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Characterization of menthe longifolia: Significant antimicrobial and antioxidant effects of ethanolic and hexane extracts

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Abstract: *Mentha longifolia*, commonly known as wild mint, has been traditionally used for its medicinal properties. This study investigates the antimicrobial properties of hexane and ethanolic extracts from *M. longifolia* against selected bacterial and fungal strains. Various methods were employed to assess the antimicrobial activity, including agar disc diffusion and broth microdilution assays, while biofilm inhibition and destruction were tested using the crystal violet assay. The antioxidant activity was evaluated using the catalase enzyme assay. Additionally, phytochemical analysis was carried out to screen for possible bioactive components of *M. longifolia* extracts. Both ethanolic and hexane extracts demonstrated significant antibacterial and antifungal activities against all tested strains. Notably, both extracts exhibited a strong dose-dependent inhibition of biofilm formation and disruption of pre-formed biofilms. Moreover, the extracts effectively reduced catalase activity in bacterial cells, leading to oxidative stress-mediated toxicity. These effects are attributable to the presence of key bioactive compounds—phenols, flavonoids, terpenoids, quinones, tannins, and saponins—identified through phytochemical screening. Overall, the hexane and ethanolic extracts of *M. longifolia* demonstrate promising antimicrobial properties, suggesting their potential as natural alternatives for treating bacterial and fungal infections.

Keywords: *Antibacterial, Antifungal, Antioxydant, Ethanolic extract, Hexane extract, Mint.*

1. Introduction

Antimicrobial resistance is a major global health challenge, caused by the overuse and misuse of antibiotics and poor infection control [1]. Mechanisms such as efflux pumps, enzymatic degradation, and horizontal gene transfer allow bacteria to evade conventional treatments [2].

Medicinal plants are increasingly recognized for their therapeutic potential, offering a cost-effective alternative with minimal side effects [3]. *Mentha longifolia*—commonly referred to as horse mint or wild mint—is a perennial herb native to Europe, Asia, and Africa. It is widely known in traditional medicine for its antiseptic, anti-inflammatory, and analgesic properties. The plant's diverse phytochemical profile, which includes essential oils, flavonoids, phenolic acids, and terpenoids, contributes to its therapeutic effects [4]. *M. longifolia* extracts and EOs were active against various bacteria [5, 6] and fungi [7].

A. baumannii, a multidrug-resistant (MDR) pathogen, is commonly associated with hospital-acquired infections, particularly in intensive care units [8]. It is one of a significant concern due to its high ability to develop resistance to a broad array of antibiotics. Additionally, *A. baumannii's* capacity to form biofilms on both living and non-living surfaces contributes to persistent infections and increased resistance to treatment [9]. This has made *A. baumannii* one of the ESKAPE pathogens that are notorious for their ability to evade the effects of most conventional antibiotics. These organisms are a major public health issue because of their robust mechanisms of antibiotic resistance, making infections caused by them exceptionally difficult to treat [10].

To tackle the growing issue of antimicrobial resistance, this study aims to assess the antibacterial and antifungal activities of *M. longifolia* ethanolic and hexane extracts against a range of microbial

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strains. Additionally, this study seeks to examine the impact of these extracts on biofilm inhibition and destruction. Furthermore, it aims to identify the phytochemicals responsible for the observed antimicrobial effects and explore their possible mode of action.

The findings highlight the potential of plant extracts as alternatives to traditional antibiotics and antifungals, which are losing their effect due to resistance. They also emphasize the effectiveness of plant extracts in combating biofilm formation, a key factor in bacterial virulence and persistence.

2. Methods

2.1. Test Microorganisms

The bacterial and fungal strains used in this study included *Acinetobacter baumannii*, *Micrococcus luteus*, *Stenotrophomonas maltophilia*, *Aspergillus nidulans*, and *Aspergillus flavus*. All strains were obtained from Hammoud Hospital, Saida. The bacterial strains were subsequently sub-cultured into fresh nutrient agar (NA) plates, and incubated at 37°C for 24 hours and maintained at 4°C. The fungal strains were also sub-cultured into Sabouraud dextrose agar (SDA) and incubated for 96 hours at 30 °C. Glycerol stock cultures were also prepared and stored at -80 °C to preserve for further use.

2.2. Plant Material Collection and Extraction

Fresh leaves of *M. longifolia* were collected from a local source. The collected leaves were thoroughly washed with distilled water and air-dried in the shade for one week. The dried leaves were subsequently processed into a fine powder using a blender. Approximately 100 g of the plant powder was soaked separately in hexane and 80% ethanol in a reagent bottle at a ratio of 1:10 (w/v) for one week with occasional stirring. The extracts were then centrifuged and filtered using Whatman filter paper No. 1. Finally, the extracts were oven-dried at 50°C to yield a crude extract that was stored at -20°C in Eppendorf tubes until further analysis.

2.3. Antimicrobial Susceptibility Testing

2.3.1. Disc Diffusion Method

The antimicrobial activity of the ethanolic and hexane mint extract was evaluated using the disc diffusion method as described by Bauer, et al. [11]. Sterile paper discs (6 mm in diameter) were impregnated with 50 µL of the extract at different concentrations (25, 50, and 100 mg/mL) and placed on MHA plates for bacteria and SDA plates for fungi and inoculated with 100 µL of 0.5 McFarland of the test microorganisms. The plates were incubated at 37°C for 24 hours for bacteria and at 30°C for 4 days for fungi. The diameter of the inhibition zones was measured in millimeters, and the results were compared with doxycycline and fluconazole discs that served as positive controls for bacteria and fungi, respectively. Discs with 10% DMSO served as negative controls, while 80% ethanol and hexane discs were added as well as solvent controls.

2.3.2. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the ethanolic and hexane mint extracts was determined using the broth microdilution method as described by Wiegand, et al. [12]. The extracts were serially diluted in a 96-well microtiter plate using 10% DMSO. In each well, 10 µL of the microbial suspension standardized at 0.5 McFarland was combined with 90 µL of NB for bacteria and SDB for fungi, making up a total volume of 200 µL. Blank, positive, and negative control wells were added. The plates were incubated at 37°C for 24 hours for bacteria and at 30°C for 4 days for fungi, after which the optical density (OD) was measured using an ELISA plate reader. The MIC was determined as the lowest extract concentration that visibly inhibited the growth of the microorganisms.

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2.3.3. Determination of the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC were determined by taking 10 µL of microbial suspension from wells that didn't show any visible growth in the MIC assay, and then subculturing it on NA plates and incubating it at 37°C for 24 hours for bacteria, and on SDA plates and incubating it at 30°C for 4 days for fungi. The MBC and MFC were defined as the lowest concentration of the extract that resulted in no growth on the agar plates [13].

2.3.4. Antibiofilm Crystal Violet Assay

The antibiofilm activity was evaluated following the method described by Famuyide, et al. [14]. The bacterial strains used for this assay were grown overnight in NB at 37°C. The cultures were adjusted to an OD of 0.1 at 600 nm, corresponding to approximately 1 × 10^8 CFU/mL.

For the biofilm inhibition assay, 10 µL of the adjusted bacterial suspension was added to each well of a sterile 96-well polystyrene microtiter plate, along with 90 µL NB. Positive and negative control wells were added. The plates were incubated at 37°C for 3 hours to allow initial biofilm formation. After this period, 100 µL of the serially diluted extracts were added to the wells containing the bacterial suspension. The plates were then incubated for an additional 24 hours at 37°C to assess the inhibitory effect of the extract on biofilm development. After 24 hours, the wells were gently washed five times with autoclaved distilled water to remove unattached cells. Then, the plates were allowed to dry in the oven at 60°C for 20 minutes. The biofilms were then stained with 100 µL of 1% crystal violet solution for 15 minutes at room temperature. Excess crystal violet was removed by washing the wells five times with autoclaved distilled water. Then, the plates were left to air-dry for 10 minutes. The stain was solubilized with 125 µL of 95% ethanol, and the absorbance was measured at 595 nm using a microplate reader.

For the biofilm destruction assay, pre-formed biofilms were used. After the initial incubation, the extracts were added to each well, and the plates were incubated again at 37°C for another 24 hours to evaluate the extract's effect in breaking down the established biofilms. After this second incubation, the wells were washed, stained with crystal violet, and the remaining biofilms were measured following the same method used for the inhibition assay.

2.3.5. Catalase Assay

The enzymatic activity of catalase (CAT) was measured to assess the oxidative stress response triggered by the extract, following the protocol outlined by Chen and Zhang [15]. MIC of the extracts was added to each tube to achieve a final volume of 1500 µL. The cultures were incubated, and samples were collected at different time points (1, 6, 18, and 24 hours) and centrifuged at 13,000×g for 5 minutes at 4°C. The pellets were re-suspended in a lysis buffer. 50 µL of tissue homogenate was added to a quartz cuvette. The cuvette was then placed in a holder, and 1 mL of the reaction solution was added. The reduction in absorbance was recorded every 15 seconds for 1 minute at 240 nm. A control blank was prepared using 1 mL of the reaction solution with 50 µL of 10 mM PBS buffer. The CAT activity was calculated using the activity formula.

2.3.6. Qualitative Phytochemical Analysis

The ethanolic and hexane extracts of *M. longifolia* were subjected to qualitative phytochemical screening for the presence of various bioactive compounds, including phenols, alkaloids, steroids, anthraquinones, flavonoids, saponins, tannins, quinones, and terpenoids, as described by Tongco, et al. [16] and Sofowora [17]. The following tests were performed:

Test for Alkaloids: Wagner's Test: 2 mL of extract was acidified with 1.5% v/v hydrochloric acid (HCL), followed by the addition of a few drops of Wagner's reagent. The formation of a brown or reddish precipitate indicated the presence of alkaloids.

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Test for Flavonoids: Shinoda Test: 2 mL of extract was combined with a few fragments of magnesium ribbon and concentrated HCL. The presence of flavonoids was indicated by the formation of a pink or red coloration.

Test for Tannins: Lead acetate test: Add 2 mL of the extracts to a few drops of 10 % lead acetate $Pb(C_2H_3O_2)_2$ solution. The presence of tannins was indicated by the formation of a white precipitate.

Test for Saponins: Froth Test: 2 mL of the extract was mixed thoroughly with 5 mL of distilled water and allowed to sit for 10 minutes. The presence of saponins was indicated by the formation of a stable foam.

Test for Terpenoids: Salkowski Test: 2 mL of the extract was combined with 2 mL of chloroform and 3 mL of concentrated sulfuric acid. The formation of a reddish-brown color at the interface indicates the presence of terpenoids.

Test for Phenols: Ferric Chloride Test: 2 mL of the extract was combined with 2 mL of distilled water, along with a few drops of 10% ferric chloride solution. The presence of phenols was indicated by the formation of a blue or green color.

Test for Anthraquinones: Borntrager's Test: 2 mL of the extract was mixed with 3 mL of benzene and then filtered. The filtrate was then mixed with 10% ammonia solution. The presence of anthraquinones was indicated by a pink, red, or violet color in the ammoniacal layer.

Test for Quinones: Sulfuric Acid Test: 2 mL of the extract was combined with concentrated sulfuric acid. The presence of quinones was indicated by the formation of a red or yellow color.

Test for Steroids: Liebermann-Burchard Test: 2 mL of the extract was mixed with a few drops of acetic anhydride, followed by the gradual addition of concentrated H_2SO_4. The presence of steroids was indicated by the formation of a reddish-brown ring and green color in the upper layer.

2.4. Statistical Analysis

All experiments were conducted in triplicate, and the data were expressed as mean ± SEM. The significance of the results was assessed using p-value, for which a p-value less than 0.05 was considered statistically significant. The statistical analysis was performed using Microsoft Excel, which helped in thoroughly evaluating the data to assess the significance of the observed effects.

3. Results

3.1. Antimicrobial Susceptibility Testing

3.1.1. M. Longifolia Extracts Exhibit Potent Antimicrobial Activity

The antimicrobial activity of *M. longifolia* hexane and ethanolic extracts was tested against three bacterial strains, *A. baumannii*, *S. maltophilia*, and *M. luteus*, and two fungal strains at different concentrations, as shown in Table 1. The results show that both extracts exhibited potent antimicrobial activity against all strains. The highest activity was recorded against *M. luteus* where the inhibition zone reached 37±0.44 mm at 100 mg/ml for the ethanolic extract, followed by *A. baumannii* (18±0.66 mm) for both extracts, and *S. maltophilia* (15.5±0.16 mm) and (15±0.33 mm) for the ethanolic and hexane extracts respectively.

Table 1.
Antimicrobial activity (mm) of *M. longifolia* ethanolic and hexane extracts. Doxycycline and fluconazole were used as positive controls against bacteria and fungi, respectively. Values shown are means of three replicates with ± SEM.

Test Microorganism	Zone of Inhibition (mm)						
	Ethanolic Extract (mg/ml)			Hexane Extract (mg/ml)			Positive Control (0.125 mg/ml)
	100	50	25	100	50	25	
A. baumannii	18±0.66	16± 0.57	11± 0.33	18±0.66	15± 0.41	11±0.33	22±0.11
S. maltophilia	15.5±0.16	15±0.33	11±0.45	15.5±0.28	15±0.33	12±0.63	30±0.18
M. luteus	37±0.44	25±0.57	20±0.83	33±0.57	25±0.57	20±0.33	49±0.21
A. flavus	20±0.33	17±0.33	11± 0.16	19±0.33	17±0.16	11±0.33	16±0.18
A. nidulans	22±0.33	20±0.33	16±0.33	20±0.33	19±0.33	16±0.33	19±0.27

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A. nidulans showed strong susceptibility for the ethanolic extract with an inhibition zone of 22 ± 0.33 mm that exceeded that of the positive control (19 mm).

The Minimum Inhibitory Concentration (MIC) assay was employed to evaluate the lowest concentration of *M. longifolia* extracts required to inhibit the growth of bacterial and fungal strains. Both ethanolic and hexane extracts exhibited an MIC of 25 mg/mL against *all tested bacteria*. Minimum Bactericidal Concentration (MBC) results revealed variations in bacterial susceptibility. For *A. baumannii* and *S. maltophilia*, the MBC was 50 mg/mL, while *M. luteus* demonstrated a lower MBC of 25 mg/mL, reflecting its higher sensitivity to the extracts. Similarly, fungal inhibition assays showed an MIC of 50 mg/mL for both extracts against *A. flavus* and *A. nidulans*. The Minimum Fungicidal Concentration (MFC) was 100 mg/mL for both fungal strains, indicating the extracts' potent fungicidal activity. The results are demonstrated in Table 2.

Table 2.
Table showing MIC, MBC, and MFC values for ethanolic and hexane extracts against the tested strains.

Test Microorganism	MIC (mg/ml)		MBC/MFC (mg/ml)	
	Ethanolic Extract	Hexane Extract	Ethanolic Extract	Hexane Extract
A. baumannii	25	25	50	50
S. maltophilia	25	25	50	50
M. luteus	25	25	25	25
A. nidulans	50	50	100	100
A. flavus	50	50	100	100

3.1.2. M. Longifolia Extracts Inhibit and Destroy Biofilms of Tested Bacterial Strains

The biofilm inhibition activity of *M. longifolia* extracts was assessed using the Crystal Violet Assay. Both ethanolic and hexane extracts of *M. longifolia* showed dose-dependent inhibition of biofilm formation (Figure 1a–b). For *A. baumannii*, maximum inhibition was 64.4% and 74.5% at 4× MIC for the hexane and ethanolic extracts, respectively. Against *M. luteus*, inhibition peaked at 87.9% for the hexane extract and 89.6% for the ethanolic extract at 4× MIC. For *S. maltophilia*, the hexane extract inhibited biofilm by 68.0% at 4× MIC, and the ethanolic extract reached 80.5% at the same concentration. The ethanolic extract generally exhibited higher activity than the hexane extract, with all results statistically significant ($p < 0.05$).

The biofilm destruction capability of *M. longifolia* extracts was further assessed (Fig. 1c–d). For *A. baumannii*, the ethanolic extract showed destruction ranging from 10.67% at 1/16 MIC to 48.13% at 4× MIC, while the hexane extract achieved slightly lower efficacy (10.45% to 43.91%). Similarly, for *S. maltophilia*, the ethanolic extract exhibited higher destruction (12.43% at 1/16 MIC to 56.42% at 4× MIC) compared to the hexane extract (11.54% to 50.80%). In *M. luteus*, the ethanolic extract caused significant biofilm destruction, starting at 22.05% (1/16 MIC) and reaching 71.49% (4× MIC). The hexane extract showed comparable activity, with destruction rates ranging from 22.06% to 66.90%. Overall, the ethanolic extract was more effective than the hexane extract across all tested bacteria.

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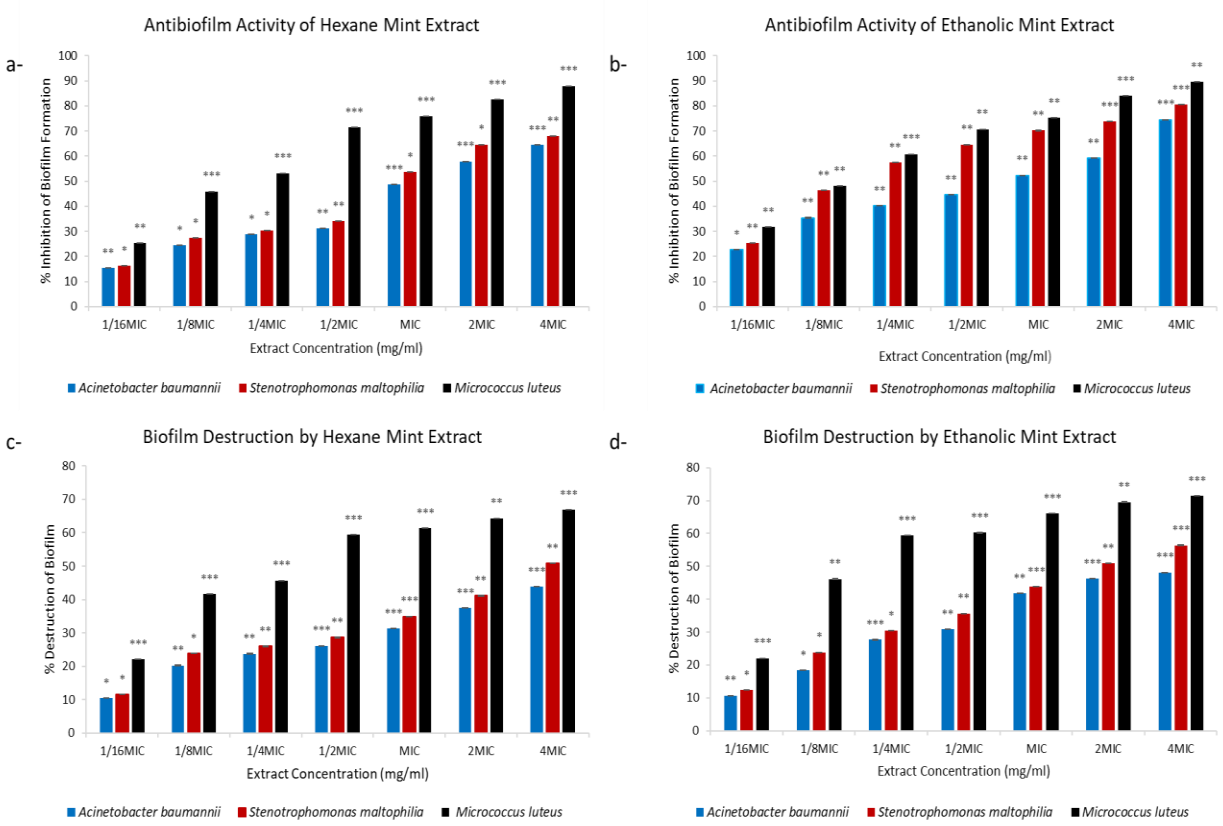

Figure 1.
Antibiofilm Activity of M. longifolia extracts showing biofilm inhibition for hexane (a) and ethanolic extracts (b), and biofilm destruction for hexane (c) and ethanolic extracts (d) in A. baumannii, S. maltophilia, and M. luteus, with results presented as means ± SEM. Significant differences ($p < 0.05$) in biofilm destruction were observed at various time points.

3.1.3. Catalase Inhibition by M. Longifolia Extracts Against the Tested Bacteria Indicates Antioxidant Activity

The following results present the impact of hexane and ethanolic extracts of *M. longifolia* on catalase (CAT) activity in *M. luteus*, *S. maltophilia*, and *A. baumannii* (Figure 2). Measurements were taken at 1, 6, 18, and 24 hours to evaluate the temporal effects of the extracts on CAT activity across the three bacterial species. The data illustrate the extent of CAT inhibition induced by the extracts, providing insight into their influence on bacterial oxidative stress mechanisms.

In *M. luteus*, the hexane extract decreased catalase activity by 17.1% at 1 hour, 40.3% at 6 hours, 76.1% at 18 hours, and 96.6% at 24 hours. The ethanolic extract exhibited a more pronounced effect, with reductions of 13.1%, 38%, 70.6%, and 89% at the corresponding time points. For *S. maltophilia*, the hexane extract resulted in catalase reductions of 26.3% at 1 hour, 52% at 6 hours, 70.6% at 18 hours, and 81.6% at 24 hours, while the ethanolic extract showed decreases of 21.5%, 57.1%, 77.3%, and 95.6%, respectively. In *A. baumannii*, the hexane extract caused reductions of 12.7% at 1 hour, 26.9% at 6 hours, 74% at 18 hours, and 89.5% at 24 hours. The ethanolic extract led to decreases of 18.1%, 33.9%, 79.4%, and 91.2% over the same time points. Overall, both extracts showed a time-dependent inhibitory effect on catalase activity, with the ethanolic extract generally producing greater reductions than the hexane extract across all bacterial species.

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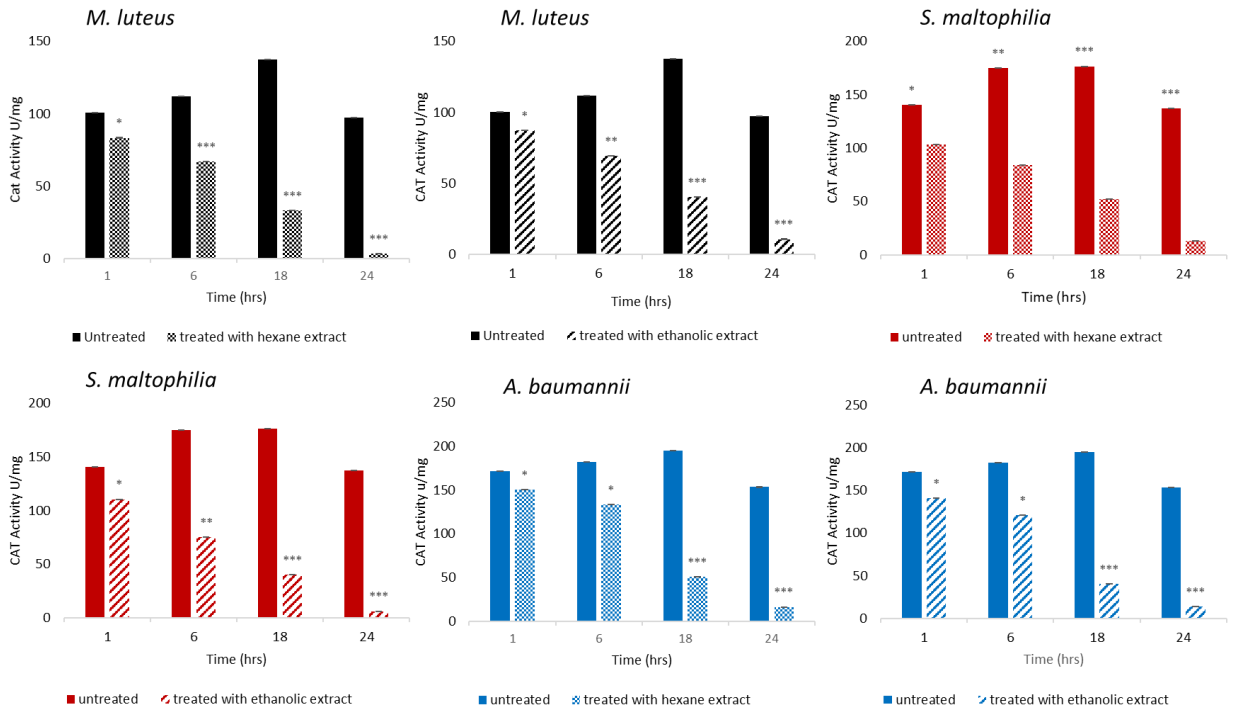

Figure 2.
The effect of hexane and ethanolic mint extracts on CAT activity in M. luteus, S. maltophilia, and A. baumannii, with results presented as means ± SEM. Significant differences ($p < 0.05$) in CAT activity were observed at various time points.

3.2. Phytochemical Screening of M. longifolia Extracts

Phytochemical screening of *M. longifolia* extracts revealed distinct patterns of secondary metabolites. The ethanolic extract demonstrated the presence of terpenoids, quinones, saponins, tannins, phenols, and flavonoids. However, the hexane extract showed a positive test for terpenoids, quinones, and flavonoids. These findings highlight the variety of bioactive compounds present in *M. longifolia* extracts, as detailed in Table 3.

Table 3.
Results of the qualitative phytochemical screening for M. longifolia ethanolic and hexane extracts.

Test	Ethanolic	Hexane
Anthraquinones	-	-
Terpenoids	+	+
Quinones	+	+
Steroids	-	-
Saponins	+	-
Tannins	+	-
Phenols	+	+
Flavonoids	+	+
Alkaloids	-	-

4. Discussion

In recent years, the urge to study natural medicine has been growing due to the threat of antibiotic resistance. More studies are exploring the effectiveness of plant extracts as alternatives to synthetic antibiotics. Mint is one of the best-known plants to have strong antimicrobial properties against various pathogens. With this in mind, this research set out to examine the antibacterial and antifungal effects of

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M. longifolia hexane and ethanolic extracts against *A. baumannii*, *S. maltophilia*, and *M. luteus*, as well as fungal species like *A. flavus* and *A. nidulans*.

The results revealed that both extracts exhibit notable antibacterial and antifungal effects against all the tested strains, with slight variations between the hexane and ethanolic extracts. The ability of both extracts to generate significant broad antimicrobial activity can be linked to bioactive compounds detected during phytochemical screening. Both extracts tested positive for phenols, flavonoids, terpenoids, and quinones, which are widely recognized for their antimicrobial effects. Phenols and flavonoids are known for disrupting bacterial cell membranes and inhibiting key enzymatic processes, leading to cell death [18, 19]. Terpenoids, found in both extracts, increase membrane permeability, causing leakage of cellular components, particularly in Gram-negative bacteria [20]. They also inhibit protein synthesis [21] and ATPase activity [22]. Quinones, also detected in both extracts, act by generating ROS that cause oxidative damage to microbial cells [23, 24]. Tannins and saponins, on the other hand, were present in the ethanolic extract and absent in the hexane extract. This difference in the phytochemical profile is explained by the enhanced antimicrobial activity in the ethanolic extract that was demonstrated by larger inhibition zones and higher antibiofilm activity. Tannins exhibit strong antimicrobial properties by precipitating microbial proteins and chelating essential metal ions required for microbial growth, inhibiting biofilm formation [25] while saponins are known to form complexes with sterols in microbial membranes, increasing permeability and disrupting cellular integrity [26].

The MIC results also demonstrated that both ethanolic and hexane extracts had a consistent inhibitory effect, while the MBC experiment indicated that both hexane and ethanolic extracts have bacteriostatic as well as bactericidal effects. The observed difference in MBC values, with Gram-positive bacteria requiring 25 mg/ml and Gram-negative bacteria requiring 50 mg/ml, aligns with Koohsari et al., who reported that Gram-negative bacteria tend to require higher concentrations of plant extracts to achieve bactericidal effects compared to Gram-positive bacteria [27]. This difference is commonly attributed to the more complex cell wall structure of Gram-negative bacteria, particularly the lipopolysaccharide (LPS) layer, which can limit the entry of bioactive compounds.

The observed strong anti-biofilm activity can be linked to the presence of phenolic compounds in *M. longifolia* that act by inhibiting biofilm formation [19]. Moreover, terpenoids, present in both extracts, are well-known for their suppression of quorum sensing and inhibition of microbial adhesion, which hinders the initial stages of biofilm formation and dismantles existing ones [28]. The results also revealed the enhanced activity of the ethanolic extract over the hexane extract in disrupting preformed biofilms, where the percentage destruction reached 71.49% against *M. luteus*. This shows that polar compounds extracted by ethanol but absent in the hexane extract, such as tannins and saponins, can be effective in penetrating and disrupting the biofilm matrix.

The catalase assay results show that both the hexane and ethanolic extracts of *M. longifolia* exhibit prooxidant effects, significantly reducing catalase activity in the bacterial strains. This observation aligns with the findings of Yanda, et al. [29] who also observed reduced catalase activity in treated strains of *Enterococcus faecalis* and *E. coli* [29]. This suggests that the extracts work by generating ROS and thus activating oxidative stress in cells. Quinones, which tested positive in both extracts, act by increasing ROS inside host cells [24]. In addition, flavonoids are also reported to induce oxidative stress in microbial cells, leading to their death [30].

Although our study offers valuable insights, several limitations should be noted. First, the absence of *in vivo* testing. The antimicrobial effects observed were based solely on *in vitro* assays, which might not fully represent how the extracts perform in a living organism. *In vivo* studies are essential to evaluate factors like bioavailability, metabolism, and overall therapeutic effectiveness.

Additionally, the study did not employ quantitative analysis, such as HPLC or GC-MS, to identify and measure the specific phytochemicals accountable for the observed results. Quantifying these compounds and understanding their mode of action could deepen our knowledge of how the extracts work. Moreover, while ethanolic and hexane extracts were studied, investigating other solvents could uncover different antimicrobial properties. Addressing these gaps in future research could provide a

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complete assessment of the antimicrobial potential of *M. longifolia* extracts and their use in treating infections.

5. Conclusion

Our findings collectively highlight the potent antimicrobial and antibiofilm effects of *M. longifolia* leaves and link them to their phytochemical composition. Both ethanolic and hexane extracts exhibited significant antibacterial and antifungal effects. Key bioactive compounds—phenols, flavonoids, terpenoids, saponins, tannins, and quinones—play a central role in these effects by disrupting cell membranes, inhibiting protein synthesis, and inducing oxidative stress through ROS generation. Among the extracts, the ethanolic extract showed superior efficacy, likely due to its enhanced solubility of polar phytochemicals like tannins and saponins. The broad-spectrum activity of the extracts, demonstrated by their efficacy against broad-spectrum bacteria, supports the potential of *M. longifolia* as a source of antimicrobial agents, particularly against biofilm-forming and resistant pathogens.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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