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Bioactivity of *Pseudomonas azotoformans* UICC B-91 as an agent with antimicrobial activity, *Salmonella typhi and Microsporum canis*

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ABSTRACT

Infectious diseases caused by bacterial and fungal pathogens remain a major public health concern, particularly in the context of increasing antimicrobial resistance. This study aimed to evaluate the in vitro antimicrobial bioactivity of *Pseudomonas azotoformans* UICC B-91 extract against *Salmonella typhi* and *Microsporum canis.* A quantitative experimental design with a completely randomized design (CRD) was applied using three extract concentrations (30%, 50%, and 100%). Antibacterial activity against *S. typhi* was assessed using the disc diffusion method, while antifungal activity against *M. canis* was evaluated using the agar well diffusion method. Inhibition zones were measured and analyzed using SPSS with one-way ANOVA. The results showed that the extract exhibited weak antibacterial activity against *S. typhi*, with inhibition zones ranging from 1.30 ± 0.28 mm to 2.00 ± 0.71 mm, and statistical significance observed only at the 50% concentration ($p = 0.039$). In contrast, moderate antifungal activity against *M. canis* was observed, with a maximum inhibition zone of 6.50 ± 0.71 mm, and ANOVA confirmed a significant effect among treatments ($F = 803.936$; $p = 0.026$). These findings indicate that *Pseudomonas azotoformans* UICC B-91 extract contains bioactive compounds with measurable antimicrobial activity under in vitro conditions, particularly against dermatophyte fungi.

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INTRODUCTION

Infectious diseases remain a major global health burden, particularly in developing countries, where bacterial and fungal pathogens continue to cause substantial morbidity and mortality. One of the most important bacterial pathogens is *Salmonella typhi*, the etiological agent of typhoid fever, a systemic infection transmitted primarily through contaminated food and water. Typhoid fever can lead to severe gastrointestinal disturbances, septicemia, and fatal complications if not adequately treated (Destiawan et

al., 2024; Khadka et al., 2021). Antibiotics have been the cornerstone of typhoid fever management; however, the increasing emergence of antibiotic-resistant *Salmonella* strains has significantly reduced treatment effectiveness and prolonged disease transmission (Narimisa et al., 2024; Kadariswantiningsih et al., 2025). In parallel, pathogenic fungi particularly dermatophytes such as *Microsporum canis* pose a serious yet often underestimated health problem. *M. canis* is a major causative agent of dermatophytosis (tinea) in animals and humans, with zoonotic transmission playing a key role in its spread (Pasquetti et al., 2017; Moriello (2020).

The prevalence and epidemiological impact of *S. typhi* and *M. canis* infections show regional variation but remain consistently high. In Europe, *M. canis* is one of the leading causes of tinea capitis in children, particularly in urban settings with close contact between humans and companion animals (Pasquetti et al., 2017). In contrast, tropical countries such as Indonesia report markedly higher dermatophytosis prevalence, reaching approximately 40–50%, with *M. canis* identified as the dominant species (Tan et al., 2021). Similarly, typhoid fever remains endemic in Indonesia and other Southeast Asian countries, driven by poor sanitation, limited access to clean water, and inadequate hygiene practices (Destiawan et al., 2024). Although antibiotics and antifungal agents are widely used, persistent infection rates and recurrent cases indicate that existing therapeutic strategies are insufficient, particularly when bacterial and fungal infections are considered separately rather than as part of a broader antimicrobial resistance (AMR) problem.

Antimicrobial resistance has emerged as one of the most critical global health threats of the 21st century. Empirical data indicate a high prevalence of extended-spectrum β-lactamase (ESBL)-producing *Salmonella* isolates in Indonesia, with pooled resistance rates approaching 46% over the last decade (Kadariswantiningsih et al., 2025). At the same time, resistance and reduced susceptibility to commonly used antifungal agents, such as azoles, have been reported in dermatophytes including *M. canis*, particularly following prolonged or inappropriate antifungal use (Moriello, 2020; Sakan & Yohanes, 2021). These findings highlight the urgent need for alternative antimicrobial agents with novel mechanisms of action. Natural antimicrobials derived from microorganisms have gained increasing attention due to their chemical diversity and lower risk of cross-resistance compared with conventional antibiotics (Bayot & Bragg, 2024). However, experimental evidence evaluating their efficacy against both bacterial and fungal pathogens within a single study framework remains limited.

One promising source of alternative antimicrobials is *Pseudomonas azotoformans*, a bacterium known to produce a variety of bioactive secondary metabolites, including rhamnolipids and alkaloid compounds with broad-spectrum antimicrobial activity (Pratiwi et al., 2022). Previous studies have reported the inhibitory effects of *P. azotoformans* against selected bacterial pathogens and opportunistic fungi; nevertheless, systematic investigations targeting clinically relevant pathogens such as *Salmonella typhi* and *Microsporum canis* are still scarce. Moreover, most existing studies focus on either antibacterial or antifungal activity alone, leaving a clear research gap in evaluating dual antimicrobial potential. Addressing this gap is crucial to advancing the development of alternative antimicrobials capable of mitigating AMR across different pathogen groups. Therefore, this study aims to evaluate the antimicrobial bioactivity of *Pseudomonas azotoformans* UICC B-91 extract against *Salmonella typhi* and *Microsporum canis* using standardized diffusion-based assays, providing empirical evidence for its potential application as a natural antimicrobial agent.

RESEARCH METHODS

This study used a quantitative experimental approach with a completely randomized design (CRD) to evaluate the antimicrobial activity of *Pseudomonas azotoformans* UICC B-91 extract. The experiment consisted of three extract concentrations (30%, 50%, and 100%), one positive control, and one negative control for each test organism. Each treatment was performed in duplicate, resulting in ten experimental units per pathogen. All treatments were randomly assigned to homogeneous experimental units to minimize bias and ensure valid statistical comparison.

The bacterial test organism was *Salmonella typhi* ATCC 14028, obtained from a certified culture collection, while the fungal test organism was *Microsporum canis*, a local clinical isolate from the Microbiology Laboratory of Universitas Indraprasta PGRI. Both organisms were selected using purposive sampling based on their clinical relevance and prevalence.

Pseudomonas azotoformans UICC B-91 was obtained from the University of Indonesia Culture Collection (UICC) and cultured on Nutrient Agar followed by Nutrient Broth incubation. Bioactive compounds were extracted using a maceration method with ethanol and ethyl acetate as solvents to obtain crude extracts containing polar and semi-polar metabolites. The solvent phase was separated using a separating funnel and concentrated by rotary evaporation. The crude extract was diluted with sterile distilled water to final concentrations of 30%, 50%, and 100% (v/v) for antimicrobial testing.

Antibacterial activity against *Salmonella typhi* was assessed using the disc diffusion method on Nutrient Agar, while antifungal activity against *Microsporum canis* was evaluated using the agar well diffusion method on Potato Dextrose Agar. Amoxicillin and ketoconazole were used as positive controls for antibacterial and antifungal assays, respectively, and sterile distilled water served as the negative control. Inhibition zones were measured in millimeters using a vernier caliper.

Data were analyzed using SPSS version 24. Normality and homogeneity assumptions were tested using the Shapiro–Wilk and Levene's tests, respectively. Normally distributed data were analyzed using one-way ANOVA, while non-parametric data were analyzed using the Mann–Whitney test. Statistical significance was determined at $\alpha = 0.05$.

The test bacteria used were *Salmonella typhi* ATCC 14028. Before being tested with *Pseudomonas azotoformans* UICC B-91 extract, *S. typhi* bacteria must first be reinnoculated using NA media inoculated with *S. typhi* through the scratch plate method and then incubated at 37°C for 24-48 hours (Rachman, 2018; Sondang et al., 2018). Furthermore, morphological characterization was carried out microscopically with a light microscope to further ensure that the bacteria used were *S. typhi*.

The test mold isolation used was *Microsporum canis*. The isolate was a local isolate of pure culture of the Microbiology Laboratory, Indraprasta PGRI University. The *M. canis* test mold originating from pure culture was taken in one loop then inoculated on Sabouraud Dextrose Agar (SDA) medium and then incubated at 37^{o}C for 24 hours. To further ensure the *M. canis* fungus, morphological identification was carried out using a light microscope. The reinnoculated fungus was then used as the test fungus.

The glass object is cleaned first to be free from fat. After that the glass object is fixed using a spirit lamp flame. Drop Lactophenol Cotton Blue on the glass object. The fungal colony is taken using an ose needle (which has been fixed beforehand) aseptically then the colony is flattened. Mix the fungal colony with lactophenol cotton blue (Shamly et al., 2014). Cover with deglass and observe under a microscope with a 10x10 magnification lens and continued with a 40x10 magnification lens.

Pseudomonas azotoformans extract is carried out using the maceration method, namely using ethanol and ethyl acetate extraction to isolate bioactive compounds (Fakhruzy, 2020). In the initial stage of extraction, *P. azotoformans* bacteria Streak is carried out to grow using NA medium for 2-3 days, then

left until the colonies of the fungus spread evenly on the NA medium. After that, the bacterial colonies are inserted into NB medium and then incubated. The addition of ethanol or ethyl acetate to NB medium containing bacteria serves to extract compounds. Next, it is separated using a separating funnel and concentrated using a rotational evaporator. This process produces an extract with a more perfect and thicker content or concentration of bioactive compounds. This maceration extraction method is very effective because it does not take a long time and this method can produce a thicker and more perfect *P. azotoformans* extract. Extraction or maceration is the process of separating a substance based on the difference in its solubility in two liquids that do not dissolve in each other. The function of extraction is to dissolve polar compounds in polar solvents and non-polar compounds in non-polar compounds (Fakhruzy, 2020) and to facilitate the transfer of compounds from solids to solvents. This method involves soaking natural materials in a solvent, usually air or an organic solvent, and allowing the solvent to stand for some time (Utami et al., 2020). This stage changes the form of the solvent from solution to vapor, to separate a solvent from a solution, thus producing an extract with a more concentrated content or concentration or according to requirements (Tetti, 2014).

Antibacterial bioactivity of *Pseudomonas azotoformans* UICC B-91 extract against *Salmonella typhi* ATCC 14028 was evaluated using a disc diffusion assay, selected due to its simplicity, reproducibility, and widespread acceptance as a preliminary screening method for antimicrobial activity. This technique enables direct comparison of inhibition zone diameters among different extract concentrations and control treatments under standardized conditions. The experiment was designed using a completely randomized design (CRD), in which all treatment conditions were randomly assigned to homogeneous experimental units (agar plates inoculated with *S. typhi*). The treatments consisted of three extract concentrations (30%, 50%, and 100%), a positive control (amoxicillin), and a negative control (sterile distilled water). Each treatment was performed in duplicate (two independent replications), resulting in ten experimental units in total for antibacterial testing. Replication was applied to account for experimental variability and to ensure data reliability for statistical analysis. A standardized bacterial suspension equivalent to 0.5 McFarland was evenly spread onto the surface of Nutrient Agar plates. Sterile paper discs were impregnated with a fixed volume of *P. azotoformans* extract and placed aseptically onto the inoculated agar surface. The plates were incubated at 37 °C for 24 hours. After incubation, antibacterial activity was quantified by measuring the diameter of the clear inhibition zone surrounding each disc using a vernier caliper. The mean inhibition zone diameter from replicate treatments was used for further statistical analysis.

Antifungal activity testing was carried out using the agar well diffusion method. Spore suspension (10^6 spores/mL) of *Microsporum canis* mold derived from a 5-day-old culture was inoculated onto the surface of PD A in a petri dish using a sterile cotton swab, then a paper disc that had been dripped with 2 drops of *Pseudomonas azotoformans* extract test solution was given with concentrations (30 % , 50 % and 100 %). For the positive control in the form of ketoconazole and its negative control in the form of distilled water and then incubated at a temperature of 28^{o}C for 72 hours. This anti-fungal activity test was carried out with 2 repetitions. Disc paper is used to measure the diameter of the inhibition zone formed around it. The diameter of the clear area formed around the paper disc was measured using a vernier caliper. The larger the diameter of the inhibition zone, the lower the concentration of the inhibitory compound (Sundari, 2022). The clear area is considered as the inhibition zone where the inhibition zone less than 5 mm is considered weak, the inhibition zone of 5-10 mm is considered moderate, the inhibition zone of 10-20 mm is considered strong, and inhibition zones greater than 20 mm are considered very strong (Figure 1).

Dm_1 = Diameter of Vertical Inhibition Zone
Dm_2 = Horizontal Bland Zone Diameter
Dm_3 = Disc/Well Diameter

$$L = \frac{(Dm_1+Dm_3)+(Dm_2+Dm_3)}{2} \quad (1)$$

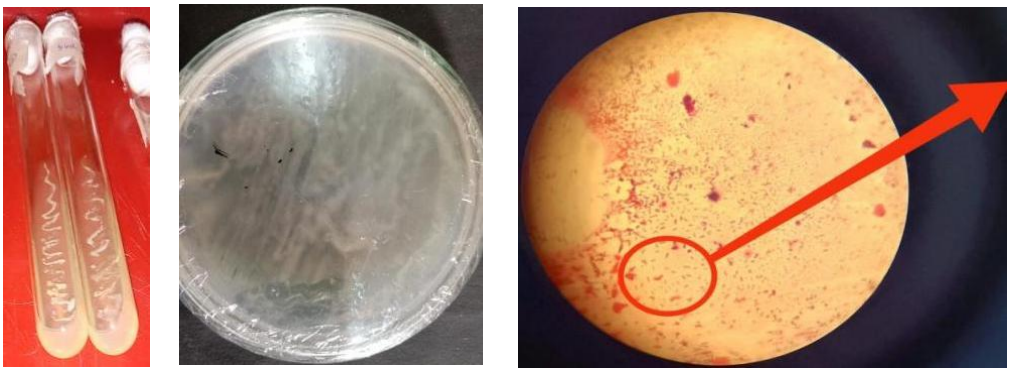
$$L = \frac{(Dm_1-6)+(Dm_2-6)}{2} \quad (2)$$

Figure 1. Obstacle Zone Measurement (Sumber: Tiwa et al., 2017)

Quantitative data analysis techniques are implemented and the results are used as accurate data collected in the final research sheet. Quantitative data were analyzed statistically using ANOVA (one-way) and Mann–Whitney tests in Statistical Product and Service Solution (SPSS) for windows versi 24 to determine the significance of inhibitory effects

FINDING AND DISCUSSION

To confirm the identity and purity of the bacterial test organism used in this study, macroscopic and microscopic observations of *Salmonella typhi* ATCC 14028 were conducted prior to antimicrobial testing.



(a) (b) (c)

Figure 2. Test Bacterial Isolate *Salmonella typhi* ATCC 14028:
(a) Pure Culture, (b) Macroscopic Appearance, (c) Microscopic Appearance

Based on the results obtained from microscopic observations (Figure 2) of the test bacteria used in this study, *S. typhi* bacteria are known to have a rod-like shape and are pinkish red in color, so the bacteria are Gram-negative. In the early stages of the observation process, samples of test bacteria were taken from the NA medium to the preparation. The Gram staining technique is used to determine the staining results of *S. typh*i bacteria. According to Utami et al., (2020) *S. typhi* is a rod-shaped bacteria and the size ranges from 0.7 - 2-5.

For microscopic structural observation, the *S. typhi* bacteria observed were pink and rod-shaped, indicating Gram-negative properties, so they were given violet, lugol, 70% alcohol, and fuchsin sequentially, with each stage interspersed with burning on Bunsen burner. These bacteria also remain red so it can be confirmed that they are bacteria. Gram-negative, which means they do not have a peptidoglycan layer like Gram- positive bacteria (Fitri et al., 2020)

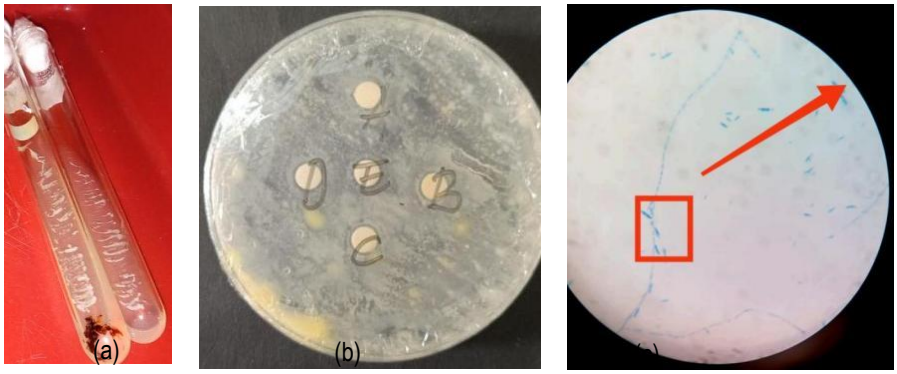


Figure 3. *Microsporum canis* Test Mold Isolate :
(a) Pure Culture, (b) Macroscopic Appearance, (c) Microscopic Appearance

Based on the results of microscopic observations of *Microsporum canis* (Figure 3) shows that when viewed microscopically, the fungus is white and shaped like a spider . There are small protrusions similar to thorns on the surface. *M. canis* has septate hyphae and many fusiform macroconidia with thick, rough walls at the tips. One macroconidia has six up to eight septa (Maniagasi, 2019).

In this observation, *M. canis* used for this study was stained with LPCB. The function of LPCB itself is as a dye that can show the structure of fungal hyphae and macroconidia. This staining is very helpful in identifying *M. canis* because it can show the unique structure of hyphae and macroconidia. Staining also helps in finding other species that are similar to *M. canis*. The technique used is the fungus that has been grown on Sabouraud Dextrose Agar (SDA) medium is then taken and dripped with aquades on a glass object. Then observed using a microscope at 40x magnification until the colony shape of the mold is visible. The use of LPCB as a dye can help determine the microscopic form of *M. canis* (Paramata, 2012).

Table 1. Inhibition Zone Diameter of *Pseudomonas azotoformans* UICC B-91 Extract Against *Salmonella typhi* and *Microsporum canis*

Treatment	Concentration	B1 (mm)	B2 (mm)	Mean ± SD (mm)	Inhibition Category
Salmonella typhii					
P. azotoformans extract	30%	1.5	1.5	1.50 ± 0.00	Weak
P. azotoformans extract	50%	1.5	2.5	2.00 ± 0.71	Weak
P. azotoformans extract	100%	1.5	1.1	1.30 ± 0.28	Weak
Positive control (Amoxicillin)	32 µg/mL	6	6	6.00 ± 0.00	Moderate
Negative control (Aquadest)	0	0	0	0.00 ± 0.00	No inhibition
Microsporum canis					
P. azotoformans extract	30%	1.5	1.5	1.50 ± 0.00	Weak
P. azotoformans extract	50%	4.5	8.5	6.50 ± 2.83	Moderate
P. azotoformans extract	100%	6	7	6.50 ± 0.71	Moderate
Positive control (Ketoconazole)	0.15%	6	6.5	6.25 ± 0.35	Moderate
Negative control (Aquadest)	0	0	0	0.00 ± 0.00	No inhibition

Tabel 2. One-way ANOVA results of antibacterial activity

Treatment	p-value (Sig.)	Interpretation
P. azotoformans 30%	0.118	Not significant
P. azotoformans 50%	0.039	Significant
P. azotoformans 100%	0.214	Not significant
Amoxicillin	0.054	Not significant

Tabel 3. One-way ANOVA results of antifungal activity

Test organism	Source of Variation	df	F value	p-value	Interpretation
Microsporum canis	Between groups	4	803.936	0.026	Significant
	Within groups	1			
	Total	5			

The results of the bioactivity study (Table 1) demonstrates that *Pseudomonas azotoformans* UICC B-91 extract exhibits measurable antimicrobial activity against both *Salmonella typhi* and *Microsporum canis*, although with different levels of inhibitory effectiveness depending on the test organism and extract concentration. This finding supports the hypothesis that secondary metabolites produced by *Pseudomonas* species possess dual antibacterial and antifungal potential.

This study provides quantitative and statistical evidence that *Pseudomonas azotoformans* UICC B-91 extract exhibits differential antimicrobial activity against *Salmonella typhi* and *Microsporum canis*. Integration of raw inhibition zone data (Table 1), graphical representations (Figures 4 and 5), and inferential statistics (ANOVA) (Table 2 and Table 3) demonstrates that the extract shows moderate and statistically significant antifungal activity, while its antibacterial effect against *S. typhi* remains weak and partially significant depending on concentration.

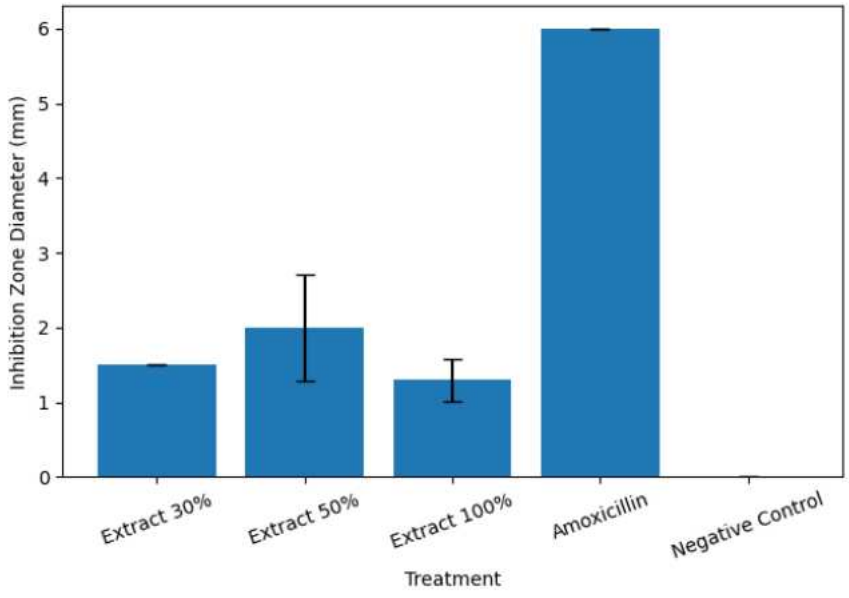


Figure 4. Antibacterial activity of *Pseudomonas azotoformans* UICC B-91 extract against *Salmonella typhi* expressed as mean inhibition zone diameter ± SD (n = 2)

Based on the raw data (Table 1), inhibition zones produced by *P. azotoformans* extract against *S. typhi* ranged from 1.30 ± 0.28 mm to 2.00 ± 0.71 mm, which fall into the weak inhibition category according to Davis & Stout (1971). Statistical analysis revealed that only the 50% extract concentration resulted in a significant effect ($p = 0.039$) (Table 2 and Figure 4), whereas other concentrations and the positive control did not reach statistical significance ($p > 0.05$). These findings indicate that the antibacterial activity of the crude extract against *S. typhi* is limited and concentration-dependent.

From a physiological perspective, the weak inhibitory effect can be explained by the structural characteristics of *S. typhi* as a Gram-negative bacterium. The presence of an outer membrane rich in

lipopolysaccharides (LPS) acts as a selective permeability barrier that restricts the diffusion of many antimicrobial compounds, particularly large or hydrophobic secondary metabolites (Pratiwi, 2017; Bayot & Bragg, 2024) As a result, crude extracts often show reduced efficacy against Gram-negative bacteria compared to Gram-positive bacteria or fungi.

Nevertheless, the statistically significant inhibition observed at 50% concentration suggests that *P. azotoformans* produces antibacterial compounds capable of interfering with *S. typhi* growth, albeit at low efficiency in crude form. Previous studies on *P. azotoformans* UICC B-91 have reported the production of antimicrobial secondary metabolites such as alkaloids and biosurfactants that can disrupt bacterial membranes or interfere with metabolic processes (Pratiwi et al., 2022). However, without fractionation or purification, the effective concentration of these compounds may remain below the threshold required to generate larger inhibition zones in diffusion-based assays.

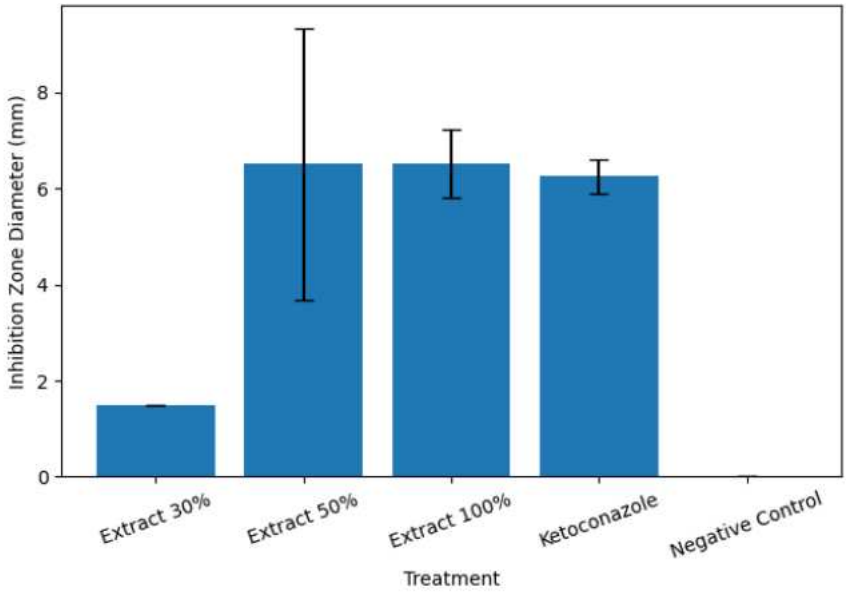


Figure 5. Antifungal activity of *Pseudomonas azotoformans* UICC B-91 extract against *Microsporum canis* expressed as mean inhibition zone diameter ± SD (n = 2)

In contrast to the antibacterial results, *P. azotoformans* extract demonstrated a clearer and more consistent inhibitory effect against *M. canis* (Table 1). Raw inhibition zone data showed an increase in inhibition diameter with increasing extract concentration, reaching 6.50 ± 0.71 mm at 100%, which is classified as moderate inhibition and comparable to ketoconazole (6.25 ± 0.35 mm). One-way ANOVA analysis confirmed that the differences among treatments were statistically significant ($F = 803.936$; $p = 0.026$), indicating that the antifungal effect was not due to random variation (Table 2 and Figure 5).

The higher susceptibility of *M. canis* can be explained by fundamental differences in fungal cell physiology. Fungal cell membranes contain ergosterol, a key structural component that is absent in bacterial membranes. Many microbial secondary metabolites, including biosurfactants and phenazine-like compounds produced by *Pseudomonas* spp., exert antifungal activity by disrupting membrane integrity, increasing permeability, and causing leakage of intracellular components (Moriello, 2020; Pratiwi et al., 2022). This mechanism is functionally similar to that of azole antifungals, which inhibit ergosterol synthesis and compromise fungal cell membrane stability.

In addition, *Pseudomonas* species are known to produce rhamnolipids, which act as surface-active molecules capable of damaging fungal hyphae, inhibiting spore germination, and interfering with cell wall

synthesis. These compounds have been reported to be particularly effective against dermatophytes, including *Microsporum* species, which rely on intact hyphal growth for colonization and infection (Sakan & Yohanes, 2021) . The moderate inhibition observed in this study is therefore biologically consistent with known antifungal mechanisms of *Pseudomonas*-derived metabolites.

When both test organisms are compared, the results clearly indicate that *P. azotoformans* UICC B-91 extract exhibits greater antifungal than antibacterial activity in its crude form. This differential response reflects differences in microbial cell envelope structure, target accessibility, and susceptibility to secondary metabolites. While the antibacterial activity against *S. typhi* was weak, the statistically significant antifungal activity against *M. canis* highlights the potential of *P. azotoformans* as a source of natural antifungal agents.

It is important to note that diffusion-based assays may underestimate the true antimicrobial potential of crude extracts due to limited compound diffusion, interaction with agar components, and concentration gradients (Bayot & Bragg, 2024). Therefore, the observed inhibition zones should be interpreted as preliminary indicators rather than definitive measures of efficacy. Further studies involving extract fractionation, identification of active compounds, and determination of minimum inhibitory concentrations (MIC) are required to better characterize the antimicrobial mechanisms and enhance bioactivity.

The present study is limited by the use of crude extracts and a small number of replicates, which may influence variability and statistical power. Despite these limitations, the integration of raw data, statistical analysis, and mechanistic interpretation provides robust preliminary evidence supporting the antimicrobial potential of *P. azotoformans* UICC B-91. Future research should focus on isolating specific bioactive compounds, evaluating synergistic effects, and testing efficacy against a broader range of clinically relevant pathogens.

CONCLUSION

The results demonstrated that the extract exhibited weak antibacterial activity against S. typhi and moderate antifungal activity against M. canis, as indicated by inhibition zone measurements and supported by statistical analysis. Statistical evaluation confirmed that the antifungal activity against M. canis was significant, while the antibacterial effect against S. typhi was limited and only significant at a specific extract concentration. These findings indicate that P. azotoformans UICC B-91 produces bioactive compounds with measurable antimicrobial effects under in vitro conditions, particularly against dermatophyte fungi.

However, the scope of this study is restricted to in vitro screening, and therefore the results do not yet support claims regarding clinical effectiveness, safety, or the ability to overcome antimicrobial resistance. The mechanisms of action of the bioactive compounds, their toxicity profiles, and their efficacy in vivo were not investigated in this study. Consequently, the extract should be regarded as a preliminary source of antimicrobial compounds rather than a confirmed natural antibiotic alternative.Further research is required to isolate and identify the active compounds, elucidate their mechanisms of action, assess cytotoxicity and safety, and evaluate antimicrobial efficacy using minimum inhibitory concentration (MIC) assays and in vivo models. Such studies are necessary before any practical or therapeutic application of P. azotoformans UICC B-91–derived compounds can be considered.

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