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https://doi.org/10.31925/farmacia.2026.2.5 **ORIGINAL ARTICLE**

COMPOSITION, ANTIOXIDANT PROPERTIES AND ANTIBACTERIAL PRESERVATION POTENTIAL OF ALGERIAN *MYRTUS NIVELLEI* ESSENTIAL OIL

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Abstract

This study investigated the chemical composition and biological properties of essential oil obtained from Algerian *Myrtus nivellei* leaves (MNEO) and evaluated its potential as a natural preservative in cosmetic formulations. The essential oil was obtained by hydrodistillation and analysed using gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). A total of 33 compounds were identified, representing 96.12% of the oil composition, with 1,8-cineole as the major component (61.9%). Antioxidant activity was assessed using DPPH radical scavenging and reducing power assays, with IC_{50} and EC_{50} values of 3.04 mg/mL and 3.08 mg/mL, respectively. Antibacterial activity was evaluated against common cosmetic contaminants and preservative efficacy was further examined using a shampoo model over 28 days. The results suggest that MNEO may represent a promising natural ingredient for cosmetic preservation.

Rezumat

Studiul a avut ca obiectiv investigarea compoziției chimice și a proprietăților biologice ale uleiului esențial obținut din frunzele speciei *Myrtus nivellei* provenite din Algeria (MNEO), precum și evaluarea potențialului acestuia de utilizare ca și conservant natural în produsele cosmetice. Uleiul esențial a fost obținut prin hidrodistilare, iar compoziția sa a fost analizată prin cromatografie de gaze (GC) și cromatografie gazoasă cuplată cu spectrometrie de masă (GC/MS). Au fost identificați 33 de compuși, reprezentând 96,12% din compoziția totală, 1,8-cineolul fiind componentul majoritar (61,9%). Activitatea antioxidantă a fost evaluată folosind testul DPPH și metoda puterii reducătoare, valorile IC_{50} și EC_{50} fiind de 3,04 mg/mL, respectiv 3,08 mg/mL. Pentru evaluarea activității antibacteriene au fost utilizate tulpini frecvent implicate în contaminarea produselor cosmetice. Eficacitatea conservantă a fost evaluată într-un model experimental de șampon pe o perioadă de 28 de zile. Rezultatele arată că MNEO prezintă proprietăți antioxidante și antibacteriene, ceea ce sugerează că poate fi utilizat ca ingredient natural în formulări cosmetice.

Keywords: *Myrtus nivellei*, essential oil, antioxidant, antibacterial, cosmetic preservative

Introduction

According to existing cosmetic regulations, customers are at danger if hazardous cosmetics contain ingredients that are restricted or forbidden. Additionally, consumers run the danger of health problems due to the contamination of cosmetic products (1). The presence of microbes or exposure to ambient oxygen can alter cosmetic goods. Two classes of chemicals are frequently employed to lessen these effects: antioxidant preservatives, which stop oxidation and the production of free radicals, and antimicrobial preservatives, which target microorganisms (2). According to regulations, a preservative is any natural or artificial ingredient used to stop the growth of microorganisms (3). The effectiveness of a preservative should encompass a broad spectrum of activity and exceed the expected shelf-life and usage time of the cosmetic product (4). Additionally, the antimicrobial activity should be potent enough to prevent microbial adaptation and resistance to the preservative system (5).

In Algeria, there are two species of *Myrtus*: *Myrtus communis* (common myrtle) and *Myrtus nivellei* (Sahara myrtle) (6). *Myrtus nivellei* is an endemic species found in the Sahara, mainly in the central region and sparsely in the northern region (7). It has two sub-

species, one in Algeria and one in Chad. *M. nivellei* grows in scattered populations in rocky and sandy areas with underground water sources, usually above 1400 m in altitude (8). It is a shrub with rough bark, lanceolate leaves, white petals and black berries as fruits (9, 10).

This species is a fragrant perennial plant that grows along the riverbeds of North Africa's deserts. It is also referred to as "rayhan Sahrawi" or "Tiflfest" in traditional medicine from Algeria. The infusion of its leaves is commonly consumed in this region as a beverage, replacing green tea (11). It is also used to treat diarrhoea, fever, diabetes and combat gonorrhoea (12, 13). Additionally, the decoction of the leaves is used by Algerian nomads in the Tassili region to address liver disorders (11). Crushed leaves, mixed with oil or butter, are utilized as a cream for the treatment of dermatosis and for hair and body care (12, 14, 15). Fresh or dried berries are consumed to alleviate mouth canker sores (15).

This study aimed to investigate the possible application of *M. nivellei* leaf essential oil as a natural preservative in cosmetic products. The study concentrated on the chemical compound of the essential oil, assessing its efficacy against three bacterial strains that are frequently present as contaminants in cosmetic products, as well as its antioxidant and antibacterial qualities.

Materials and Methods

Collection of plant material

M. nivellei leaves were collected in October 2017 from Tassili n'Ajjer, a large plateau in southeast Algeria located at 25°30'0" N and 9°0'0" E. The plant material was botanically identified by Dr. Noureddine Halla, and a voucher specimen bearing the code LBPVBP-MN2017 was added to the Herbarium of the Department of Biology, University of Saida's Laboratory of Biotoxicology and Biological Valorisation of Plants. Following their collection, the plant samples were preserved for later use by drying them in the shade.

Essential oil extraction

Three *M. nivellei* plant samples, each weighing 100 g, were hydro-distilled in 300 mL of distilled water for a period of three hours. The Clevenger-type apparatus was used to carry out this process. The oil that was obtained was dried with anhydrous sodium sulphate and kept in sealed glass vials at 4°C to 5°C until further analysis is conducted.

GC and GC/MS analyses

For gas chromatography-flame ionization detection (GC-FID) analysis, an Agilent 6890 plus instrument equipped with a nonpolar separation column (HP-5 MS, 30 m × 0.25 mm × 0.25 µm film thickness) was employed. The column temperature program initiated at 60°C for 8 minutes, increased at a rate of 2°C *per* minute to 250°C and was maintained at 250°C for 10 minutes. At 250°C, a volume of 0.2 µL was injected into the GC inlet. Helium served as the carrier gas with a flow rate of 0.5 mL/min. The flame ionization detector (FID) temperature was set at 280°C. In addition, MNEO was evaluated by GC/MS with an Agilent 5973 instrument equipped with a silica-capillary column HP5MS (30 m x 0.25 mm x 0.25 m film thickness). The GC-MS conditions included Helium as the carrier gas with a flow rate of 0.5 mL/min, a split mode of 1:20, injection of 0.2 µL at an inlet temperature of 250°C. After starting at 60°C for 8 minutes, the oven temperature program raised it by 2°C *per* minute to 250°C, and then held it at 250°C for 10 minutes. The scan range of 30 - 550 atomic mass units was covered by electronic impact ionization at 70 eV.

Identification of components

The proportions of the constituent components in the extracted oil were quantified as a percentage of the peak area compared to the total peak area. The components were identified by comparing their relative retention times (Rt) and mass spectra with data from NIST and WILLY library, as well as literature, using GC/MS equipment (16). The components of the extracted oil were also identified from their retention indices as described by Van den Dool and Kratz (17) relative to n-alkanes (C_8-C_{29}).

Antioxidant activity

DPPH radical scavenging activity. Different concentrations of the MNEO sample, totalling fifty microliters, were combined with 1950 µL of a methanolic solution containing DPPH at a concentration of 6.34×10^{-5} M (18). A negative control (blank) was prepared by mixing 50 µL of methanol with 1950 µL of the DPPH methanolic solution. After incubation in the dark for 30 minutes at room temperature, the reduction of DPPH was indicated by a colour change in the solution from violet to yellow. The absorbance of the solution was then measured at 515 nm using a spectrophotometer. Ascorbic acid was used as a positive control (19). The following formula was used to determine percent inhibition (PI), which was based on the colour intensity reduction, and was used to express the results:

$$PI = ((OD_{control} - OD_{essential\ oil})/OD_{control}) \times 100.$$

In the given equation, "$OD_{control}$" represents the optical density or absorption of the negative control, whereas "$OD_{essential\ oil}$" represents the absorbance associated with the essential oil.

Reducing power assay. MNEO (1 mL) at varying concentrations was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution (20). For twenty minutes, the resultant mixture was incubated at 50°C. To halt the reaction, 2.5 mL of 10% trichloroacetic acid was

applied after incubation. After that, the mixture was centrifuged for 10 minutes at room temperature at 650× g. The resulting supernatant was then mixed with 0.5 mL of 0.1% (w/v) iron chloride and 2.5 mL of distilled water. The absorbance of this solution at 700 nm was compared to that of a blank solution. The activity of MNEO was assessed by contrasting it with butylhydroxyanisole (BHA), the positive control.

Analysing the antibacterial capacity

The antibacterial efficacy of MNEO was evaluated against three strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25933 and *Staphylococcus aureus* ATCC 25923, known contaminants in cosmetics (21). Methods sanctioned by the National Committee for Clinical Laboratory Standards were used to determine the minimum inhibitory concentration (MIC) for MNEO, with some minor adjustments (22). The initial inoculum was established at 106 colony forming units (CFU)/mL. After 16 - 20 hours of incubation, the MIC for each sample was determined after incubating in a humid, dark environment at 37°C. In comparison to control wells without MNEO, which did not contain MNEO, the end point was defined as the lowest concentration of the antibacterial agent that completely inhibited visible growth. Furthermore, the minimum bacterial concentration (MBC) for MNEO was determined. After MIC determination, Boekel Scientific, Feasterville, PA, USA used a 96-pin replicator to draw 50 µL samples from each microliter tray well and placed them on nutrient agar plates. Before assessing the MBC, which is the lowest MNEO concentration that completely inhibits visible growth (23, 24), the plates had to be incubated at 37°C for 24 hours.

Evaluating the efficacy of antibacterial characteristics in a cosmetic product using a "challenge test"

The antibacterial efficacy test adhered to the requirements set by the European Pharmacopoeia (21). Briefly, a "standard" shampoo was initially formulated by combining five fundamental components: sodium lauryl sulphate (15%), cocamidopropyl betaine (1%), glycerine cocamide diethanolamine (3.5%), ethylenediamine tetra acetic acid (0.2%), sodium chloride (1%) and water (q.s. 100%). Subsequently, three variants of shampoo were formulated: one devoid of preservatives, one containing a synthetic preservative (formaldehyde), and one incorporating MNEO in accordance with the previously determined MIC. An assessment was conducted to determine the antibacterial effectiveness of several shampoos against *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25933 and *Staphylococcus aureus* ATCC 25923. Bacteria were harvested in physiological solution and the inoculum was adjusted to 10^8 cells/mL. Series of containers have been inoculated of the product to be examined, each with a suspension of one of the test organisms to give an inoculum of 10^5 to 10^6 microorganisms *per* millilitre of the preparation. After, we maintained the inoculated product at 20 - 25°C, protected from light. Samples were collected at various time intervals (0, 2, 7, 14, 21 and 28 days post-inoculation) and the quantity of living cells was ascertained using plate enumeration employing a designated inactivating agent. The antibacterial preservative efficacy was evaluated and compared with the logarithmic reduction criteria established by the European Pharmacopoeia for preservative efficacy testing. Criterion A represents the recommended level of antimicrobial efficacy, while Criterion B may be accepted in justified cases when Criterion A cannot be achieved due to safety considerations related to potential adverse reactions.

Results and Discussion

GC/MS analysis

The EOMN average yield was ranged from 0.8 ± 0.14% (w/w). This value was identical to that obtained by Bouzabata *et al*. (12) (0.5 to 0.9%) and in a previous study by Ramdane *et al*. (25) (0.75%). The findings from the GC/MS analysis of MNEO are succinctly presented in Table I.

Table I

Chemical composition of the essential oil extracted from *M. nivellei* leaves

Nb	Rt	Compound	Formula	RI[a]	RI[b]	%
1	9.161	α-Pinene	$C_{10}H_{16}$	933	936	3.70
2	10.110	Camphene	$C_{10}H_{16}$	949	953	0.31
3	12.733	Myrcene	C_9H_{16}	993	991	0.19
4	13.893	Phellandrene	$C_{10}H_{16}$	1010	1005	0.23
5	16.580	1,8-Cineole	$C_{10}H_{18}O$	1032	1031	61.90
6	17.563	4-Thujanol	$C_{10}H_{18}O$	1062	1067	0.11
7	18.592	Terpinene	$C_{10}H_{16}$	1065	1062	0.21
8	19.357	α-Terpinolene	$C_{10}H_{16}$	1087	1087	0.12
9	19.918	p-Cymenene	$C_{10}H_{12}$	1095	1089	0.13
10	20.758	Linalool	$C_{10}H_{18}O$	1106	1098	0.32
11	26.467	Terpinene-4-ol	$C_{10}H_{18}O$	1185	1180	0.54
12	27.965	α-Terpineol	$C_{10}H_{16}$	1206	1198	6.20
13	31.108	Piperitone	$C_{10}H_{16}O$	1251	1251	0.09

Nb	Rt	Compound	Formula	RI[a]	RI[b]	%
14	31.783	Geraniol	$C_{10}H_{18}O$	1255	1255	0.20
15	31.914	Linalyl acetate	$C_{12}H_{20}O_2$	1262	1259	0.34
16	34.412	α-Terpinen-7-al	$C_{10}H_{14}O$	1286	1282	0.11
17	37.361	2-Acetoxy-1,8-cineole	$C_{12}H_{20}O_3$	1343	1345	0.64
18	38.110	α-Terpinylacetate	$C_{12}H_{20}O_2$	1354	1350	8.10
19	38.721	Neryl acetate	$C_{12}H_{20}O_2$	1363	1365	0.20
20	40.219	Gerany lacetate	$C_{12}H_{20}O_2$	1386	1383	2.90
21	40.539	9-Octadecene, 1,1-dimethoxy-, (Z)-	$C_{20}H_{40}O_2$	1391	-	0.13
22	41.894	Phenol, 3-ethoxy-	$C_8H_{10}O_2$	1412	-	0.11
23	43.317	2-Pentanone, 4-cyclohexylidene-3,3-diethyl-	$C_{15}H_{26}O$	1435	-	0.25
24	44.997	Benzofuran, 2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-2-(1-methylethenyl)-, cis-	$C_{11}H_{16}O_2$	1462	-	0.33
25	47.632	2H-Pyran-5-carboxylic acid, 2-oxo-	$C_6H_4O_4$	1495	-	0.13
26	48.118	2,4-Hexadiene, 3,4-dimethyl-, (E,Z)-	C_8H_{14}	1512	-	0.43
27	48.958	1-Methylcycloheptene	C_8H_{14}	1526	-	0.25
28	49.495	2,6-Octadiene, 2,4-dimethyl-	$C_{10}H_{18}$	1536	-	0.25
29	50.290	Alantolactone, 4alpha,4Aalpha-epoxy-	$C_{15}H_{20}O_3$	1549	-	0.08
30	50.644	Tricyclo[4.2.1.1(2,5)]decane	$C_{10}H_{16}$	1555	-	0.07
31	50.804	Presilphiperfolan-9-.beta.-ol	$C_{15}H_{26}O$	1558	-	0.15
32	51.450	Propanoicacid, 2-oxo-, ethyl ester	$C_5H_8O_3$	1569	-	1.50
33	53.147	Cyclohexen, 1,5,5-trimethyl-6-acetomethyl-	$C_{12}H_{20}O$	1597	-	5.90
		Total				96.12%
Monoterpene hydrocarbons: 11.41% (No. 1-4, 7-9, 12, 28, 30) Oxygenated monoterpenes: 75.45% (No. 5-6, 10-11, 13-20) Oxygenated sesquiterpenes: 0.48% (No. 23, 29, 31) Others: 8.78% (No. 21-22, 24-27, 32, 33)						

Rt = Retention time; RI[a] = Retention index relative to C_8-C_{29} n-alkanes determined following sample passage through an HP5MS nonpolar column; RI[b] = Comparison the retention index with the literature's documentation [16,17]; "-" = RI[b] not available

A total of thirty-three constituents, accounting for 96.12% of the entire essential oil composition, were successfully identified. The predominant group of components was oxygen-containing monoterpenes, accounting for 75.45% of the total. Monoterpene hydrocarbons comprised 11.41%, while oxygenated sesquiterpenes made up 0.48%. 1,8-cineole was identified as the main components of MNEO, accounting for 61.90 % of the total composition. The predominance of 1,8-Cineole in essential oils of *M. nivellei* was reported in studies carried out such as Bouzabata *et al*. (12) (33.6-50.4%) and Ramdane *et al*. (25) (53,44%).

Multiple investigations have verified that 1,8-cineole is the primary constituent found in different members of the *Myrtaceae* family (26). This chemical has exhibited notable biological properties, including antibacterial activity against (27), antifungal activity against (28), anti-inflammatory effects against (29) and antioxidant effects against (30).

Antioxidant activity

MNEO was assessed for its antioxidant power *via* two conventional strategies: radical scavenging activity with DPPH and reducing power assay. The ability of MNEO to scavenge DPPH radicals was compared to that of the positive control (ascorbic acid), and the concentrations required for inhibiting DPPH radicals are illustrated in Figure 1.

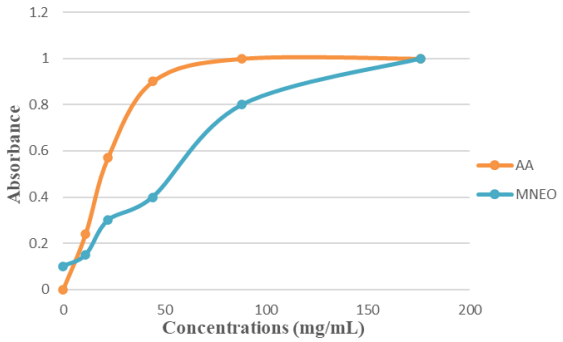

Figure 1.
Percentage of inhibition of the free radical DPPH as a function of different concentrations of ascorbic acid (AA) and essential oil of *M. nivellei* (MNEO) leaves

The measurement of MNEO's antiradical activity was done by determining the IC_{50}, which is the concentration that causes 50% inhibition. A lower IC_{50} value indicates higher efficiency, with the MNEO exhibiting an IC_{50} value of 3.04 mg/mL. This aligns with the findings of Ramdane *et al*. (25), who reported a value of 2.25 mg/mL. However, the standard antioxidant ascorbic acid (AA) showed a significantly lower IC_{50} value (0.01 mg/mL), confirming its much stronger radical scavenging capacity compared to the essential oil. In the reducing power assays, an elevation in absorbance signifies an increase in the reducing power of the essential oil under examination. In order to assess MNEO's anti-

oxidant activity, the EC_{50} was calculated by determining the concentration of the essential oil where the absorbance was 0.5 (24). As shown in Figure 2, the EC_{50} value was 3.08 mg/mL.

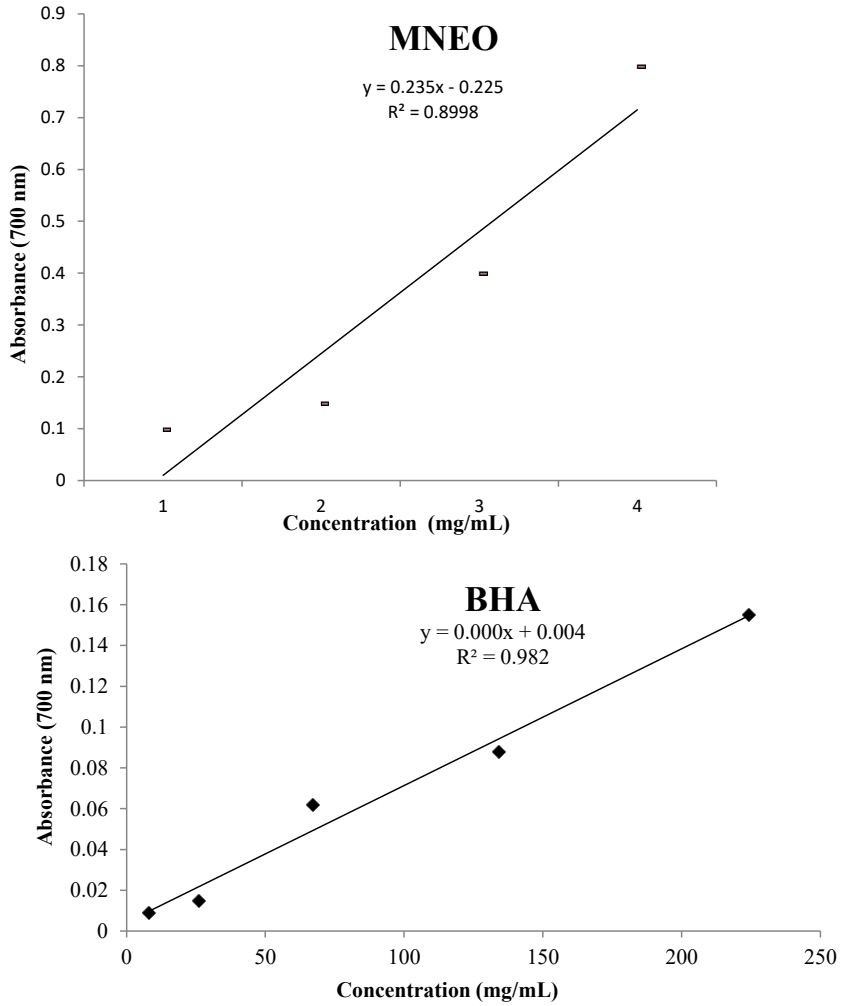

Figure 2.
Reducing power of the different concentrations of BHA and essential oil of *M. nivellei* (MNEO) leaves

Analysing the antibacterial capacity

Table II outlines the antibacterial activity of MNEO, characterized by MIC and MBC values. Among the tested bacteria, *S. aureus* displayed the highest sensitivity, with an MIC of 1.5 mg/mL, while *P. aeruginosa* exhibited the highest resistance, with an MIC of 9.5 mg/mL. The MIC for *E. coli* was approximately 3.75 mg/mL. These findings suggest moderate antibacterial efficacy and should be regarded as initial antimicrobial screening evidence.

It's noteworthy that Gram-positive bacteria tend to be more sensitive compared to Gram-negative bacteria, attributable to the simpler architectural structure of their cell wall. This structural distinction potentially renders Gram-positive bacteria more vulnerable to the penetration and activity of essential oils, which consist of nonpolar molecules, in comparison to Gram-negative bacteria (31).

Table II
MIC and MBC in mg/mL for the essential oil extracted from *M. nivellei* leaves

Verified strains	MIC[a]	MBC[b]
Pseudomonas aeruginosa ATCC 27853	9,5	19
Escherichia coli ATCC 25933	3.75	3.75
Staphylococcus aureus ATCC 25923	1,5	1,5

MIC[a] = Minimum Inhibitory Concentrations; MBC[b] = Minimum Bactericidal Concentrations

The primary component of MNEO, 1,8-cineole, is probably what gives it its antibacterial action against all strains tested. In fact, 1,8-cineole has shown anti-quorum sensing (QS) and broad-spectrum antibacterial activity against a variety of pathogenic bacteria. By rupturing the integrity of the bacterial cell

membrane (32, 33), 1,8-cineole has been shown in studies to be effective against methicillin-resistant strains of *S. aureus* (34). Furthermore, by inhibiting the expression of the corresponding gene in *E. coli* (35), this chemical can influence the formation of bacterial biofilms and their pathogenicity. Furthermore, it has been documented that 1,8-cineole prevents *P. aeruginosa* from growing and interferes with the development of bacterial biofilms (36). However, it is crucial to recognize that additional small molecules can potentially be involved in the inhibitory effect (37, 38).

Antibacterial Performance in the Cosmetic "Challenge Test"

Figure 3 demonstrates the inhibition of *P. aeruginosa*, *E. coli* and *S. aureus* bacteria after being exposed to MNEO at the minimum inhibitory concentration (MIC) for a period of 28 days in a simulated shampoo environment.

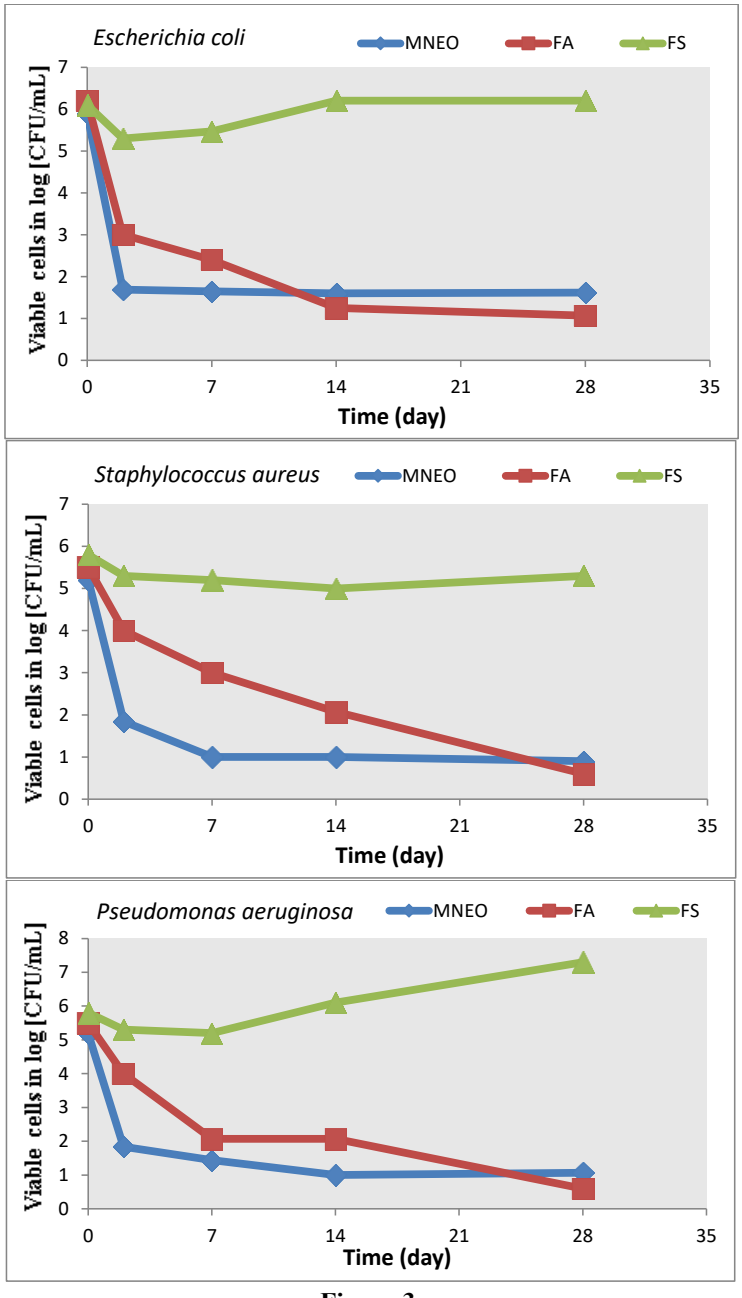

Figure 3.

Growth inhibition of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in formulation shampoo (FS) alone or in SF containing *M. nivellei* essential oil (MNEO) or formaldehyde (FA)

Observations after 28 days showed a decrease in log10 values of 5.13 (*E. coli*), 5.6 (*P. aeruginosa*) and 4.9 (*S. aureus*), indicating that formaldehyde effectively controlled all examined pathogens. In contrast, the shampoo free of formaldehyde and MNEO had comparable antibacterial action, with

log_{10} decreases of roughly 0.5 (*S. aureus*), 0.6 (*P. aeruginosa*) and 0.9 (*E. coli*) after just two days of incubation. Nevertheless, on the 14th day of incubation, bacterial proliferation had resumed. The studied bacteria's apparent sensitivity may have resulted from a combination of intrinsic features and the shampoo's formulation, which decreased the bacteria's survivability. The considerable drop shown in the control groups may be caused by elements in the shampoo formulation, such as anaerobic conditions, the presence of EDTA and an acidic pH. These conditions can enhance the permeability of microbial cell membranes, rendering them more susceptible to antimicrobial agents like essential oils (19, 39, 40).

Conclusions

The findings of this study unequivocally demonstrate that MNEO's exceptional antioxidant activity is primarily due to its high concentration of 1,8-cineole (61.9%). Additionally, the challenge test conducted using a shampoo formulation containing MNEO demonstrated a consistent inhibitory effect on all three bacterial strains tested, underscoring their heightened susceptibility to MNEO. These encouraging findings suggest that MNEO could represent a potential natural preservative in shampoo formulations, providing a promising alternative for maintaining product quality and limiting bacterial growth. However, further studies, including stability and safety evaluations, are required to confirm its suitability for cosmetic applications.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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