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Determination of Biological Activity and Biochemical Composition of *Polytrichastrum formosum* (Hedw.) G.L.Sm.

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Highlights

- GC–MS identified bioactive compounds such as 2,4-di-tert-butylphenol.
- Ethanol extract showed notable activity against multidrug-resistant strains.
- High phenolic content supported strong antioxidant potential of the extract.

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Abstract

This study examines the chemical profile and bioactivity of *Polytrichastrum formosum* (Hedw.) G.L. Sm., a moss species, to explore its potential therapeutic applications, focusing on antibacterial, antioxidant, and anti-quorum sensing activities. The moss specimen was collected and extracted using ethanol. Various tests, including disk diffusion, minimum inhibitory concentration (MIC), Cupric reducing antioxidant capacity (CUPRAC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Folin-Ciocalteu methods, were employed to assess its antibacterial, antioxidant, and phenolic content. Quorum sensing inhibition was evaluated by testing pyocyanin - motility inhibition in *Pseudomonas aeruginosa,* and violacein inhibition in *Chromobacterium violaceum*. The components of the *P. formosum* ethanol extract were analysed using gas chromatography (GC) and mass spectrometry (MS). The ethanol extract of *P. formosum* exhibited notable antibacterial effects against 19 strains, including multidrug-resistant bacteria. It also showed robust antioxidant capacity, as evidenced by substantial DPPH and CUPRAC radical scavenging activities. The concentration of phenolic compounds was measured at 190.05 mg GAE/mL. Moreover, the extract moderately suppressed pyocyanin production in *P. aeruginosa* and slightly reduced its twitching motility, indicating potential anti-quorum sensing activity. GC-MS analysis revealed the presence of bioactive constituents, including 2,4-di-tert-butylphenol and linoleic acid, which are associated with various biological effects. This study emphasizes the notable antibacterial, antioxidant, and anti-quorum sensing activities of *P. formosum* extract, indicating its value as a natural candidate for addressing antibiotic resistance and oxidative stress. Further investigation into its secondary metabolites may contribute to the development of innovative therapeutic agents.

1. INTRODUCTION

During the "Golden Age" of pharmaceutical sciences, between 1930 and 1970, more than 160 new types of antibiotics were developed [1]. The antibiotics produced during this period achieved significant success in controlling the mortality and morbidity caused by infectious bacteria [2]. However, due to the inappropriate, irregular, and uncontrolled use of antibiotics worldwide, antimicrobial resistance has emerged, rendering many currently used antibiotics ineffective [3]. The World Health Organization recognizes antimicrobial resistance as one of the ten most critical threats to global public health [4].

The resistance developed by multidrug-resistant (MDR) strains against currently used antibiotics, along with their ability to form biofilms, plays an important role in complicating the treatment of chronic

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infectious diseases [5]. Biofilm structures protect bacteria from the effects of nutrient depletion, blood pH, and osmotic pressure. Additionally, they create a stable homeostasis environment by preventing antibiotics and host immune cells from penetrating the biofilm community. By providing a physical barrier, biofilms enable bacteria to communicate and coexist even under harsh conditions [6]. Data from the U.S. National Institutes of Health indicate that biofilm formations contribute to over 80% of infectious diseases and account for more than 60% of nosocomial infections. Similarly, the U.S. Centers for Disease Control and Prevention reports biofilms are a leading factor in approximately 60% of persistent infectious conditions [7].

It is known that bacteria form biofilm structures under the control of the quorum sensing (QS) system [8]. The term quorum sensing (QS) refers to a system that allows bacteria to detect when they are at a critical concentration within a confined space and respond to their environment by activating certain genes responsible for producing virulence factors, such as toxins or enzymes [9]. It has been reported that biofilm structures formed through the QS system increase bacterial resistance to antibiotics by approximately 1,000-fold [10]. Since the QS system controls various mechanisms, including biofilm formation and virulence potential, targeting QS offers a different strategy for managing bacterial infections. [11].

Another serious issue faced today is oxidative stress, which arises as a result of changing lifestyles. Oxidative stress arises when the equilibrium between oxidants and antioxidants is disrupted, typically as a result of elevated levels of reactive nitrogen species (RNS) and reactive oxygen species (ROS). High levels of oxidative stress increase susceptibility to disease and accelerate both the onset and progression of illness [12]. Therefore, external antioxidants are needed to prevent oxidative damage caused by free radicals [13].

Antibiotic resistance and oxidative stress are major causes of many significant diseases today, including cardiovascular disorders, cancer, diabetes, and inflammation [14]. Consequently, the development of novel antibiotics has become crucial to address the growing global threat of antibiotic resistance [15]. The scarcity of new antibiotics has encouraged the development of alternative therapies, including herbal treatments. Plants are rich sources of important secondary metabolites, such as alkaloids, terpenoids, and phenolic compounds, which have therapeutic value and have been used since ancient times due to their availability and low cost [16]. Bryophytes have historically been overlooked as a source of secondary metabolites, largely due to challenges in their identification and their small size [17]. Although research on the medicinal uses of bryophytes has only emerged in the past few decades, they have been used as natural remedies in various parts of the world for centuries [18,19].

Polytrichastrum formosum (Hedw.) G.L. Sm., belonging to the Polytrichaceae family, is a dioicous and acrocarpous moss species that typically grows to a height of 50-200 mm, forming dense tufts. This species is distributed in temperate regions of the Northern Hemisphere, particularly in moist, slightly acidic, and humus-rich soils. *P. formosum* can be found in forests, on rocky surfaces, or directly on the ground, as well as in well-drained bogs and lowland heathlands [20]. The studies conducted to date in the literature have focused on the systematic and morphological characteristics of *P. formosum*. With this study, the biological activity and biochemical content of the species *P. formosum* have been revealed for the first time.

2. MATERIAL METHOD

2.1. Plant Sample

The *P. formosum* specimen was collected from Bozdağ, Izmir (38˚25'55.837"N 28˚09'12.573"E) by Dr. Atakan Benek. The voucher specimen is preserved at the Dokuz Eylül University, Fauna and Flora Research and Application Center.

2.2. Microbial Culture and Inoculum Setup

This research employed a total of 17 reference bacterial strains as well as 5 standard yeast strains, all sourced from the Microbiology Laboratory at the Dokuz Eylül University, Department of Biology.

Additionally, 7 bacterial strains isolated from food samples and 13 clinical isolates were included. Moreover, 11 multidrug-resistant bacterial isolates were also tested. All bacterial cultures were incubated at 37°C for 24 hours, and fungal strains were incubated at 27°C for 48 hours on Nutrient Agar (Oxoid). The cell densities were adjusted to approximately 10^{-8} CFU/mL for bacterial suspensions and 10^{-7} CFU/mL for yeasts, following the 0.5 McFarland standard using sterile 0.9% NaCl (Merck) solution [21].

2.3. Plant Extraction Method

P. formosum was ground into a fine powder using a laboratory grinder. A total of 40 grams was weighed and placed in a glass Erlenmeyer flask, and then 300 mL of 99.9% absolute ethanol was added. The prepared flask was then placed on an orbital shaker (WilkeShake) and shaken at 160 rpm for 3 days at room temperature in a dark room. After shaking, the *P. formosum* extract was filtered through Whatman No. 1 filter paper and transferred to a glass evaporating flask. The flask was connected to a rotary evaporator (Buchi), and the ethanol in the extract was evaporated under vacuum at 40 °C. An extract was prepared with 99% ethanol for disk diffusion test and GC/MS analysis. Repeat extraction was performed for extracts - in minimum inhibitory concentration (MIC), antibiofilm, and anti-quorum sensing detection tests. In the extraction process, which has the same first stage, 2% dimethyl sulfoxide (DMSO) was used to dissolve the dry residue remaining on the inner surface after weighing the glass evaporation vessels in which ethanol was evaporated in the second stage [22].

2.4. Antibacterial and Antifungal Activity Test

The antibacterial and antifungal activities of the ethanol extract of *P. formosum* were evaluated using the disk diffusion assay in accordance with the protocol of Andrews [23]. Oxoid brand antimicrobial susceptibility test disks with 6 mm diameter were loaded with 50 µL (931 mg), 100 µL (1860 mg) and 200 µL (3720 mg) of extract. The disks were then incubated at 30°C under sterile conditions for 24 h to facilitate complete evaporation of ethanol and thus prevent solvent interference in the biological test [24]. Mueller Hinton Agar (MHA-Oxoid) was utilized as the culture medium for antimicrobial susceptibility testing. Microbial inoculate were adjusted to 0.5 McFarland standard and uniformly spread onto MHA plates using sterile cotton swabs under aseptic conditions. Bacteria and yeasts were incubated under optimal conditions, and inhibition zones were measured millimeters post-incubation. Negative controls included both sterile blank disks and disks containing ethanol only. Gentamicin (10 mg) was employed as the positive control for Gram-negative bacterial strains. Tobramycin (10 mg) served as the reference antimicrobial for Gram-positive bacteria and fungi, as outlined by Canli et al. [25].

The MIC test for the *P. formosum* extract was adapted from the method described by Balouiri et al. [26]. The tests were conducted in 96-well U-bottom microplates made of polystyrene (PS), and Mueller Hinton Broth (MHB) was used as the test medium. The moss extract was loaded into the first well at 100 µL (2.445 mg) and then serially diluted. The extract concentrations were determined based on the amount of substance present on the disc that produced the largest inhibition zone in the disc diffusion assay. Strains standardized at 0.5 McFarland were added to all wells. The test was repeated three times, the results were visually analyzed and recorded in mg/mL. As a positive control, bacterial suspension was inoculated onto the medium without moss extract. As a negative control, moss extract was applied to the medium without the addition of bacterial suspension.

2.5. Antioxidant Activity Test

The antioxidant capacity of the *P. formosum* extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, quantitatively assessed by Mensor et al. [27]. To prepare the DPPH radical solution, 0.0039 g of DPPH was weighed and dissolved in 50 mL of ethanol. The *P. formosum* extract was prepared at a concentration of 1 g/mL in ethanol, and 200 µL was loaded into the first well, followed by serial dilutions. Ascorbic acid, used as a positive control, was prepared at a concentration of 1 g/mL in ethanol. The reaction mixtures were incubated at room temperature in the dark for 30 minutes to prevent photodegradation.

Post-incubation, absorbance readings were taken at 515 nm using a microplate reader spectrophotometer (Biotek). All measurements were performed in triplicate to ensure reproducibility and statistical reliability. Ascorbic acid was employed as a standard antioxidant control [28]. The percentage of DPPH radical scavenging activity was calculated using the appropriate equation

DPPH radical scavenging (%) = $[(A_0 - A_1)/A_0] \times 100$.

Where A_0 represents the absorbance of the control (DPPH solution without extract) and A_1 represents the absorbance in the presence of the extract.

The antioxidant activity of the *P. formosum* extract was evaluated using the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assay, adapted from the protocol described by Apak et al. [29]. Moss extracts were tested at concentrations of 10, 7.5, 5, 2.5, and 1 mg/mL. A 10^{-2} M copper (II) chloride solution was prepared. The neocuproine (Nc) solution (7.5×10^{-3} M) was obtained from 96% ethanol. To prepare the ammonium acetate buffer in distilled water. Ascorbic acid was used as the reference standard at ten different concentrations ranging from 0.1 to 1 mM. For the assay, 1 mL each of Cu (II), Nc, and ammonium acetate buffer solutions was transferred into the test tube. The sample extract and distilled water were added. After 60 minutes of incubation, absorbance was measured at 450 nm.

2.6. Assessment of Total Phenolics

The total phenolic content of the *P. formosum* extract was determined according to the Folin-Ciocalteu phenol reaction. This method was adapted from studies by Singleton et al. [30], and Ainsworth and Gillespie [31], with absorbance measurements conducted using a UV spectrophotometer to quantify the total phenolic compounds. Gallic acid was used as the standard phenolic compound in the tests. The *P. formosum* extract was prepared at a concentration of 7.5 mg/mL. The Folin-Ciocalteu reagent (FCR) was diluted with distilled water to 10% (v/v). Sodium carbonate was prepared in distilled water. Each sample was added to glass tubes. Then 10% (v/v) FCR reagent was added and vortexed. Following this step, 800 µL of 700 mM Na_2CO_3 was added to each reaction tube, and the mixtures were incubated at ambient temperature for 2 hours. Then, each sample absorbance was recorded at 765 nm. The phenolic content of the extract was quantified and expressed in milligrams of gallic acid equivalent (mg GAE).

2.7. Anti-Quorum Sensing Activity Test

Pyocyanin and violacein inhibition tests and motility tests were performed to see the inhibitory effects of *P. formosum* extract on the quorum sensing activities of bacteria.

The pyocyanin inhibition test method was adapted from the study conducted by Üreyen Esertaş et al. [32]. To determine the inhibition of pyocyanin by moss extract, *Pseudomonas aeruginosa* PA01 strain was incubated in Luria-Bertani (LB) Broth at 37°C for 9 hours with shaking at 220 rpm. At the end of the incubation period, the strain was diluted 1:100 in LB Broth. The diluted culture was transferred into a sterile glass tube, and moss extract was added. Sterile distilled water was used as a positive control. The prepared tubes were incubated for 24 hours at 37°C with shaking. After incubation, each tube was centrifuged for 10 minutes. Following centrifugation, the supernatant was transferred to clean glass tubes. Then, 0,9 mL of chloroform was added to the tubes and vortexed for 30 seconds. After vortexing, the tubes were left for phase separation, and the lower blue phase was transferred to clean tubes. After the transfer, 0.3 mL of 0.2 N hydrochloric acid was added to the tubes and vortexed again for 30 seconds. The resulting pink phase was transferred, and its absorbance was measured at 520 nm using a UV spectrophotometer.

The violacein inhibition test method was adapted from the study conducted by Üreyen Esertaş et al. [32]. To determine the inhibition of violacein by moss extract, *Chromobacterium violaceum* ATCC 12472 strain was incubated in LB Broth for 24 hours at 37°C with shaking at 175 rpm. After incubation, the prepared bacterial culture was plated onto LB Agar. The violacein inhibition effect of the moss extract was assessed using the agar well diffusion method. 6 mm diameter wells were made in the agar plates where bacteria were cultured, and moss extract was 50 µL added to each well. The prepared plates were incubated for 24

hours at 37°C. The diameters of the inhibition zones around the wells were measured with a ruler, and the results were expressed in millimeters.The motility tests consist of three tests: Swimming, Swarming, and Twitching, and the *Pseudomonas aeruginosa* PA01 strain was used.

The method used to determine swimming inhibition was adapted from the study by Déziel et al. [33]. To assess the inhibition of swimming motility by moss extract, the *P. aeruginosa* PA01 strain was streaked onto LB Agar and incubated at 37°C for 24 hours prior to the test. To analyze the inhibition of swimming motility, a medium containing 1% tryptone, 5% NaCl, and 3% agar was prepared. Once the media cooled to 40°C, moss extracts, prepared at a concentration of MIC/2 (2.445 mg), were added to the medium, which was then poured into glass petri dishes. The enriched strain was inoculated with a sterile toothpick onto the extract-containing media. As a positive control, inoculation with a sterile toothpick was performed on media without extracts. As a negative control, media containing extracts but without bacterial inoculation were used. All media were incubated at 37°C for 18-24 hours. After incubation, bacterial growth was measured from the inoculation center outward using a ruler, and the results were expressed in millimeters.

The method used to determine swarming inhibition was also adapted from the study by Déziel et al. [33]. To determine the inhibition of swarming motility by moss extract, the *P. aeruginosa* PA01 strain was streaked onto LB Agar and incubated at 37°C for 24 hours prior to the test. To observe swarming motility inhibition, a medium containing 8 g/L Nutrient Broth, 5 g/L dextrose, and 5% agar was prepared. Once the media cooled, moss extracts, prepared at a concentration of MIC/2 (2.445 mg), were added to the medium, which was then poured into glass petri dishes. The enriched strain was inoculated with a sterile toothpick onto the extract-containing media. As a positive control, inoculation with a sterile toothpick was performed on media without extracts. As a negative control, media containing extracts but without bacterial inoculation were used. All media were incubated at 37°C for 18-24 hours. After incubation, bacterial growth was measured outward from the inoculation center using a ruler, and the results were expressed in mm.

The method used to determine twitching inhibition was adapted from the study by Déziel et al. [33]. To assess the inhibition of twitching motility by moss extract, the *P. aeruginosa* PA01 strain was streaked onto LB Agar and incubated at 37°C for 24 hours prior to the test. To analyse twitching inhibition, a medium containing 20 g/L LB Broth and 1% agar was prepared. Once the media cooled, moss extracts, prepared at a concentration of MIC/2 (2.445 mg), were added to the medium, which was then poured into glass petri dishes. The enriched strain was inoculated with a sterile toothpick onto the extract-containing media, ensuring the toothpick touched the bottom of the petri dish, unlike in the swimming and swarming tests. As a positive control, inoculation with a sterile toothpick was performed on media without extracts. As a negative control, media containing extracts but without bacterial inoculation were used. All media were incubated at 37°C for 18-24 hours. After incubation, the media in the petri dishes were removed using a sterile lab spatula. The bottom of the petri dishes was stained with crystal violet and left for 15 minutes. Afterward, the crystal violet was discarded, and the petri dishes were rinsed with distilled water. The stained bacterial growth on the bottom of the petri dishes was measured from the inoculation center outward using a ruler, and the results were expressed in mm.

2.8. Gas Chromatography–Mass Spectroscopy Method (GC-MS)

Gas chromatography was employed to separate the chemical constituents present in the ethanol extract of *P. formosum*, while mass spectrometry (Agilent 8890 GC-MS instrument) was used for their identification. The analysis was carried out under the same conditions as described in the study by Tunca-Pinarli et al. [34].

2.9. Statistics

All biological activity assays of *P. formosum* moss were performed in triplicate. Statistical evaluations were conducted using R Studio v.2024.12.0. For each assay, the arithmetic mean of the three replicates was calculated. Differences among groups were analyzed using one-way ANOVA, considering a p-value below 0.05 as statistically significant. To assess the relationship between extract concentration and antimicrobial response in the disk diffusion test, the Pearson correlation coefficient was applied [35]. Antioxidant activity

results are presented as mean ± standard deviation (SD), based on three independent replicates. EC_{50} values were estimated using a Four-Parameter Logistic Regression model, with a 95% confidence interval.

3. THE RESEARCH FINDINGS AND DISCUSSION

3.1. Results of Antibacterial and Antifungal Activity

P. formosum ethanol extract was effective against 3 standards, 1 food isolate, 9 clinical isolates, and 6 multidrug-resistant strains. The results are given in Tables 1-8.

Table 1. *Results of P. formosum extract against standard strains (mm)*

Microorganisms	50 µL	100 µL	200 µL	Gentamicin (10 µg)	Tobramycin (10 µg)
Bacillus subtilis DSM 1971	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	30.00 ± 0.00	26.00 ± 0.00
Candida albicans DSM 1386	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00
Enterobacter aerogenes ATCC13048	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.00 ± 0.00	18.00 ± 0.00
Enterococcus faecalis ATCC 29212	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12.00 ± 0.00	8.00 ± 0.00
Escherichia coli ATCC25922	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 0.00	20.00 ± 0.00
Listeria monocytogenes ATCC 7644	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28.00 ± 0.00	24.00 ± 0.00
Pseudomonas aeruginosa DSM 50071	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.00 ± 0.00	22.00 ± 0.00
Pseudomonas fluorescens P1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 0.00	12.00 ± 0.00
Salmonella enteritidis ATCC 13076	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21.00 ± 0.00	15.00 ± 0.00
Salmonella typhimurium SL1344	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.00 ± 0.00	15.00 ± 0.00
Staphylococcus aureus ATCC 25923	8.00 ± 0.47	9.00 ± 0.00	0.00 ± 0.00	21.00 ± 0.00	14.00 ± 0.00
Staphylococcus epidermidis DSMZ 20044	9.00 ± 0.00	10.00 ± 0.47	0.00 ± 0.00	22.00 ± 0.00	20.00 ± 0.00
Staphylococcus hominis ATCC 27844	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 0.00	16.00 ± 0.00
Staphylococcus warneri ATCC 27836	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.00 ± 0.00	18.00 ± 0.00
Bacillus cereus RSKK 863	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.00 ± 0.00	18.00 ± 0.00
Shigella flexneri RSKK 184	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 0.00	17.00 ± 0.00
Acinetobacter baumannii CECT 9111	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 0.00	22.00 ± 0.00
Lactobacillus reuteri CECT 925	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00	26.00 ± 0.00
Pichia anomola HUT 7083	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	27.00 ± 0.00	27.00 ± 0,.00

Debaryomyces hansenii PYCC 2971	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	19.00 ± 0.00	21.00 ± 0.00
Kluyveromyces marxianus PYCC 2948	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17.00 ± 0.00	19.00 ± 0.00
Yarrowia lipolytica PYCC 4936	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 0.00	20.00 ± 0.00

Using the agar well diffusion method, the antibacterial activity of *P. formosum* methanol extract against six distinct microorganisms was assessed in the study by Akatın et al. [36]. The findings demonstrated that *P. formosum* methanol extract was efficient against four strains, with *S. epidermidis* exhibiting the strongest effect, with an inhibition zone measuring 13.26 mm in diameter. In contrast, as shown in Tables 1–4, this study used the disc diffusion method to test an ethanol extract of *P. formosum* against 53 distinct bacteria. Nine clinical isolates, three standard bacteria, one food-isolated bacterium, and six multidrug-resistant bacteria were among the 19 strains against which the *P. formosum* ethanol extract was effective. An inhibitory zone of 10 mm against the multidrug-resistant *S. aureus* MRSA strain was the best outcome. Differences between the studies can be attributed to factors such as the region of sample collection, variations in biochemical composition, differing extraction solvents, and the methodologies used. Despite these differences, the data from both studies support each other and demonstrate that *P. formosum* possesses antimicrobial activity.

Although *S. epidermidis* was traditionally regarded as a low-virulence organism, it is now widely acknowledged as an important opportunistic pathogen. The species is most commonly associated with hospital-acquired infections, particularly those linked to the use of medical devices. The most problematic infections arise on foreign materials -such as long-term catheters and implanted biomedical devices-where *S. epidermidis* readily establishes itself. The clinical management of such device-related infections is further complicated by the organism's ability to generate robust biofilms, a topic that will be examined in more depth below [37]. Notably, staphylococci constitute the predominant etiological agents of implant-associated infections (representing nearly 80% of cases), with *S. aureus* and *S. epidermidis* together responsible for approximately two-thirds of these incidents [38]. In light of the increasing clinical importance of *S. epidermidis*, the antibacterial activity observed for *P. formosum* presents a promising avenue for potential therapeutic applications.

Table 2. *Results of P. formosum extract against food isolate strains (mm)*

Microorganisms	50 µL	100 µL	200 µL	Gentamicin	Tobramycin
Enterococcus durans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.00	13.00 ± 0.00
Enterococcus faecium	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28.00 ± 0.00	15.00 ± 0.00
Klebsiella pneumoniae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	19.00 ± 0.00	23.00 ± 0.00
Listeria innocua	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	13.00 ± 0.00	15.00 ± 0.00
Salmonella infantis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17.00 ± 0.00	14.00± 0.00
Salmonella kentucky	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12.00 ± 0.00	16.00 ± 0.00
Escherichia coli	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00	0.00 ± 0.00

L. innocua is a Gram-positive bacterium with a ubiquitous presence, commonly found in diverse natural habitats, urban settings, and various food products [39]. This species is phylogenetically close to *L. monocytogenes*, a major foodborne pathogen responsible for human listeriosis an uncommon yet frequently

life-threatening infection [40]. Unlike *L. monocytogenes*, however, *L. innocua* is generally regarded as non-pathogenic in mammals. Despite this classification, sporadic occurrences of *L. innocua* associated septicemia and meningitis have been documented in humans [41] as well as in ruminants [42]. Within this framework, the antibacterial properties observed for the *P. formosum* extract suggest that it may serve as a promising candidate for addressing infections caused by *L. innocua* strains.

Table 3. *Results of P. formosum extract against clinic isolate strains (mm)*

Microorganisms	50 µL	100 µL	200 µL	Gentamicin	Tobramycin
Staphylococcus aureus	9.00 ± 0.47	10.00 ± 0.00	10.00 ± 0.00	22.00 ± 0.00	18.00 ± 0.00
Streptococcus mutans	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.00	22.00 ± 0,00	24.00 ± 0.00
Staphylococcus hominis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.00 ± 0.00	11.00 ± 0.00
Staphylococcus haemolyticus	0.00 ± 0.00	7.00 ± 0.47	7.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
Staphylococcus lugdunensis	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	17.00 ± 0.00	18.00 ± 0.00
Shigella boydii	8.00 ± 0.00	7.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00	18.00 ± 0.00
Acinetobacter baumannii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 0.00	16.00 ± 0.00
Shigella flexneri	7.00 ± 0.00	8.00 ± 0.47	9.00 ± 0.47	16.00 ± 0.00	14.00 ± 0.00
Staphylococcus aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 0.00	16.00 ± 0.00
Enterococcus faecalis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12.00 ± 0.00	10.00 ± 0.00
Klebsiella pneumoniae	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	18.00 ± 0.00	18.00 ± 0.00
Candida tropicalis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Candida glabrata	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.47	7.00 ± 0.00	8.00 ± 0.00

Shigellosis, an infectious disease caused by *S. flexneri* strains, is responsible for approximately 125 million diarrhoea cases worldwide each year and contributes significantly to the global burden of diarrhoea-related morbidity and mortality, resulting in approximately 160,000 deaths [43,44]. The infection occurs when the bacteria invade and proliferate within the epithelial cells of the colon, leading to symptoms such as fever, abdominal cramps, nausea, vomiting, and tenesmus. In light of this serious condition, as shown in Table 3, the antimicrobial activity exhibited by *P. formosum* extract against *S. flexneri* suggests its potential as an antimicrobial agent for the treatment of shigellosis, a growing global health concern in the coming years.

Human infections have been attributed to more than 17 different *Candida* species identified to date [45]. The pathogenic capabilities of these yeasts stem from several virulence traits, including mechanisms that enable evasion of host immune responses, strong adhesion and biofilm-forming capacity, as well as the secretion of hydrolytic enzymes -such as proteases, phospholipases, and hemolysins- that damage host tissues [46]. Notably, during the past twenty years, infections caused by Non-*Candida albicans Candida* species have risen markedly, with *C. glabrata* emerging as the most prominent contributor to this increase [47]. Clinically, *C. glabrata* represents a growing challenge, being responsible for mucosal infections and accounting for nearly 15% of systemic bloodstream infections linked to *Candida* species [48]. In view of

the escalating clinical relevance of *C. glabrata*, the antibacterial activity demonstrated by *P. formosum* in this study highlights its potential as a promising antimicrobial candidate.

Table 4. *Results of P. formosum extract against multi-drug resistance strains (mm)*

Microorganisms	50 µL	100 µL	200 µL	Gentamicin	Tobramycin
Escherichia coli	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00
Klebsiella pneumoniae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.00 ± 0.00	20.00 ± 0.00
Acinetobacter baumannii	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Enterobacter aerogenes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16.00 ± 0.00	18.00 ± 0.00
Serratia odorifera	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00
Proteus vulgaris	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 0.00	11.00 ± 0.00
Streptococcus pneumoniae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	8.00 ± 0.00
Staphylococcus aureus MRSA	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.47	0.00 ± 0.00	7.00 ± 0.00
Staphylococcus aureus MRSA + MDR	8.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.47	22.00 ± 0.00	21.00 ± 0.00
Providencia rustigianii	8.00 ± 0.47	9.00 ± 0.00	9.00 ± 0.00	16.00 ± 0.00	19.00 ± 0.00
Achromobacter sp.	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00	0.00 ± 0.00

There has been a significant global increase in infections caused by *Staphylococcus* bacteria. According to reports from the USA Centers for Disease Control and Prevention (CDC), *S. aureus* is now recognised as the second most common bacterial pathogen causing disease, following *E. coli*. The widespread and uncontrolled use of antibiotics has led to the emergence of various pathogenic bacteria exhibiting antimicrobial resistance [49]. Deaths caused by antibiotic-resistant bacteria have surpassed those attributed to the leading known causes of mortality worldwide. Given its role in clinical treatment challenges and prolonged hospital stays, the antibiotic resistance observed in *S. aureus* poses a serious public health threat on an international scale [50]. In this context, as presented in Table 4, the antimicrobial activity demonstrated by *P. formosum* extract against resistant *S. aureus* strains is of great significance.

Table 5. *MIC results of P. formosum extract against standard strains (mg/mL)*

Microorganisms	*P. formosum*
Candida albicans DSM 1386	0.064 ± 0.00
Enterococcus faecalis ATCC 29212	0.509 ± 0.00
Listeria monocytogenes ATCC 7644	0.127 ± 0.00
Pseudomonas fluorescens P1	1.019 ± 0.00
Staphylococcus aureus ATCC 25923	0.016 ± 0.00

Staphylococcus epidermidis DSM 20044	1.019 ± 0.00
Lactobacillus reuteri CECT 925	4.075 ± 0.00
Pichia. anomola HUT 7083	1.019 ± 0.00
Debaryomyces hansenii PYCC 2971	0.127 ± 0.00
Kluyveromyces marxianus PYCC 2948	0.255 ± 0.00
Yarrowia lipolytica PYCC 4936	0.064 ± 0.00

In the study conducted by Akatın et al. [36], the MIC test was also used to determine the antimicrobial activity of the moss *P. formosum*. The test results showed that the *P. formosum* extract was effective at concentrations of 33.3 mg/mL against *S. epidermidis* and *S. aureus* strains and 66.6 mg/mL against *B. cereus* strains. According to the MIC test results performed in this study, *P. formosum* extract showed activity against the standard strain of *S. aureus* at 0.012 mg/mL (Table 5), against the clinical strain at 0.096 mg/mL (Table 7), and against the multidrug-resistant strain at concentrations of 0.774 mg/mL and 0.096 mg/mL (Table 8).

Table 6. *MIC results of P. formosum extract against food isolate strains (mg/mL)*

Microorganisms	*P. formosum*
Enterococcus durans	0.127 ± 0.00
Enterococcus faecium	0.032 ± 0.00
Listeria innocua	0.127 ± 0.00
Escherichia coli	0.127 ± 0.00

In the conducted antimicrobial activity tests, it was determined that the *P. formosum* extract exhibited the lowest concentration of effect against *E. faecium* among food-derived strains. Although enterococci are normally considered low pathogenic, they become significant pathogens in hospital infections due to the easy transfer of virulence factors, resistance genes, and genes on plasmids [51]. *E. faecium*, an opportunistic pathogen, is a microorganism with a steadily increasing frequency of causing infections. It is known that enterococci cause endocarditis, urinary tract infections, bloodstream infections, wound infections, and surgical site infections [52-54]. The strong result obtained against *E. faecium*, a global public health problem, as shown in Table 6, may make *P. formosum* a significant antimicrobial agent in the future.

Table 7. *MIC results of P. formosum extract against clinic isolate strains (mg/mL)*

Microorganisms	*P. formosum*
Staphylococcus aureus	0.127 ± 0.00
Staphylococcus mutans	0.255 ± 0.00
Staphylococcus hominis	2.038 ± 0.00

Staphylococcus haemolyticus	0.096 ± 0.00
Enterococcus faecalis	0.127 ± 0.00

As presented in Table 7, in the MIC assays performed on clinically obtained isolates, the extract from *P. formosum* showed variable levels of antimicrobial activity against all examined *Staphylococcus* strains. *Staphylococcus* species are considered among the most significant human pathogens and are notably capable of swiftly acquiring resistance to numerous antimicrobial agents [55]. They also exhibit a broad spectrum of resistance strategies, which contributes to the ongoing rise in multidrug resistance seen in this genus [56]. Their pathogenic success is largely attributed to their efficient use of various virulence elements and immune evasion tactics, allowing them to cause both short-term and persistent infections across different tissues and organs [57]. Therefore, the antimicrobial potential observed in the *P. formosum* extract is of considerable significance.

Table 8. *MIC results of P. formosum extract against multi-drug resistance isolate strains (mg/mL)*

Microorganisms	*P. formosum*
Proteus vulgaris	2.038 ± 0.00
Streptococcus pneumoniae	0.509 ± 0.00
Staphylococcus aureus MRSA	1.019 ± 0.00
Staphylococcus aureus MRSA + MDR	0.127 ± 0.00
Providencia rustigianii	0.032 ± 0.00

As shown in Table 8, in the conducted study, the *P. formosum* extract exhibited antimicrobial efficacy against multidrug-resistant strains. Globally, drug resistance is escalating due to the indiscriminate use of antimicrobial agents. The treatment of resistant microorganisms is challenging, necessitating alternative or higher doses of antimicrobials or encountering a deficiency/scarcity of effective antimicrobials, which adversely impacts nations across all levels of development. According to the World Health Organization (WHO), multidrug-resistant (MDR) pathogens, also known as 'superbugs,' constitute one of the most significant public health threats, causing several million deaths annually worldwide [58, 59]. *P. rustigianii* is commonly found in wounds, respiratory tracts, urinary tracts, the perineum, axillae, and human blood and faeces [60]. *Providencia* species naturally exhibit resistance to tigecycline and polymyxins, two antibiotics commonly regarded as final treatment options for combating drug-resistant pathogens [61]. In this context, the effect demonstrated by the *P. formosum* extract presents potential as a significant antimicrobial agent against *P. rustigianii* in the future.

3.2. Results of Antioxidant Activity

The DPPH and CUPRAC methods were used to determine the antioxidant activity of the moss samples. In both methods, *P. formosum* extract exhibited high antioxidant activity. The results are given in Figure 1 and Table 9.

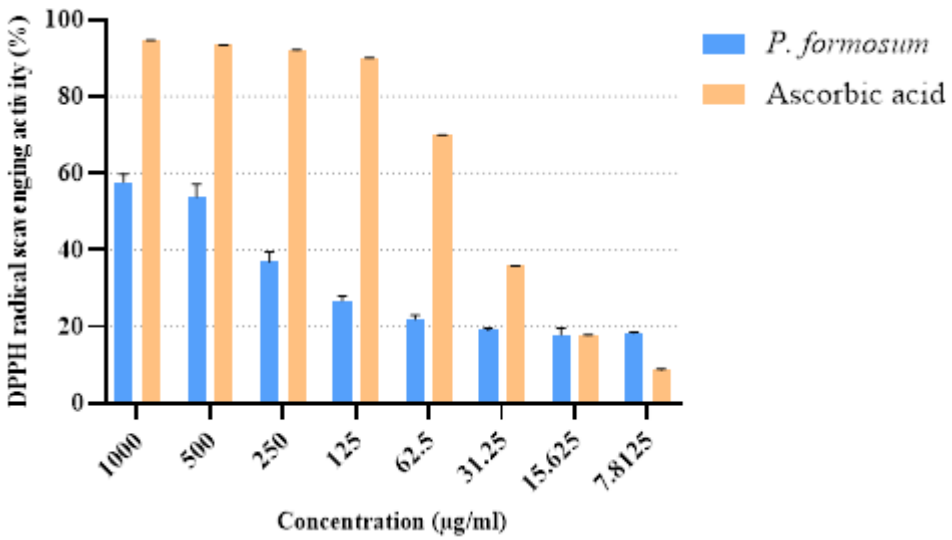

Figure 1. *Results of P. formosum and ascorbic acid's DPPH radical scavenging activity (%)*

In the study conducted by Smolińska-Kondla et al. [62], the antioxidant activities of water and ethanol extracts from five moss samples, including *P. formosum*, were determined. The calibration curve was prepared using Trolox, and the results were expressed in µmolTE/L. The ethanol extract of *P. formosum* was found to scavenge 50% of the DPPH radicals at a value of 128.47±0.45 µmolTE/L and 90% at a value of 23.00±0.57 µmolTE/L. In this study, the ethanol extract of *P. formosum* was found to scavenge 50% of the DPPH radicals at a value expressed in micrograms, which was determined to be 0.2718 µg. Both studies demonstrate that the *P. formosum* species possess antioxidant activity and support each other.

The CUPRAC method was used to determine the antioxidant activity of *P. formosum* extract. The test results were expressed in terms of ascorbic acid equivalents (AAE). A literature review revealed that there is no previous study demonstrating the antioxidant activity of *P. formosum* using the CUPRAC method. This study is the first to be conducted in this area.

Table 9. *CUPRAC results of P. formosum*

Concentration (mg/mL)	*P. formosum* (AAE)
10.00	1.578 ± 0.00
7.5	1.263 ± 0.00
5.0	0.948 ± 0.06
2.5	0.633 ± 0.01
1.0	0.398 ± 0.01

In the conducted CUPRAC antioxidant activity assay, the notable antioxidant potential of the *P. formosum* extract was elucidated. The assay results demonstrated a linear increase in antioxidant activity with increasing concentrations of the *P. formosum* extract. Notably, the exhibition of significant antioxidant activity even at low concentrations (0.398 mM at 1.00 mg/mL) suggests that this extract could serve as a robust antioxidant source. Ascorbic acid, utilised as a comparative standard, confirmed its well-established high antioxidant capacity, displaying potent antioxidant activity across a broad concentration range (0.1 mM to 10.00 mM). However, the impressive performance of the *P. formosum* extract as a natural source underscores its potential as an alternative to synthetic antioxidants. The observation of 1.578 mM

antioxidant activity at a concentration of 10.00 mg/mL for the *P. formosum* extract indicates its potential to occupy a significant position among natural antioxidants. Consequently, the *P. formosum* extract can be considered a promising candidate in the pursuit of natural antioxidants and presents substantial potential for future research.

3.3. Results of Total Phenolic Substance

The total phenolic content of the *P. formosum* extract (7.5 mg/ml) was calculated as milligrams of gallic acid equivalent (mg GAE) and was determined to be 190.05 ± 8.79 mg GAE.

3.4. Results of Anti-Quorum Sensing Activity

The results of the tests conducted to determine the anti-quorum sensing activity of *P. formosum* extract are presented in Figures 2 and 3.

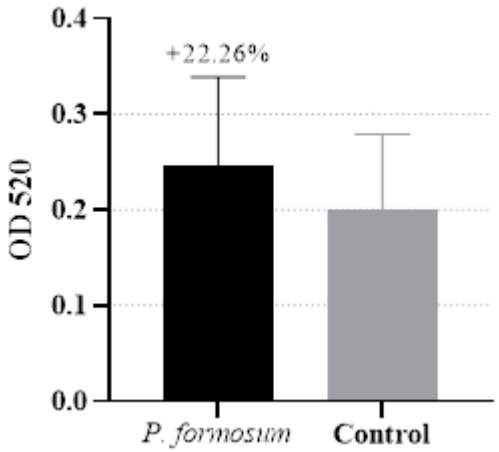

Figure 2. *Pyocyanin result of P. formosum*

Pyocyanin, a characteristic blue phenazine pigment secreted by a significant majority (90-95%) of *P. aeruginosa* strains, is closely associated with and augments the expression of virulence factors in this bacterium [63]. Furthermore, strains capable of pyocyanin production exhibit a more pronounced pathogenic potential and increased resistance to various therapeutic agents compared to their non-producing counterparts [64]. Given that the pyocyanin-eDNA complex influences cellular surface hydrophobicity, thereby facilitating the development of robust biofilm architectures, pyocyanin plays a substantive role in biofilm formation [65]. Consequently, the capacity of *P. formosum* to inhibit pyocyanin biosynthesis holds critical significance in combating *P. aeruginosa* infections.

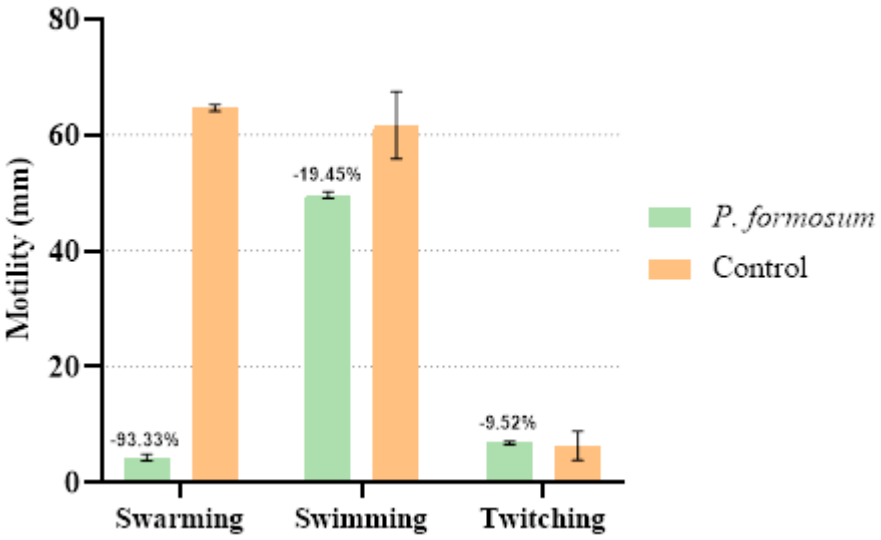

Figure 3. *Motility results of P. formosum*

Bacteria possess a diverse array of virulence factors implicated in infection processes, among which motility plays a critical role, particularly in the pathogenesis of skin infections [66]. Motility can undertake various functions during host-microbe interactions. For instance, in the initial stages of biofilm formation, planktonic bacteria utilize their flagella for movement and pili for surface attachment, enabling them to swim freely near the surface [67, 68]. *P. aeruginosa*, a prevalent opportunistic pathogen in wound infections, exacerbates the cutaneous inflammatory response and enhances resistance to antimicrobial agents through its capacity for biofilm formation. In this context, our study investigated the effects of *P. formosum* extract on different motility types of *P. aeruginosa.* The findings revealed that the *P. formosum* extract did not exhibit a significant effect on swarming and swimming motility, while a slight reduction was observed in twitching motility. The effect of *P. formosum* extract on *P. aeruginosa*'s twitching motility suggests a potential for hindering early biofilm formation and dissemination.

The conducted study determined that *P. formosum* extract did not exhibit an inhibitory effect on violacein production in the *P. aeruginosa* strain.

3.5. Results of Biochemical Composition

The data obtained from GC-MS analysis, conducted to determine the biochemical composition of *P. formosum* extract, are presented in Table 10, and the corresponding chromatogram is shown in Figure 4.

Table 10. *P. formosum biochemical content list*

Retention Time	Area (%)	Compound Name	Formula
5.05	0.57	Isopropoxycarbamic acid, ethyl ester	$C_6H_{13}NO_3$
6.358	7.07	Pyrrolidine-alpha.alpha.alpha.alpha'-D4	$C_4H_5D_4N$
14.637	0.64	6-amino-3-methyl-5-nitroso-1H-pyrimidine-2,4-dione	$C_4H_4N_4O_3$
22.583	1.25	E-11,13-Tetradecadien-1-ol	$C_{14}H_{26}O$
23.262	0.63	Terpinolen	$C_{10}H_{16}$

24.946	0.72	1,5-Anhydro-d-fructose	$C_6H_{12}O_5$
25.866	8.24	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$
26.495	0.33	Phosphoric acid, diethyl octyl ester	$C_{12}H_{27}O_4P$
30.109	2.78	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4 methylimidazolium ion	$C_{24}H_{45}N_2O_3$
30.928	0.84	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	$C_{10}H_{12}O_4$
32.426	1.28	Tridecane, 3-methylene-; 1-Dodecene, 2-ethyl-; 2 Ethyl-1-dodecene #	$C_{14}H_{28}$
32.593	0.33	Eicosyltrichlorosilane	$C_{20}H_{41}ClSi$
32.872	0.55	Epoxypseudobisoeugenyl-2-methylbutyrate	$C_{15}H_{20}O_4$
32.938	1.13	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, ethyl ester	$C_{11}H_{14}O_4$
33.87	4.38	3-Eicosyne	$C_{20}H_{38}$
34.064	0.80	Oxirane, tetradecyl-	$C_{16}H_{32}O$
34.557	0.57	1-Tridecyne	$C_{13}H_{24}$
34.794	0.51	Diisobutyl phthalate	$C_{16}H_{22}O_4$
35.058	1.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$
36.257	0.91	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8 dione	$C_{17}H_{24}O_3$
37.429	0.65	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$
38.264	4.60	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$
39.533	1.27	4-Hexadecen-6-yne, (E)-	$C_{16}H_{28}$
39.704	1.07	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$
41.614	0.80	2-Hexadecen-1-ol	$C_{20}H_{40}O$
42.929	3.18	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$
43.131	12.96	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$
43.267	4.27	13-Tetradece-11-yn-1-ol; 13-Tetradecen-11-yn-1-ol #	$C_{14}H_{24}O$
44.521	0.39	Dipentene diepoxide	$C_{10}H_{16}O_2$
46.652	0.38	Pyridinium, 1-hexadecyl-, chloride, monohydrate	$C_{21}H_{38}N$
52.878	0.50	Diazoprogesterone	$C_{21}H_{30}N_4$

59.344	1.92	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$
62.108	15.45	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	$C_{14}H_{24}$
71.33	3.45	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$

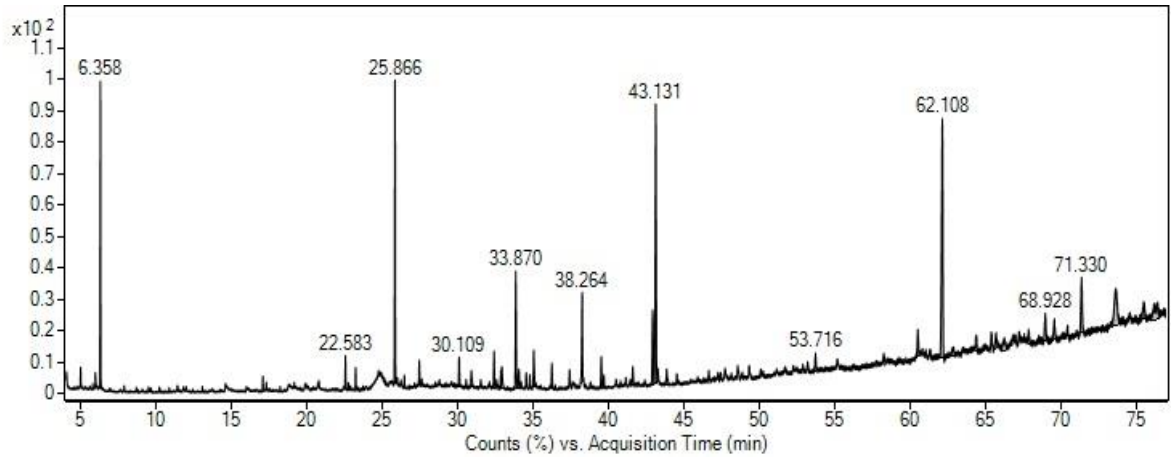

Figure 4. *GC-MS chromatogram of P. formosum*

The GC-MS analysis of the ethanol extract of *P. formosum* revealed several compounds with known biological activities, supporting the observed bioactivities in this study. Notably, phenol, 2,4-bis(1,1-dimethylethyl)- has been shown to inhibit biofilm formation regulated by quorum sensing in *Serratia marcescens*, a uropathogenic bacterium. This compound significantly reduced the production of protease, hemolysin, lipase, prodigiosin, and extracellular polysaccharides in *S. marcescens*, leading to an 85% decrease in biofilm formation [69]. Linoleic acid has also been shown to interfere with quorum sensing-mediated virulence and biofilm development in *P. mirabilis* and *S. marcescens* [70]. Moreover, 1,5-anhydro-D-fructose exhibits inhibitory effects on Gram-positive bacterial growth and *S. aureus* MRSA biofilm formation. [71]. dl-α-Tocopherol (Vitamin E) has also been found to disrupt quorum sensing mechanisms in *Pseudomonas aeruginosa*, leading to a reduction in biofilm formation and virulence factor production. This compound significantly decreased the levels of pyocyanin, pyoverdine, and proteases [72].

Additionally, linoleic acid ethyl ester is reported as an antibacterial and quorum sensing inhibitor, capable of disrupting bacterial cell membranes and preventing biofilm formation. In terms of antioxidant function, dl-α-tocopherol (Vitamin E) is essential for safeguarding cellular membranes against oxidative stress and supporting the overall antioxidant defence mechanisms. This compound is known to neutralise free radicals, thereby reducing cellular stress [73].

Overall, the predominance of polyunsaturated alcohols, fatty acid esters, phenolic compounds, and terpenoid-like structures suggests that the antimicrobial activity observed in P. formosum may arise from the combined or synergistic effects of these bioactive chemical classes. Such compounds are well documented for their ability to interfere with microbial membrane stability and metabolic processes [74-77].

However, the biological activities of some other detected compounds remain poorly documented in the literature. Specifically, E-11,13-Tetradecadien-1-ol, 3-Eicosyne, and 9,12,15-Octadecatrien-1-ol (Z,Z,Z) are long-chain hydrocarbons and oxygenated compounds whose antibiofilm and quorum sensing inhibitory properties require further investigation. Nevertheless, the observed antimicrobial, antibiofilm, and antioxidant activities of *P. formosum* could be directly linked to its phenolic and fatty acid derivatives.

Therefore, expanding research on the biological effects of secondary metabolites in bryophytes will contribute to a better understanding of the pharmacological potential of natural compounds.

In the study conducted by Okan [78] in 2025, the fatty acid profile of *Polytrichum formosum* was characterized by GC–MS, with oleic acid (36.6%) and palmitic acid (35.5%) identified as the predominant constituents. In contrast, the present investigation employed GC–MS to analyze the ethanol extract of *Polytrichastrum formosum*, reporting specific bioactive compounds associated with biological activities. Although both studies utilized the GC–MS technique and emphasized the relevance of fatty acids, Okan's work primarily addressed quantitative fatty acid distribution and nutritional aspects, whereas the current study identified distinct bioactive metabolites (e.g., 2,4-di-tert-butylphenol) and correlated them with antimicrobial and antioxidant activities. In addition, despite examining the same taxon, Okan's study did not adopt the updated genus designation *Polytrichastrum*.

4. CONCLUSION

This study highlights the significant biological activities and therapeutic potential of *P. formosum*. The ethanol extract demonstrated notable antibacterial, antifungal, and antioxidant activities, along with anti-quorum sensing properties, suggesting its potential as an alternative to conventional antibiotics in the face of rising antimicrobial resistance. Antioxidant tests using DPPH and CUPRAC methods revealed that *P. formosum* could serve as a promising natural antioxidant source, with results comparable to ascorbic acid. Additionally, GC-MS analysis identified the presence of compounds with known biological activities, providing insights into the components responsible for their observed bioactivities. Overall, *P. formosum* shows promising potential as a natural source for the development of novel antimicrobial and antioxidant agents, and further research into its secondary metabolites is needed to better understand its pharmacological potential.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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