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ANTIBIOFILM EFFECTS OF *Fomes fomentarius* (L.) FR. EXTRACTS ON SOME MICROORGANISMS

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Abstract

Biofilms, structured microbial communities, are a significant focus of research due to their nature as they provide protection against environmental stressors but also cause substantial medical and industrial problems. These communities, embedded in an extracellular matrix, are implicated in persistent infections, corrosion in infrastructure, and food spoilage, while also holding potential in beneficial applications like biofuel production and wastewater treatment. Consequently, there is growing interest in modulating biofilm formation, with natural products emerging as promising candidates. This study assessed the impact of Fomes fomentarius (L.) Fr. extracts on some microorganisms. The impact of ethanol (EtOH) and chloroform extracts on biofilm formation was evaluated using crystal violet staining, with SEM and AFM imaging used for confirmation. A comprehensive chemical analysis of the extracts was performed via gas chromatography-mass spectrometry (GC/MS). The EtOH extract was found to contain compounds such as stearic acid and oleic acid, while the chloroform extract contained compounds like methyl stearate and octadecadienoic acid. The key finding was that the F. fomentarius-EtOH extract significantly inhibited biofilm formation in S. aureus MRSA between 30.90-47.06%. The chloroform extract, however, showed no discernible effect on biofilm development. The effectiveness of the EtOH extract was compared using Halamid® as a positive control. Inhibition was observed for the S. aureus MRSA strain, as 54.21% with 125 µg/mL of the Halamid® concentration. This suggests that F. fomentarius extracts may offer a natural source of compounds with the potential to control and manage biofilm formation.

1. INTRODUCTION

Biofilms are structured microbial communities embedded within a self-produced extracellular polymeric substance (EPS) matrix. This matrix acts as a protective shield, enhancing microbial resistance to environmental stressors and antimicrobial agents [1-8]. The advantages and disadvantages of biofilm structure vary depending on the microorganism's species, its pathogenic potential, the environment in which it forms, and the intended application. The controlled modulation of biofilm formation, encompassing both its stimulation and inhibition, holds significant scientific and practical value across a wide range

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of fields, from scientific research to industrial applications. Therefore, the ability to control biofilm formation is a critical tool for various disciplines. For example, it is possible to reduce the risk of infection by inhibiting biofilm formation on the surfaces of medical devices, ensure product safety in the food industry by preventing unwanted biofilm formation, or remediate environmental pollution by promoting the formation of specific biofilms in bioremediation applications [9,10]. Biofilms offer numerous advantages, including their applications in biodegradable packaging, nutraceutical supplements, biofertilizer and biofuel production, enhancing the energy efficiency of high-energy batteries, and wastewater treatment systems. This diverse range of uses highlights the importance of identifying compounds that stimulate biofilm formation and introducing novel biofilm inducers into the scientific literature [11-13]. However, in environments where hygiene is vital, such as hospitals and food production facilities, the formation of biofilms by pathogenic microorganisms poses a serious risk to human and public health. Moreover, biofilms contribute to the development of antimicrobial resistance, thereby exacerbating a global public health crisis. Therefore, preventing biofilm formation is crucial for safeguarding public health. In this regard, the identification of biofilm-inhibiting compounds and the development of novel antibiofilm agents are essential [14,15].

Nature offers a vast and valuable reservoir of resources for the discovery of such compounds, providing researchers with a diverse array of natural materials, including macrofungi. Macrofungi contribute significantly to ecosystem stability by actively participating in biogeochemical cycles [16-21]. While some species are edible and consumed as food, others contain pharmacologically active components and are used in traditional medicine. Recent studies on fungi have revealed the presence of a multitude of bioactive molecules. Research has shown that various compounds isolated from species belonging to the phylum Basidiomycota, in particular, exhibit antibacterial, antifungal, phytotoxic, cytotoxic, antiviral, and other pharmacological activities [20,22,23].

The discovery of bioactive compounds in macrofungi with potential therapeutic applications has made them an increasingly valuable resource for developing new pharmaceutical, therapeutic, industrial, and biotechnological products. Despite the existence of numerous studies on the antimicrobial activities of macrofungi, studies specifically evaluating their effects on microbial biofilm formation remain limited. To address this literature gap and to discover novel bioactive molecules, more comprehensive research on macrofungi is required [20,24,25].

Fomes fomentarius (L.) Fr., a fungus within the Basidiomycota division, is a medicinal mushroom species with a long history of use in traditional medicine owing to its diverse array of bioactive compounds. Research into the antimicrobial and antibiofilm properties of this fungus offers potential avenues for alternative therapeutic strategies. Bioactive constituents present in *F. fomentarius,* including triterpenes, polysaccharides, and phenolic compounds,

have demonstrated both antimicrobial activity and the ability to inhibit biofilm formation or disrupt pre-existing biofilms. Recent investigations corroborate the antimicrobial effects of *F. fomentarius* extracts against a range of pathogenic microorganisms. These studies underscore the potential of macrofungi to exhibit activity against both Gram-positive and Gram-negative bacteria, yeasts, and certain viruses. The capacity of *Fomes fomentarius* extracts to impede biofilm development and eradicate established biofilms positions this fungus as a compelling candidate, particularly in the context of biofilm-associated infections such as those related to catheters and implants [14,15,24,26].

This study was designed to evaluate the *in vitro* effects of chloroform and ethanol (EtOH) extracts derived from *Fomes fomentarius*on biofilms formed by a methicillin-resistant *Staphylococcus aureus* (MRSA) strain, renowned for its robust biofilm production. The impact of these extracts on biofilm formation was quantitatively assessed via crystal violet staining and qualitatively corroborated through scanning electron microscopy (SEM) and atomic force microscopy (AFM) imaging. The findings were benchmarked against those obtained with Halamid®, a recognized biofilm inhibitor. Furthermore, the chemical constituents of the extracts were characterized utilizing gas chromatography-mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1 Macrofungus sample

Fomes fomentarius (L.) Fr. sample was obtained from the personal collection of Prof. Dr. Ilgaz AKATA. The fungi were collected from the Istanbul Belgrad Forest and identified by Prof. Dr. Ilgaz AKATA.

2.2 Extraction procedure

This study aimed to extract and quantify the active components present in *Fomes fomentarius* samples. The mushroom samples were pulverised using a blender. The ground sample was extracted separately in ethanol (EtOH) and chloroform solvents for three days at 140 rpm to release the active components. After the extraction process, the obtained extracts were filtered using 125 mm diameter filter paper (Sigma-Aldrich, USA). The filtrates were concentrated using a rotary evaporator at a temperature range of 40-50°C, ensuring the complete removal of solvents. The residues obtained after evaporation were dissolved in a mixture of sterile distilled water (sdH_2O) and dimethyl sulfoxide (DMSO) in varying proportions based on their solubility properties. The *F. fomentarius*-EtOH extract stock was prepared at a ratio of 50:50 (sdH_2O:DMSO) and a concentration of 0.419 g/4 mL and the *F. fomentarius*-Chloroform extract stock was prepared at a ratio of 40:60 (sdH_2O:DMSO) and a concentration of 0.537 g/5 mL. The DMSO concentration in the obtained extracts was reduced to 2% to minimise the cytotoxic effect on microorganisms. Finally, the extract concentrations applied to the initial wells were calculated as 2095 µg/mL (*F. fomentarius*-EtOH) and 1790 µg/mL (*F. fomentarius*-Chloroform), respectively.

2.3 Microorganisms used

A total of twenty microorganisms were tested, including *Escherichia coli* isolates (2, 3, 4, 5, 6, 7, 8, 9, 10, 11) and the *E. coli* ATCC 25922 (1) standard strain, *P. mirabilis*, *S. pneumoniae*, *S. flexneri*, *A. baumannii*, two strains of *S. aureus*, one of which is MRSA, and three yeast strains (*Candida albicans* DSMZ1386, *Candida glabrata*, and *Candida tropicalis*). Six microorganisms exhibiting high biofilm formation (*E. coli* 7, 9, 11, *S. aureus*, *S. aureus* MRSA, and *C. albicans* DSMZ1386) were selected for further study.

2.4 Inoculum preparation

Bacterial strains were cultured at 37°C for 24 h, whereas *Candida albicans* was incubated at 27°C for 48 h. Inocula were prepared by suspending morphologically similar colonies of each microorganism in a sterile 0.9% saline solution, and the cell density was adjusted to approximately 1×10^8 CFU/mL, corresponding to a 0.5 McFarland standard. Mueller-Hinton agar (Merck, Germany) served as the culture medium for bacterial strains, while Sabouraud dextrose agar (Merck, Germany) was utilized for *C. albicans* [27,28].

2.5 MIC method

To ascertain the sub-lethal concentrations of extracts exhibiting antibiofilm activity against the target microorganisms, the Minimum Inhibitory Concentration (MIC) was determined via a two-fold serial microdilution assay, following the methodology outlined by [29]. The MIC was defined as the lowest concentration of the extract at which no visible microbial growth was observed. All experiments were conducted in triplicate.

2.6 Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) method

Although the MIC test results were higher than the initial well concentration, as previously described by Norrby and Jonsson [30], samples from the initial wells were transferred to Nutrient Agar (NA) for bacterial cultures to perform the MBC test. Similarly, the *Candida* strain was transferred to Sabouraud Dextrose Agar (SDA) for the MFC test and incubated under optimal growth conditions, considering the appropriate time and temperature for each microorganism.

2.7 Biofilm detection method with Congo Red Agar (CRA)

Following the protocol described by Freeman et al. [31], a specialized medium was prepared utilizing Congo Red (CR), an azo dye. Bacterial strains were incubated at 37°C, whereas *Candida* strains were incubated at 27°C for 24 h on Congo Red Agar (CRA). Biofilm-producing microorganisms were phenotypically identified using the CRA method.

2.8 Antibiofilm activity

Consistent with the findings of Ozturk et al. [32], the crystal violet assay was employed to quantify biofilm production in microorganisms previously identified via the CRA method.

The antibiofilm activity assay, adapted from the method originally described by Karaca et al. [33], comprised two primary stages: first, optimization of biofilm formation conditions and second, evaluation of the antibiofilm activity of the prepared extracts.

To establish optimal biofilm formation conditions, all microorganisms were standardized to a 0.5 McFarland turbidity. Each strain was inoculated into culture media supplemented with varying glucose concentrations (0%, 0.5%, 1.5%, 2%, and 2.5%) and incubated at 37°C for 24 h and 48 h. Following incubation, 200 µL of crystal violet solution was added to each well and allowed to incubate for 30 min. The wells were then rinsed with distilled water and air-dried. Subsequently, 200 µL of a 70:30 ethanol/acetone solution was added to each well and incubated for 15 min. The contents of each well were then carefully transferred to a new microplate, and the absorbance was measured at 550 nm using a microplate reader.

Based on these results, the optimal biofilm formation conditions were determined to be 48 h of incubation in a medium supplemented with 0.5% glucose for *E. coli* 7, *E. coli* 9, *E. coli* 11, *E. coli* 12, *S. aureus*, *S. aureus* MRSA, and *C. albicans* DSMZ 1386 strains. Consequently, subsequent biofilm activity assays were performed under these optimized conditions.

During the biofilm activity assays, 100 µL of each extract was added to the wells in row A of a microplate, followed by two-fold serial dilutions down to row H. Inocula, standardized to a 0.5 McFarland turbidity in physiological saline, were then transferred into the wells. Halamid® served as a positive control, and all cultures were incubated at 37°C for 48 h. Following incubation, the crystal violet staining, washing, and ethanol/acetone elution steps were repeated, and absorbance measurements were obtained at 550 nm [32,34,35].

2.9 Biofilm SEM and AFM analysis

Based on the results obtained from the crystal violet biofilm detection method, the microorganism for which biofilm inhibitory effects were observed was selected for SEM and AFM analysis.

To perform these analyses, cell suspensions, culture medium, and extracts were prepared in 24-well microplates containing sterile metal coupons and incubated for 5 days, considering the optimum growth temperatures of the microorganisms. Imaging was performed with SEM and AFM at the end of the incubation period [36,37].

2.10 Statistical analysis

All experiments in this study were performed in triplicate, and statistical significance was assessed using one-way analysis of variance (ANOVA) in R Studio (v3.3.2). A *p*-value of < 0.05 was considered statistically significant. Furthermore, Pearson's correlation coefficient was calculated to evaluate the relationship between extract concentration and observed effect [38].

3. RESULTS AND DISCUSSION

3.1 Extraction yield

The preparation of ethanol and chloroform extracts of *F. fomentarius* used in the study has been described in detail previously. The extract yield obtained according to the amount of extract was calculated as 20.160% and 26.25%, respectively. When the extraction yield was examined, it was observed that the ethanol extract had a lower yield than the chloroform extract.

3.2 MIC and MBC tests

To mitigate potential concentration-dependent loss of microbial viability in subsequent antibiofilm experiments, the antimicrobial activity of *F. fomentarius* ethanol and chloroform extracts was assessed against all tested microorganisms via Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) assays. At the end of the conducted research, it was found that the MIC result was >1790 µg/mL for *F. fomentarius*-EtOH, >2095 µg/mL for *F. fomentarius*-Chloroform due to the application of extracts at low doses. MBC/MFC tests could not be applied due to the MIC test results being higher than the initial well concentration. Given that the primary objective of this study was to identify sub-lethal extract concentrations for subsequent use in antibiofilm assays, rather than to conduct a comprehensive assessment of antimicrobial activity, the absence of a quantifiable MIC value is not considered particularly consequential. It is plausible that a MIC result could be obtained through the utilization of higher initial concentrations of the macrofungal extract.

3.3 Biofilm experiments

3.3.1 Congo Red Agar (CRA) method

In this study, the microorganisms were first phenotypically determined to produce biofilms by applying the CRA method. The results obtained using this method are shown in the photographs given below. Biofilm production was observed in the microorganisms that appeared black, whereas it was not detected in the strains lacking the black pigmentation.

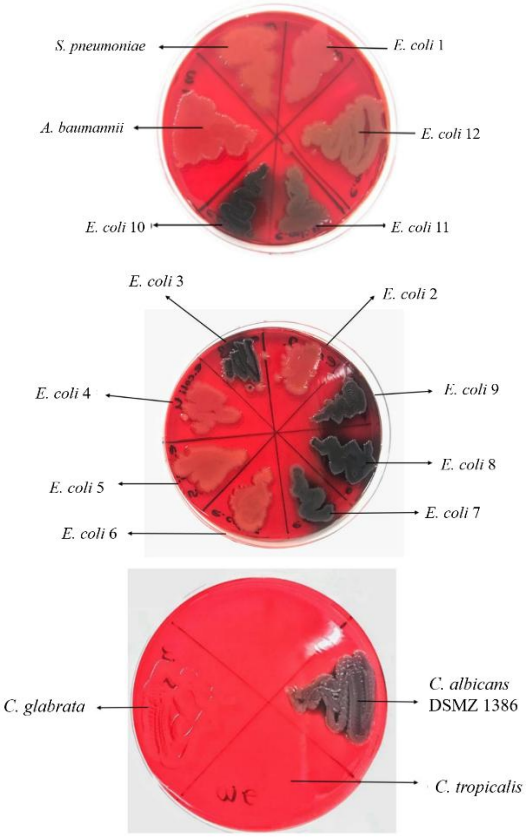


FIGURE 1. CRA results

3.3.2 Determination of optimum biofilm formation parameters

In this study, six different glucose concentrations (0.0%, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) and two different incubation times (24 and 48 hours) were tested. The results indicated that the optimum conditions for all microorganisms were a 48-hour incubation in a medium containing 0.5% glucose ($p < 0.05$). The statistical analysis revealed no significant differences between the parallel studies conducted ($p > 0.05$).

3.3.3 Antibiofilm activity

The antibiofilm activities of the microorganisms, for which optimum biofilm formation parameters were determined, were assessed using the crystal violet method, as previously described. The antibiofilm activity assays demonstrated that the *F. fomentarius*-EtOH extract significantly inhibited biofilm formation by *S. aureus* MRSA (Figure 2). No statistically significant inhibitory or activating effects were observed for the *F. fomentarius*-Chloroform extract against the tested strains. Halamid®, used as a positive control, also demonstrated an inhibitory effect.

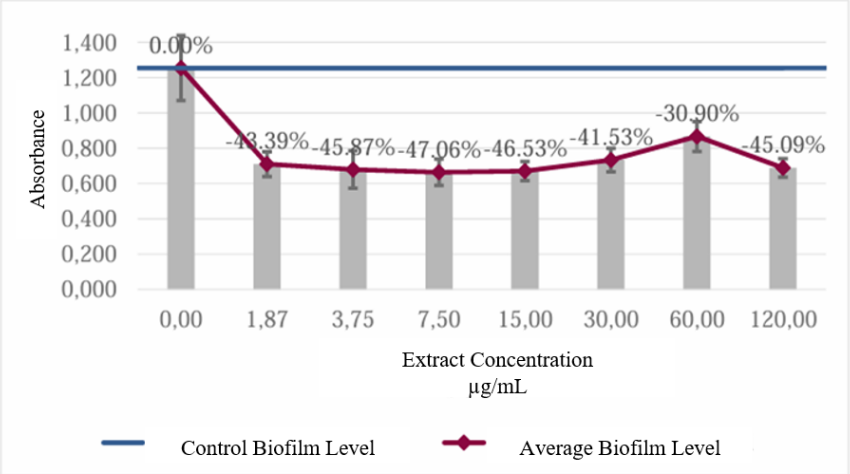


FIGURE 2. Effects of the *F. fomentarius*-EtOH extract *on S. aureus* MRSA biofilm formation (bars indicated extract concentration (µg/mL))

3.3.4 SEM images

Examination of the SEM images revealed a clear reduction in biofilm production when *F. fomentarius*-EtOH extract was applied to the *S. aureus* MRSA strain (Figure 3b). Comparison of the *F. fomentarius*-EtOH extract images (Figure 3b) and negative control (Figure 3a) demonstrated that the environment with the most biofilm formation was the one lacking the *F. fomentarius*-EtOH extract. It was observed that the Halamid® also reduced biofilm formation (Figure 3c), which was consistent with the spectrophotometric results.

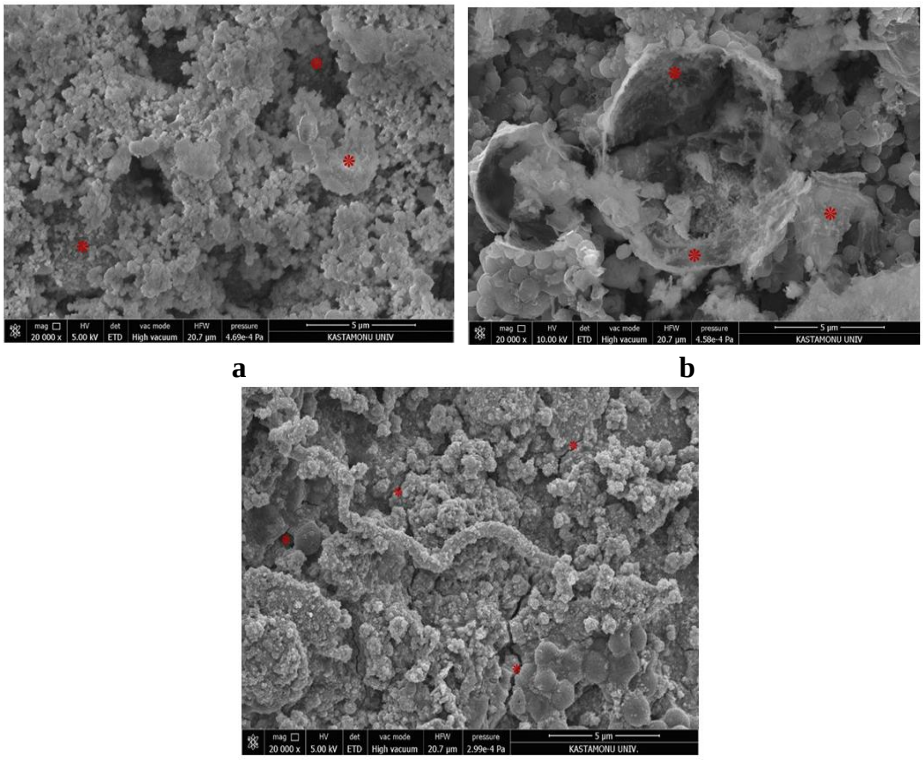


FIGURE 3. **a)** presents SEM images of control sample of *S. aureus* MRSA (20.000x) **b)** *F. fomentarius*-EtOH-treated/*S. aureus* MRSA (20.000x) **c)** Halamid-treated /*S. aureus* MRSA (20.000x)

3.3.5 AFM images

Analysis of the AFM images revealed that the *S. aureus* MRSA strain treated with the *F. fomentarius*-EtOH extract exhibited significantly reduced biofilm production (Figure 4a). A comparison between the extract-treated sample (Figure 4a) and the negative control without the extract (Figure 4b) clearly indicated that the extract application resulted in lower biofilm formation, thereby inhibiting biofilm development. These findings are consistent with the SEM results and spectrophotometric data.

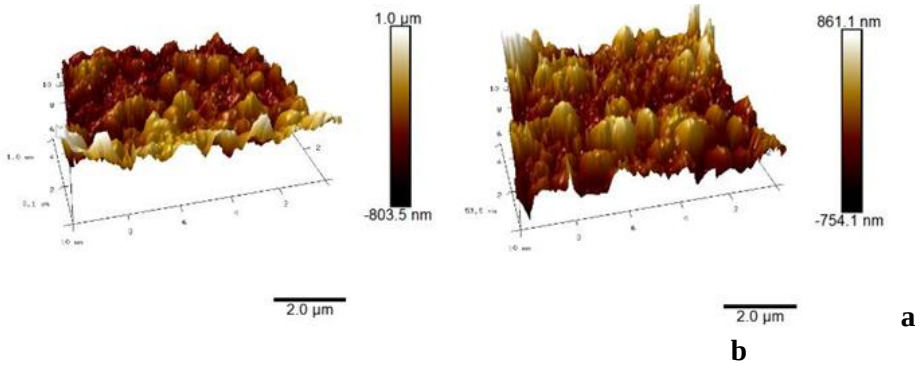


FIGURE 4. **a)** *F. fomentarius*-EtOH-treated/*S. aureus* MRSA, **b)** Negative control/*S. aureus* MRSA

3.3.6 GC/MS

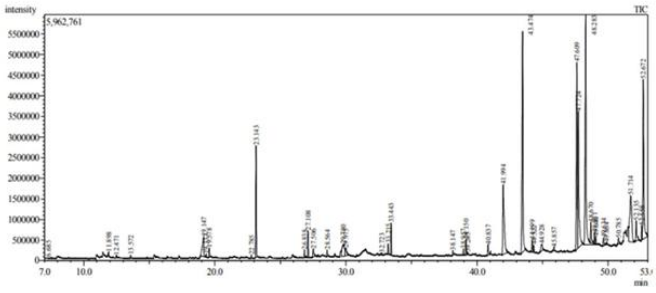
Content analysis of both extracts obtained from the *F. fomentarius* mushroom was performed using GC/MS. The chromatograms for *F. fomentarius*-EtOH and *F. fomentarius*-Chloroform are given in Figure 5a and 5b, respectively.

In addition, the major components of *F. fomentarius*-EtOH and *F. fomentarius*-Chloroform are given in Table 1.

GC/MS analysis revealed that the *F. fomentarius*-EtOH extract contained 15.86% stearic acid, 14.28% palmitic acid, 11.28% alpha-linoleic acid, 9.64% Oleic acid, 6.99 % linocaine hydrochloride, 6.95% methyl tetracosanoate, 4.58% anethole, 2.29% benzoic acid, and 2.15% D-allose, along with other minor components. Stearic acid is a recognized anti-inflammatory lipid with significant and multifaceted effects on hepatic metabolism [39,40]. Numerous fatty acids are known to exhibit antibacterial, antifungal, antioxidant, and antibiofilm properties to varying degrees across different microbial strains, including palmitic acid and stearic acid [41–44].

The GC/MS analysis revealed that the *F. fomentarius*-Chloroform extract contained 21.44% methyl stearate, 18.71% 9,12-octadecadienoic acid, and 18.70% methyl palmitate, along with other minor components. Methyl palmitate is used in the production of detergents, plastics, and animal feed. It possesses anti-inflammatory, antimicrobial, and antifungal properties [45-49].

a)



b)

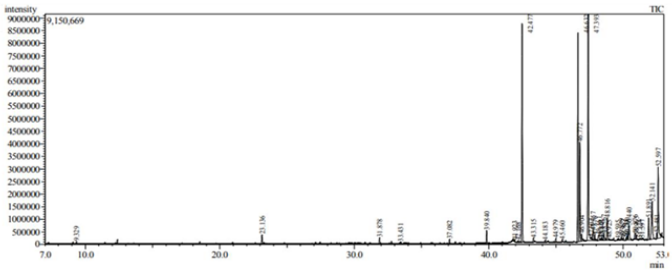


FIGURE 5. **a)** presents chromatogram illustrating the GC/MS results of *F. fomentarius*-EtOH **b)** presents chromatogram illustrating the GC/MS results of *F. fomentarius*-Chloroform

TABLE 1. Major Components of *F. fomentarius*-Chloroform and *F. fomentarius*-EtOH Extracts

***F. fomentarius*-Chloroform**		***F. fomentarius*-EtOH**	
Major Components	**Percentage (%)**	**Major Components**	**Percentage (%)**
Methyl stearate	21.44	Stearic acid	15.86
9,12-Octadecadienoic acid	18.71	Palmitic acid	14.28
Methyl palmitate	18.70	Alpha-linoleic acid	11.28
9-Octadecenamide	7.79	9-Octadecenamide	10.54
Ethyl stearate	5.23	Oleic acid	9.64
9,12-Octadecadienoic acid, methyl ester	2.39	Linocaine hydrochloride	6.99
Methyl 18-methylnonadecanoate	2.07	Methyl tetracosanoate	6.95
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	3.49	Anethole	4.58
Methyl pentadecanoate	1.06	Benzoic acid	2.29
		D-Allose	2.15
		Tetradecylcyclohexane	1.29
		Octadecanamide	1.29
		Hexadecane	1.26
		Tetradecane	1.09

Irez et al. [50] investigated the antibiofilm activity of various extracts derived from *F. fomentarius*. Their findings indicated that the *F. fomentarius*-EtOH extract significantly inhibited biofilm formation in the tested strains, with observed reductions exceeding 80% for *E. coli* and *S. aureus*, and approaching 80% for *C. albicans*. The *F. fomentarius*-Chloroform extract showed an inhibitory effect against the tested strains, but this effect was lower compared to the *F. fomentarius*-EtOH extract, with values below 20% for *E. coli* and *S. aureus*, and approximately 40% for *C. albicans*.

The *F. fomentarius*-EtOH extract exhibited an inhibitory effect on the *S. aureus* MRSA strain. In contrast, the *F. fomentarius*-chloroform extract showed neither inhibitory nor activator effects. This result may be associated with variations in extraction concentrations and the resistance profile of the tested microorganisms.

In a previous study, Halamid® was used as a positive control during the antibiofilm experiments. This study investigated the effects of Halamid® on *E. coli* 9, *S. aureus* MRSA, and *C. albicans*. The results obtained in that study were calculated as 62.91% (3.90 μg/mL) for *S. aureus* MRSA, 45.66% (0.41 μg/mL) for *E. coli* 9, and 68.70% (1.95 μg/mL) for *C. albicans* [13].

In this study, Halamid® was also used as a positive control in all biofilm analyses, demonstrating an inhibition of 54.21% for 125 μg/mL of Halamid® concentration against the *S. aureus* MRSA strain. Furthermore, the scanning electron microscopy (SEM) and atomic force microscopy (AFM) images corroborate the biofilm inhibition findings of this study. While these results align with the existing literature regarding Halamid®'s efficacy, discrepancies exist between the specific inhibition percentages and active concentrations reported herein and the data presented by Zurnaci et al. [13]. Therefore, in light of these differences, it is advisable to support any biofilm study conducted with spectroscopic methods with imaging techniques such as SEM and/or AFM for enhanced validation.

In addition to the discrepancies in biofilm inhibition percentages in this study and the studies in the literature, there are also some inconstancies in terms of the antimicrobial activity of *F. fomentarius* extracts compared to the previous studies. Such as Dokhaharani et al. [51], who reported a Minimum Inhibitory Concentration (MIC) of 0.7 mg/mL and a Minimum Bactericidal Concentration (MBC) of 12.5 mg/mL for a *F. fomentarius* methanol extract against *S. aureus* ATCC 25923 using a microdilution assay, the present study observed MIC values for *F. fomentarius* ethanol and chloroform extracts that exceeded the highest concentration tested (*F. fomentarius*-EtOH >1790 μg/mL, *F. fomentarius*-Chloroform >2095 μg/mL). This inconstancy may be attributed to differences in extraction solvents or *S. aureus* strains used.

Pavić et al. [26] synthesized silver nanoparticles (AgNPs) using a *F. fomentarius* methanol extract and subsequently evaluated the antibacterial activity of both the AgNPs and the extract against *Bacillus subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* using a Minimum Inhibitory Concentration (MIC) assay. The MIC

values for the *F. fomentarius* methanol extract were determined to be 20.83 μg/mL, 10.41 μg/mL, 2.63 μg/mL, and 20.83 μg/mL, respectively, against the aforementioned bacterial species. The corresponding MIC values for the AgNPs were 12.69 μg/mL, 6.34 μg/mL, 12.69 μg/mL, and 12.69 μg/mL. In the present study, extracts of the same macrofungus were prepared using different solvents, and the MIC assay was performed. The discrepancies between the findings of these two studies are attributed primarily to the use of different extraction solvents.

4. CONCLUSIONS

In this study, the Congo Red Agar (CRA) assay was initially employed to screen twelve *E. coli* isolates, two *S. aureus* strains, *P. mirabilis*, *S. pneumoniae*, *S. flexneri*, *A. baumannii*, *C. tropicalis*, *C. glabrata*, and *C. albicans* strains for biofilm production. This screening revealed that *E. coli* 7, *E. coli* 9, *E. coli* 11, *E. coli* 12, *S. aureus*, and *S. aureus* MRSA were capable of producing biofilms.

The *F. fomentarius*-EtOH extract exhibited an inhibitory effect on biofilm production against the *S. aureus* MRSA strain.

Biofilms are recognized as significant contributors to infections associated with vascular catheters, Foley catheters, and cerebrospinal shunts, as well as various tissue-related infections affecting the skin and teeth. Furthermore, biofilms enhance the resistance of biofilm-forming microorganisms to both antibiotics and antifungal agents. Consequently, preventing biofilm formation is of paramount importance for public health and in industrial settings [1,3,24].

Based on the biofilm data obtained in this study, it is evident that spectrophotometric assays alone may not always provide conclusive results and require corroboration from scanning electron microscopy (SEM), atomic force microscopy (AFM), and/or other complementary techniques. Therefore, for future biofilm investigations, it is recommended that spectrophotometric assays be combined with SEM and/or AFM to obtain more robust and reliable data. Given the importance of biofilm inhibition, further investigation of the *F. fomentarius*-EtOH extract, which demonstrated significant biofilm inhibitory effects in this study, is critically warranted.

In addition to the analyses performed herein, future research should focus on the purification of major constituents identified through compositional analysis or the acquisition of commercially available compounds to elucidate whether these components are responsible for the biofilm inhibitory effects of the extracts. Such studies would provide valuable insights.

Data Availability The data used in this study are available from the corresponding author upon reasonable request.

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Author Contribution Statements Conceptualisation, E.M.A.; data curation, U.M.S. and E.M.A.; methodology, U.M.S. and E.M.A.; supply and identification of the macrofungi, I.A.; writing, U.M.S. and E.M.A.; supervision, E.M.A.; project administration, E.M.A.; funding acquisition, E.M.A. All authors have read and agreed to the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

Ethical Statement This research did not involve human participants or animals. Therefore, no ethical approval was required.

Use of Artificial Intelligence No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Impact Statement This study demonstrates the potential of *Fomes fomentarius* extracts for targeted modulation of biofilms, offering a foundation for developing novel strategies to combat biofilm-related challenges in both clinical and industrial settings.

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