant to third-generation cephalosporins and carbapenems, *Escherichia coli* is resistant to third-generation cephalosporins and fluoroquinolones, *Staphylococcus aureus* is resistant to methicillin, *Streptococcus pneumoniae* is resistant to penicillin and *Salmonella* is resistant to fluoroquinolones [14].

Nigella sativa, often called black cumin seed and Habbat al-Barakah in Saudi Arabia, is an annual herbaceous plant of the Buttercup family cultivated in various regions of the world especially in warm, humid places as well as chilly, dry ones with little snowfall [15]. It can reach heights of 20 cm to 30 cm and has finely divided linear foliage [16], has grown significantly in value on the market as a result of its high concentration of vital bioactive phytoconstituents [17]. Recently, research on *N. sativa* seeds, attributed to their traditionally recognised medicinal qualities, has aroused great global interest. An old-fashioned sweet meal can be made using these seeds, enjoyed with honey and syrup, or sprinkled on bread to enhance the flavour of various foods, especially baked goods and cheese [18]. In addition, they play a role in traditional medicine as a natural treatment for many conditions, including asthma, high blood pressure, diabetes, inflammation, cough, bronchitis, headache, fever, dizziness and flu and are recognised as diuretic, carminative, lactagogue and antihelminative agents [19, 20]. According to Ketenoglu *et al*. [21] the oil of *N. sativa* has shown various pharmacological effects, including antiparasitic, antihypertensive, analgesic, antineoplastic and antibacterial activities, in particular with regard to hepatotoxicity and nephrotoxicity, attributed to the abundance of active compounds present.

Many Middle Eastern and African nations utilise the *Salvadora persica*, a desert plant belonging to the *Salvadoraceae* family, as a stick for cleaning their teeth [22]. It has three lignin glycosides Akhtar *et al*. [23], carbohydrates, volatile oils, flavonoids, alkaloids, steroids, terpenoids and saponin [24, 25]. It also has minerals like flavonoids and volatile oils [26]. The pharmacological studies have indicated that *S. persica* has antimicrobial, antioxidant, antiplastic, aphrodisiac, alexiteric, analgesic, anti-inflammatory, antipyretic, astringent, anticancer, diuretic and bitter stomach activities [27, 28]. About 80% of the world's population uses it as a natural remedy for the treatment of various diseases [29, 30].

The objective of the present investigation is to evaluate the chemical constituents, along with the antioxidant and antimicrobial characteristics, of essential oils extracted from *Nigella Sativa* and *Salvadora persica*.

Materials and Methods

Chemicals and reagents

All the chemicals employed in the research were obtained from Sigma Aldrich, demonstrating a superior level of purity equal to or exceeding 98%.

Plant material

Nigella sativa L. The seeds of *Nigella Sativa* L. were collected from a local market in Kenitra-Morocco, based on botanical characteristics. The seeds were meticulously washed before being dried at 60°C.

Salvadora persica L. Miswak "sothiou" is obtained from a local Moroccan market. Because of its botanical characteristics, the product is identified and confirmed as *Salvadora persica* L.

Extraction of N. sativa and S. persica essential oils.

The seeds of *N. Sativa* and the sticks of *S. persica* were ground using a mechanical mixer until a powder was formed, and 100 g of the latter were weighed for essential oil extraction through hydro-distillation, as reported by Sudhir *et al*. [31]. *N. Sativa* and *S. persica* powders (100 g) were placed in separate balloons containing one litter of distilled water, and the flask

was heated to 55°C. The essential oils are then recovered in a glass container before being separated in a separating funnel. The extract is then dehydrated by adding 20 mL of dichloromethane and trace amounts of $NaSO_4$ to eliminate any residual water in the oils before being filtered and kept at 4°C in a refrigerator.

Gas chromatography-mass spectrometry analysis

The essential oils were analysed by gas chromatography-mass spectrometry under the described conditions by Aouji *et al*. [32].

Antibacterial activity of Nigella Sativa L. and Salvadora persica L. essential oils

Bacterial strains' antibiotic resistance profiles. Pseudomonas aeruginosa, Listeria monocytogenes (ATCC 7644)*, Staphylococcus aureus (*ATCC 29213) and *Escherichia coli* (ATCC 35218) were subjected to antibiotic sensitivity tests using Kirby Bauer's disk diffusion method [33] to ampicilline, cefuroxime sodium, cefotamine sodium, erythromycine, netilmicin, piperacillin and tetracycline (Table I).

Table I

Used antibiotics and their families

Antibiotics	Antibiotic family
Ampicilline (10 µg)	Beta – Lactame
Cefuroxime sodium (30 µg)	Cephalosporine
Cefotamine sodium (30 µg)	Beta – Lactames 3rd generation
Erythromycine (25 µg)	Macrolides
Netilmicin (30 µg)	Aminoside
Tetracycline (30 µg)	Tetracycline
Piperacilline (100 µg)	Beta – Lactame

In vitro antibacterial activity of N. Sativa and S. persica

1000 µL of bacterium suspension with a concentration of 10^8 CFU/mL was obtained from a 24-hour culture using a micropipette and inoculated in the Mueller Hinton agar culture medium, followed by the removal of excess of the bacterial suspension. The antibacterial activity of the essential oils of *N. sativa* and *S. persica* was determined using the Sudhir *et al*. [31] method with some modifications, which involved diluting the extract with dimethylsulfoxide (DMSO) to obtain solutions of 100 µg/disc and then soaking the 5 mm diameter Whatman N°1 filter paper discs onto the culture medium. As a control, DMSO is utilised. After incubation for 24 hours at 37°C, inhibition zones were recorded.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of N. sativa and S. persica extracts

The MIC and BMC were determined using the broth macro-dilution technique in accordance with the Institute for Clinical and Laboratory Standards (CLSI M100-30th Edition). Bacteria were cultivated in Mueller Hinton Broth.

Antioxidant activity

Free radical scavenging activity (DPPH). Following the absorption of DPPH radicals at specific wavelengths, the optical density decreases. A series of 500 µL ethanol solutions containing various concentrations of extracts were extracted from a series of tubes. A total of 2500 µL of DPPH solution (0.2 mM) was added to each of the tubes. The contents were vigorously agitated and left to undergo a thirty-minute reaction at ambient temperature. At 517 nm, the absorbance of the samples was measured in comparison to the control. In parallel with the test sample, a negative control was prepared by combining 500 µL of methanol with 2500 µL of DPPH solution, the methanol served as a blank Aouji *et al*. [34].

The percentage inhibition and calculated as follows equation (1):

$$\%\ \text{Activity} = \frac{\text{Abs Cn} - \text{Abs Ech}}{\text{Abs Cn}} \times 100, \quad (1)$$

where, Abs_{Ech} = absorbance of the sample; Abs_{Cn} = absorbance of negative control.

Ferrous ion reducing antioxidant power assay (FRAP).

The formation of pearlescent Prussian blue at 700 nm was utilised to quantify the formation of the ferrous form of the iron/ferricyanide complex. Twenty minutes were permitted for the reaction of 200 µL of extracts of variable concentrations in 250 µL of phosphate buffer (0.2 M, pH = 6.6) and 250 µL of potassium ferricyanide (1%, w/v) at 50°C. Following this, 2500 µL of TCA (10% w/v) was added to the mélange, which was centrifuged for 10 minutes at 3000 rpm. A mixture of 2500 µL distilled water, 500 µL ferric chloride (0.1% w/v) and 2500 µL supernatant was prepared. At 700 nm, the absorbance was measured in relation to the negative control Aouji *et al*. [33]. An identical methodology was adhered to when preparing the standard solution of L-ascorbic acid. The anti-oxidant activity was calculated using the following equation (2):

$$\%\ \text{Reduction power} = \frac{(\text{Asample} - \text{Ablank})}{\text{Asample}} \times 100. \quad (2)$$

Statistical analysis

The statistical data were evaluated using statistical software applying a variance analysis (ANOVA) with the Tukey test at $\alpha = 0.05$. The data are presented as means ± SD from triplicates (n = 3).

Results and Discussion

Nigella sativa L. and Salvadora persica L. extraction yield

We collected 1% of the essential oils after hydro-distilling 100 g of powder from *Nigella sativa* L. seeds. Alrashidi *et al*. [35] confirmed this yield by obtaining average values of aqueous extracts of *Nigella sativa* L. ranging from 0.047 to 1.7 %.

Concerning the *Salvadora persica* L. sticks, the generated essential oils have a brownish colour, a very strong odour and a 9% essential oil yield. These findings are consistent with those of Khalil *et al*. [36], who reported a yield of 6% after methanol extraction.

In the Ramadan and Alshamrani, investigation, this yield was 20.54% using the same type of solvent [37]. In general, these differences in extraction yields are attributed to various factors, including the extraction process, the employed solvent and plant-specific parameters such as species origin, growing location, soil type, harvest time, method/extraction time of the essential oils [37].

Chromatographic analyses

Chromatographic analysis of *S. persica* essential oil revealed the presence of several compounds, with the main ones being oleic acid (39.31%) and palmitic acid (23.59%), followed by b-sitosterol (8.82%) and eugenol (1.10%). Other fatty acids were also identified, such as stearic acid (2.22%), hexadecenoic acid (1.80%) and heptadecanoic acid (0.26%), but at lower concentrations.

In addition to fatty acids, hydrocarbons were detected, including dodecane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane and triacontane, as well as sterols such as cholesterol, campesterol and cholesta-22,24 dien-5-ol,4,4-dimethyl.

Table II

Chromatographic analysis of the essential oil of *S. persica*

Name of the compound	RT (min)	Peak area %
Eugenol	07.50	01.10
Asarone	10.22	00.24
Oleic acid	16.16	39.31
Palmitic acid	14.51	23.59
Hexadecenoic acid	14.21	01.80
Cholesterol	23.29	00.34
b-sitosterol	24.62	08.82
Squalene	21.29	00.18
Stearic acid	16.29	02.22
heptadecanoic acid	15.34	00.26
pentanoic acid	13.41	00.40
Dodecane	05.42	00.34
Tetradecanoic acid	12.39	00.31
Tricosane	17.27	00.12
Tetracosane	18.08	00.37
Pentacosane	18.87	00.39
Hexacosane	19.31	00.33
Heptacosane	20.37	00.19
Octacosane	21.06	00.10
Nonacosana	21.78	00.14
Triacontane	22.45	00.12
Campesterole	24.01	00.29
Cholesta-22,24-dien-5-ol,4,4-dimethyl	24.22	01.29
Not Identified	-	17.75

The chromatographic analysis of *N. sativa* essential oil revealed a diverse array of compounds present in varying concentrations. The predominant compounds include n-hexadecanoic acid (29.47%), eicosanoic acid (10.50%) and α-thujene (5.48%). Other notable components include α-pinene (1.20%), p-cymene (8.94%), carvacrol (3.78%) and caryophyllene (5.10%). These compounds contribute to the characteristic aroma and potential therapeutic properties of *N. sativa* essential oil.

Additionally, fatty acids such as oleic acid (0.64%), palmitic acid (2.82%) and tetradecanoic acid (0.23%) were identified, albeit in lower concentrations compared to the dominant compounds (Table III).

Table III

Chromatographic analysis of *N. sativa* essential oil

Name of the compound	RT (min)	Peak area (%)
α-Thujene	03.25	05.48
α-Pinene	03.54	01.20
π-Cymene	05.48	08.94
5-Hydroxy methyl furfural	05.75	00.15
Thymoquinine	06.48	00.29
Thymol	06.91	00.26
2-Isopropylidene-5-methylhex-4-enal	08.19	00.02
Limonene	08.97	00.81
Longifolene	09.12	00.33
Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	09.78	00.15
p-tert-Butyl catechol	11.26	00.42
Tetradecanoic acid	13.45	00.23
Carvacrol	13.87	03.78
n-Hexadecanoic acid	16.17	29.47
Caryophyllene	16.80	05.10
Oleic acid	17.98	00.64
Eicosanoic acid	18.79	05.29
9, 12-Octadecadienoic acid (Z, Z)	19.40	10.50
cis-13, 16-Docasadienoic acid	22.50	02.59
cis-11,14-Eicosadienoic acid, methyl ester	22.76	03.98
Methyl 19-hexacosenoate	23.08	01.06
Cholestan-3-ol, 2-methylene-, (3β, 5α)-	23.40	00.87
9, 12, 15-Octadecatrienoic acid	23.79	01.08
Palmitic acid	24.80	02.82
Not Identified	-	**14.54**

The comparison of these two oils reveals distinct chemical profiles. In the essential oil of *S. persica*, the main compounds identified are fatty acids, particularly oleic acid, palmitic acid and stearic acid, which represent significant percentages of the peak area. In contrast, the essential oil of *N. sativa* presents a greater diversity of compounds, including a variety of terpenes such as α-thujene, p-cymene, carvacrol and caryophyllene, in addition to fatty acids such as n-hexadecanoic acid and eicosanoic acid. This diversity of compounds characterises the more complex chemical composition of *N. sativa* oil compared to that of *S. persica.*

The differences in the chemical composition of the two essential oils also reflect their potential uses. *S. persica* oil, rich in fatty acids, could find applications in the cosmetic, pharmaceutical and food industries due to its beneficial properties for skin and health. Conversely, *N. sativa* oil, with its diversity of the

compounds, including terpenes with aromatic and potentially therapeutic properties, could be used in areas such as aromatherapy, traditional medicine and pharmaceutical research.

Antioxidant activity

The antioxidant activity can be due to different mechanisms, such as scavenging of free radicals and reducing capacity [38]. It is therefore important to use multiple analysis methods and different substrates to evaluate the efficacy of antioxidants. The chosen methods are commonly used for determining the anti-oxidant activities of plant extracts and essential oils. The activity of the different essential oils (EOs) against the DPPH• radical was evaluated. Both oils successfully reduced the stable DPPH• to yellow diphenylpicryl-hydrazine. This reduction capacity was determined by a decrease in absorbance induced by the antiradical substances present in the tested EOs. Based on the obtained IC_{50} values, both EOs exhibited significant antiradical activity compared to ascorbic acid (IC_{50} = 41.23 ± 0.67 µg/mL). The degree of this activity decreased as follows: ascorbic acid > *N. sativa* > *S. persica* (Figure 1). Therefore, the strongest effect was found for *N. sativa* EO (IC_{50} = 58.30 ± 0.46 µg/mL) and the weakest for S. *persica* EO with IC_{50} = 62.03 ± 0.99 µg/mL. We note that our studied oils showed a significant capacity to eliminate the DPPH• radical. This interesting property could be related to their chemical composition [39].

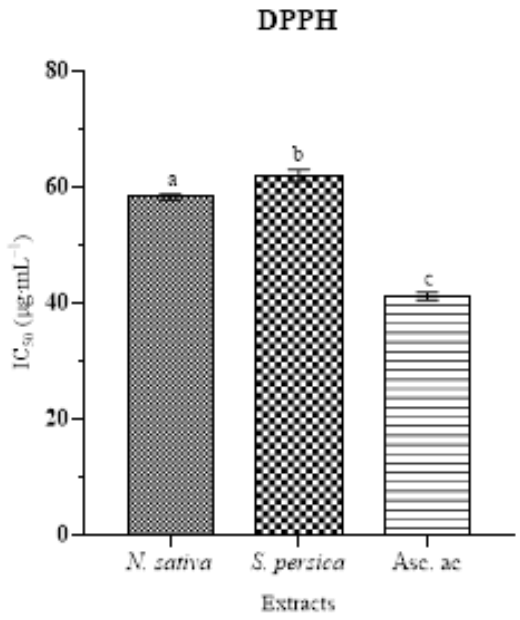

Figure 1.
Anti-radical activity of EOs against DPPH
The significant difference ($p < 0.05$) is illustrated by the letters a, b and c.

Figure 2 illustrates the iron reduction activity by the essential oils of *N. sativa* and *S. persica*. As can be seen, the reducing power of the EOs of the two tested species is close to each other. The essential oil of *N. sativa* exhibited the strongest iron reduction activity (20.857 ± 0.52 µg/mL), and the lowest was found for *S. persica* (IC_{50} = 40.27 ± 0.55 µg/mL). The potential for scavenging synthetic antioxidant free radicals was determined (30.083 ± 0.92 µg/mL).

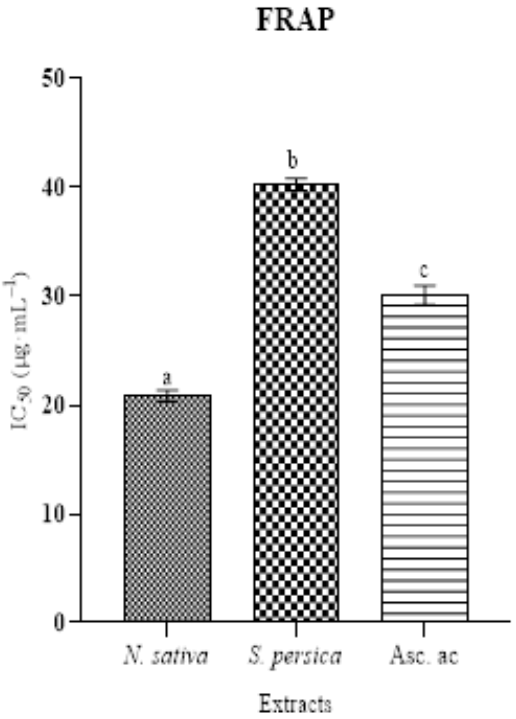

Figure 2.
Reducing activity of EOs with respect to FRAP
The significant difference ($p < 0.05$) is illustrated by the letters a, b and c.

The modification of the antioxidant potential of essential oils can be attributed to the presence of certain molecules with antioxidant activity [40]. Although the quantities of these compounds are relatively low in the oil, possible synergistic and antagonistic effects of the compounds can also be considered [41].

The reducing potential may be due to di- and mono-hydroxyl substitutions in the aromatic ring which possess powerful hydrogen donating abilities [42].

Antibiotic resistance profile of tested bacterial strains

According to the antibiogram, it appears that *Escherichia coli* is sensitive to tree of seven antibiotics, including cefotamine sodium, netilmicin and tetracycline. Furthermore, erythromycin, netilmicin and tetracycline, are the most effective antibiotics on *Staphylococcus aureus*. However, *Pseudomonas aeruginosa* present a resistance to ampicilline, piperacillin and tetracycline. Contrary to *Listeria monocytogenes*, which is resistant to ampicilline and tetracycline (Table IV).

Table IV

Comparison between the effects of antibiotics and *N. sativa* L. and *S. persica* L. extracts on pathogenic bacteria

	P. aeruginosa		***E. coli* (ATCC 35218)**		***L. monocytogenes* (ATCC 7644)**		***S. aureus* (ATCC 29213)**	
	IZ (mm)	**Pr**	**IZ (mm)**	**Pr**	**IZ (mm)**	**Pr**	**IZ (mm)**	**Pr**
Ampicilline	00.00^{a}	R	00.00^{a}	R	00.00 ± 0.00^{a}	R	00.00 ± 0.00^{a}	R
Cefotamine sodium	09.60 ± 0.12^{b}	S	17.00 ± 0.00^{c}	S	10.00 ± 0.09^{b}	S	00.00 ± 0.00^{a}	R
Cefuroxime sodium	10.00 ± 1.02^{b}	S	00.00^{a}	R	28.00 ± 0.16^{d}	ES	00.00^{a}	R
Erythromycine	19.00 ± 0.24^{c}	S	00.00^{a}	R	25.00 ± 0.0^{cd}	ES	20.00 ± 0.00^{bc}	S
Netilmicin	19.67 ± 0.10^{cd}	S	13.50 ± 0.00^{b}	S	30.00 ± 0.37^{de}	ES	18.00 ± 0.00^{b}	S
Piperacillin	00.00^{a}	R	00.00^{a}	R	17.00 ± 0.20^{bc}	S	00.00^{a}	R
Tetracycline	00.00^{a}	R	12.00 ± 0.00^{b}	S	00.00^{a}	R	15.00 ± 0.00^{b}	S
N. sativa	19.58 ± 0.14^{cd}	S	00.00^{a}	R	36.38 ± 0.13^{f}	ES	25.67 ± 0.00^{c}	ES
S. persica	20.00 ± 0.10d^{e}	S	28.00 ± 0.31^{c}	ER	15.00 ± 0.09^{bc}	S	32.00 ± 0.00^{d}	ES

Means in the same column with the same letter do not differ significantly from each other at the 5% level of significance according to the Tukey's test. ZI = Inhibition zone; R = Resistant; S = Sensitive; ES = Extremely Sensitive; ER = Extremely Resistant

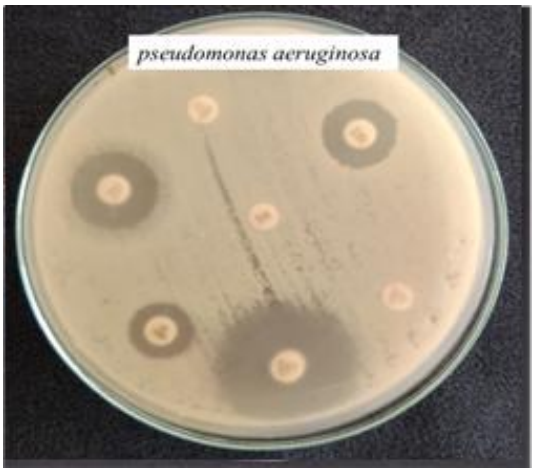

Figure 3.
Antibiogram of *P. aeruginosa* under antibacterial effect of *N. sativa* L. extract

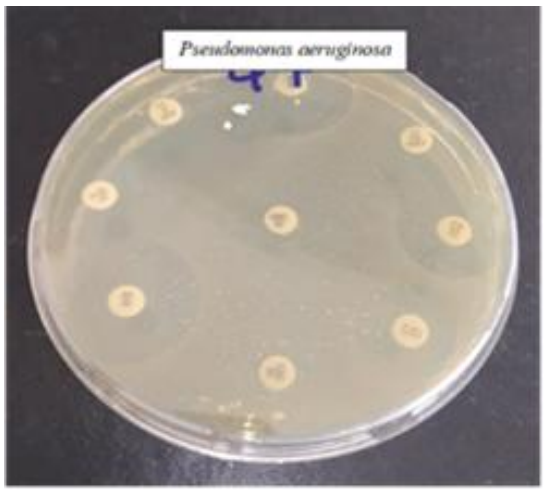

Figure 4.
Antibiogram of *Pseudomonas aeruginosa* under antibacterial effect of *S. persica* L. extract

Antibacterial activity of Nigella sativa L. and Salvadora persica L. extracts

Based on Table IV, we can conclude that *Listeria monocytogenes* is the most sensitive to the action of *Nigella sativa* L. extract (100 µg), with an inhibitory diameter of 36.38 ± 0.13 mm, followed by *S. aureus* with 25.67 ± 0.00 mm and *P. aeruginosa* with 19.58 ± 0.14 mm. However, no antibacterial activity has been shown on *E. coli* (Figure 3 and Figure 4).

In terms of antibacterial activity of *Salvadora persica* L. extract, the treatment was effective against *S. aureus* (32 ± 0.00 mm) and *E. coli* (28 ± 0.31 mm), followed by *P. aeruginosa* (20 ± 0.10 mm) and *L. monocytogenes*, which has the smallest inhibitory diameter (15 ± 0.09 mm). Furthermore, we discovered that *P. aeruginosa* was the only bacteria in which the antibacterial activity of *Salvadora persica* L. extract had the same inhibitory diameter as netilmicin antibiotic (19.67 ± 0.10 mm). Haloci *et al*. [43] demonstrated that *N. sativa* L. extract has antibacterial effects that are effective against both Gram-positive and Gram-negative bacteria. Indeed, the medicinal properties of *N. sativa* L. are attributed to the presence of the combination of compounds containing secondary metabolites; it is an effective method of overcoming the major problem of resistance to conventional antibiotherapy [44]. Similarly, Darout *et al*. discovered that *Salvadora persica* L. extracts contain a variety of anionic compounds with potential antibacterial activity, such as chlorures, sulfates, sulfocyanate and nitrates [45]. Other compounds discovered in this plant species include vitamin C, traces of tannins, saponines and sterol [46].

Determination of the MIC and MBC of N. sativa L. and S. persica L. extracts

The macro-dilution broth method was used to determine the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values of the extracts. These values indicate whether antimicrobial agents exhibit bacteriostatic (MIC only) or bactericidal (MIC and MBC close in value) activity.

The extract of *N. sativa* L. showed a wide range of MIC values, ranging from 1000 µg/mL to 2000 µg/mL, depending on the bacterial species tested. For instance, *S. aureus* (ATCC 29213) exhibited the highest MIC at 2000 µg/mL, whereas *E. coli* (ATCC 35218) showed the lowest MIC at 1000 µg/mL (Table V).

In contrast, MBC values ranged from 2500 µg/mL to 4000 µg/mL, with 2500 µg/mL being the lowest concentration resulting in complete bacterial death for certain strains (Table VI). This aligns with the findings of Rahman and Roy [46], who reported a MIC of 1000 µg/mL and an MBC of 3000 µg/mL for an ethanolic extract of *N. sativa* L. against *E. coli*. On *S. aureus*, our results showed that 1500 µg/mL inhibited growth (MIC), while 3500 µg/mL was required to achieve full bacterial eradication (MBC). With *S. persica* L. extract, variations in antimicrobial efficacy were observed. The MIC ranged between 1000 µg/mL and 2500 µg/mL, while MBC values spanned from 2500 µg/mL to 4500 µg/mL. The lowest inhibitory concentration was recorded against *Listeria monocytogenes* (ATCC 7644), with an MIC of 1000 µg/mL, and total bacterial elimination occurred at 2500 µg/mL, which was the lowest bactericidal concentration observed in this set of experiments.

These results highlight the significant inhibitory activity of both extracts against all tested pathogens, underscoring their potential as natural antimicrobial agents. Upon comparing the two extracts, it is evident that *N. sativa* L. demonstrates superior efficacy against *P. aeruginosa* and S. *aureus*, with generally lower MIC and MBC values than those *of Salvadora persica* L. Conversely, *S. persica* L. extract showed better performance against *E. coli* and L. *monocytogenes*, with correspondingly lower MIC and MBC values.

These differences may be attributed to the distinct chemical compositions and mechanisms of action of the two plant-derived extracts. Clinically, these findings are promising, suggesting that both extracts could serve as potential candidates for treating bacterial infections. Further studies should explore the underlying molecular mechanisms of their antimicrobial activities, which may aid in developing novel therapeutic agents against antibiotic-resistant pathogens.

Table V

MIC and MBC of *N. sativa* L. extract

Pathogens	Bacterial growth in MH broth (Extract concentration µg/mL)					MIC (µg/mL)	MBC (µg/mL)	Effect
	500	1000	1500	2000	2500			
P. aeruginosa	+	+	-	-	-	1500	3500	Bactericidal
E. coli (ATCC 35218)	+	-	-	-	-	1000	2500	Bactericidal
L. monocytogenes (ATCC7644)	+	+	-	-	-	1500	4000	Bactericidal
S. aureus (ATCC 29213)	+	+	+	-	-	2000	3000	Bactericidal

(+) indicates presence of growth and (-) means absence of growth.

Table VI

MIC and MBC of *S. persica* L. extract

Pathogens	Bacterial growth in MH broth (Extract concentration µg/mL)					MIC (µg/mL)	MBC (µg/mL)	Effect
	500	1000	1500	2000	2500			
P. aeruginosa	+	+	+	-	-	2000	4000	Bactericidal
E. coli (ATCC 35218)	+	+	-	-	-	1500	3000	Bactericidal
L. monocytogenes (ATCC 7644)	+	-	-	-	-	1000	2500	Bactericidal
S. aureus (ATCC 29213)	+	+	+	+	-	2500	4500	Bactericidal

(+) indicates presence of growth and (-) means absence of growth.

Conclusions

Plant extracts have long been considered as chemical molecules capable of limiting the establishment and advancement of multidrug-resistant pathogenic bacteria while remaining non-toxic to the body. The antioxidant test showed that both essential oils have a higher antioxidant power. The oils of *Nigella sativa* L. and *Salvadora persica* L. have been proven in this study to have a high antibacterial effect against multi-resistant strains that are widely known in the world population. Various chemical profiles were seen in the oils that were analysed: the essential oil of *N. sativa* is very rich in eicosanoic acid, n-hexadecanoic acid and p-cymene while the essential oil *S. persica* is richer in oleic acid, palmitic acid and b-sitosterol. Overall, these relevant results encourage in-depth studies on the factors that influence the biosynthesis and biological efficacy of essential oils, which have been widely used in the food and pharmaceutical fields. Further study and experimentation are also required to find formulations that take advantage of these essential oils' antibacterial qualities.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Diop SM, Gueye MT, Ndiaye EHB, Thiam A, Cissokho PS, Sanghare CH, Activités antioxydante et insecticide

d'huiles essentielles de *Mentha arvensis* L. du Sénégal. *Int J Biol Chem Sci*., 2021; 15: 966-975, (available in French).
2. Hosni H, Salama A, Abudunia A, Cherrah Y, Ibrahimi A, Alaoui K, Toxicité aiguë, cytotoxicité et effet antiradicalaire de l'extrait méthanolique des feuilles de l'asphodèle, *Asphodelus microcarpus*. *Phytothérapie*, 2020; 18: 284-290, (available in French).
3. Fatima EH, Akka FB, Dahmani J, Belahbib N, Zidane L, Étude ethnobotanique des plantes médicinales utilisées dans le traitement des infections du système respiratoire dans le plateau central marocain. *J Anim Plant Sci*., 2015; 25: 12, (available in French).
4. Boutkhil S, El Idrissi M, Amechrouq A, Chbicheb A, Chakir S, EL Badaoui K, Chemical composition and antimicrobial activity of crude, aqueous, ethanol extracts and essential oils of *Dysphania ambrosioides* (L.) Mosyakin & Clemants. *Acta Bot. Gall*., 2009; 156: 201-209.
5. Bouyahya A, Bakri Y, Et-Touys A, Talbaoui A, Khouchlaa A, Charfi S, Résistance aux antibiotiques et mécanismes d'action des huiles essentielles contre les bactéries. *Phytothérapie*, 2017, (available in French).
6. Cock IE, Selesho MI, van Vuuren SF, A review of the traditional use of southern African medicinal plants for the treatment of selected parasite infections affecting humans. *J Ethnopharmacol*., 2018; 220: 250-264.
7. Bouzidi LE, Larhsini M, Markouk M, Abbad A, Hassani L, Bekkouche K, Antioxidant and Antimicrobial Activities of *Withania frutescens*. *Nat Prod Commun*., 2011; 6: 1934578X1100601009.
8. Drioiche A, Amine S, Boutahiri S, Saidi S, Ailli A, Rhafouri R, Mahjoubi M, El Hilali F, Mouradi A, Eto B, Zair T, Antioxidant and Antimicrobial Activity of Essential Oils and Phenolic Extracts from the Aerial Parts of *Ruta montana* L. of the Middle Atlas Mountains-Morocco. *J Essent OilBear Plants*, 2020; 23: 902-917.
9. Frigui S, Messadi AA, Thabet L, Colonisation Et Infection À *Pseudomonas Æruginosa* Dans Un Service De Réanimation des Brûlés: Étude Sur 8 Ans. *Ann Burns Fire Disast.*, 2020; 33(4): 304, (available in French).
10. Ainane A, Mohamed Abdoul-Latif F, Jalludin M, Boujaber N, Oumaskour K, Benaziz M, Ainane T, Chemical Composition and Antimicrobial Activity of The Essential Oil of *Pistacia lentiscus* L. *PhOL-Pharmacol OnLine*, 2021; 2: 518-526.
11. Nicolaou KC, Rigol S, A brief history of antibiotics and select advances in their synthesis. *J Antib.*, 2018; 71(2): 153-184.
12. Regea G, Review on antibiotics resistance and its economic impacts. *J Pharmacol Clin Res*., 2018; 5: 555675.
13. Ghernaout D, Elboughdiri N, Antibiotics resistance in water mediums: background, facts, and trends. *Applied Engineering*., 2020; 4(1): 1-6.
14. Tariq S, Wani S, Rasool W, Shafi K, Bhat MA, Prabhakar A, Rather MA, A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microb Pathogen*, 2019; 134: 103580.
15. Datta AK, Saha A, Bhattacharya A, Mandal A, Paul R, Sengupta S, Black cumin (*Nigella sativa* L.) – A review. *J Plant Dev Sci*., 2012; 4: 1-43.
16. Ismail N, Ismail MA, Latiff L, Mazian M, Mariod AA, Black cumin seed (*Nigella Sativa* Linn.) oil and its fractions protect against beta amyloid peptide-induced toxicity in primary cerebellar granule neurons. *J Food Lipids*, 2008; 15: 519-533.
17. Kabir Y, Akasaka-Hashimoto Y, Kubota K, Komai M, Volatile compounds of black cumin (*Nigella sativa* L.) seeds cultivated in Bangladesh and India. *Heliyon*, 2020; 6: e05343.
18. Takruri HMH, Dameh MAF, Study of the nutritional value of black cumin seeds (*Nigella sativa* L.). *JSFA*., 1998; 76: 404-410.
19. Hossein H, Fazly BS, Maryam MH, Antibacterial activity of total extracts and essential oil of *Nigella Sativa* L. seeds in mice. *Pharmacolgyonline*, 2007; 2: 429-435.
20. Gál E, Banciu HL, Ionescu MI, Nastasă CM, Antibacterial synergistic and antagonistic effects of commercial essential oils from *Thymus vulgaris* L. and *Syzygium aromaticum* L. in combination with *Nigella sativa* L. *Farmacia*., 2023; 71(1): 97-108.
21. Ketenoglu O, Kiralan S, Kiralan M, Ozkan G, Ramadan M, Cold pressed black cumin (*Nigella Sativa* L.) seed oil. *Cold Pressed Oils*, 2020; 53-64.
22. Anand GT, Nithiyavathi R, Ramesh R, Sundaram SJ, Kaviyarasu K, Structural and optical properties of nickel oxide nanoparticles: Investigation of antimicrobial applications. *Surf Interfaces*, 2020; 18: 100460.
23. Sabouri Z, Akbari A, Hosseini HA, Hashemzadeh A, Darroudi, M, Eco-friendly biosynthesis of nickel oxide nanoparticles mediated by okra plant extract and investigation of their photocatalytic, magnetic, cytotoxicity, and antibacterial properties. *J Clust Sci*., 2019; 30: 1425-1434.
24. Rheima A, Anber AA, Shakir A, Salah HA, Hameed S, Novel method to synthesis nickel oxide nanoparticles for antibacterial activity. *Iran J Phys Res*., 2020; 20: 51-55.
25. Akhtar J, Siddique KM, Bi S, Mujeeb M, A review on phytochemical and pharmacological investigations of miswak (*Salvadora persica* Linn). *J Pharm Bioallied Sci*., 2011; 3: 113.
26. Ahmad H, Ahamed N, Dar JM, Mohammad UJ, Ethnobotany, pharmacology and chemistry of *Salvadora persica* L. A review. *Res Plant Biol*., 2012; 2: 2565.
27. Gupta A, Verma S, Kushwah P, Srivastava S, Rawat A, Phytochemical and antioxidant studies of *Salvadora persica* L. stem and twig. *Indian Pharm Educ Res*., 2015; 49: 71-75.
28. Iyer D, Patil U, Evaluation of antihyperlipidemic and antitumour activities of isolated coumarins from *Salvadora indica*. *Pharm Biol*., 2014; 52(1): 78-85.
29. Sofrata A, Lingstrom P, Baljoon M, Gustafsson A, The effect of miswak extract on plaque pH: An *in vivo* study. *Caries Res*., 2007; 41(6): 451-454.
30. Noumi E, Snoussi M, Trabelsi N, Hajlaoui H, Ksouri R, Valentin E, Bakhrouf A, Antibacterial, anticandidal and antioxidant activities of *Salvadora persica* and *Juglans regia* L. extracts. *J Med Plants Res*., 2011; 5(17): 4138-4146.

31. Sudhir SP, Deshmukh P, Verna HN, Comparative study of antimicrobial effect of *Nigella Sativa* L. seed extracts from differents geographies. *IJ Pharmacog.*, 2016; 3(6): 257-264.
32. Aouji M, Rkhaila A, Bouhaddioui B, Zirari M, Harifi H, Taboz Y. Lrhorfi LA, Bengueddour R, Chemical composition, mineral profile, anti-bacterial and wound healing properties of snail slime of *Helix aspersa* Müller. *Biomedicine*, 2023; 13(4): 9-19.
33. Aouji M, Imtara H, Rkhaila A, Bouhaddioui B, Alahdab A, Parvez MK, Alzahrani MS, Lrhorfi LA, Bengueddour R, Nutritional Composition, Fatty Acids Profile, Mineral Content, Antioxidant Activity and Acute Toxicity of the Flesh of *Helix aspersa* Müller. *Molecules*, 2023; 28(17): 6323.
34. Aouji M, Zirari M, Imtara H, Rkhaila A, Bouhaddioui B, Mothana RA, Noman OM, Tarayrah M, Bengueddou, Exploring the Chemical Composition, Antioxidant, and Antibacterial Properties of *Helix aspersa* Müller Flesh Crude Extract: A Comprehensive Investigation. *ACS Omega*., 2024; 9(32): 34754-34764.
35. Alrashidi M, Derawi D, Salimon J, Yusoff MF, An investigation of physicochemical properties of *Nigella sativa* L. Seed oil from Al-Qassim by different extraction methods. *J King Saud University - Sci*., 2020; 32(8): 3337-3342.
36. Khalil M, El-Sabbagh M, El Naggar E, El-Erian R, Antibacterial activity of *Salvadora persica* against oral pathogenic bacterial isolates. *NJCP*., 2019; 22(10): 1378-1378.
37. Ramadan K, Alshamrani S, Phytochemical analysis and antioxidant activity of *Salvadora persica* extracts. *JBAR.*, 2016; 2(3): 390-395.
38. Mao LC, Pan X, Que F, Fang XH, Antioxidant properties of water and ethanol extracts from hot air-dried and freeze-dried daylily flowers. *Eur Food Res Technol*., 2006; 222: 236-241.
39. Ruberto G, Baratta MT, Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem*., 2000; 69: 167-174.
40. Laghmouchi Y, Belmehdi O, Senhaji NS, Abrini J, Chemical composition and antibacterial activity of *Origanum compactum* Benth. essential oils from different areas at northern Morocco. *S Afr J Bot.*, 2018; 115: 120-125.
41. Abbaci H, Nabti E-H, Al-Bekairi AM, Hagras SAA, Salem-Bekhit MM, Adjaoud A, Alzahrani HA, Houali K, Comparative Bioactivity Evaluation of Chemically Characterised Essential Oils Obtained from Different Aerial Parts of *Eucalyptus gunnii* Hook. f. (*Myrtaceae*). *Molecules*, 2023; 28(6): 2638.
42. Shimada K, Fujikawa K, Yahara K, Nakamura T, Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem*., 1992; 40: 945-948.
43. Haloci E, Manfredini S, Toska V, Vertuani S, Ziosi P, Topi I, Kolani H, Antibacterial and antifungal activity assesment of *Nigella sativa* essential oils. *Int J Pharm Pharm Sci*., 2013; 6(6): 270-272.
44. Bessedik A, Allem R, Molecular composition and antibacterial effects of essential oil of *Nigella Sativa* L. *AJB.*, 2013; 12(20): 3006-3012
45. Darout IA, Skaug N, Ali RW, Albandar JM, Subgingival microbiota levels and their associations with periodontal status at the sampled sites in an adult Sudanese population using miswak or toothbrush regularly. *Acta Odontol Scand.*, 2003; 61: 115-122.
46. Rahman MS, Roy T, Antibacterial and Antibiofilm Activities of *Nigella sativa* L. Seed Extracts. *BJM*., 2021; 38(1): 7-13.