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Unveiling the Ancient Wisdom: Antibacterial and Antifungal Properties of *Pavetta indica*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antibacterial and antifungal properties of *Pavetta indica* leaves using an ethanolic extract (PI). The agar well diffusion method revealed dose-dependent inhibition zones against various bacterial and fungal strains. Notably, the extract demonstrated promising antifungal activity against *Cryptococcus neoformans*, comparable to the positive control (Amphotericin B) for fungal activity. All tested bacterial strains (*E. coli, Streptococcus faecalis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*) and fungal strains (*Candida albicans, Aspergillus* fumigatus, *Sporothrix schenckii*) exhibited susceptibility to PI, with inhibition zones increasing proportionally with extract concentration. Overall, this study suggests that *P. indica* leaves possess promising potential as a natural source of broad-spectrum antimicrobial and antifungal agents.

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Keywords: P. indica; antibacterial activity; antifungal activity; ethanolic extract; broad-spectrum.

1. INTRODUCTION

The emergence of multidrug-resistant (MDR) bacteria and fungi poses a significant threat to global public health. These pathogens are increasingly resistant to conventional antibiotics and antifungals, rendering current treatment options ineffective and highlighting the urgent need for novel antimicrobial agents [1,2]. Natural products derived from plants have historically played a crucial role in infectious disease management and continue to be a promising source for the discovery of new antimicrobial agents [3]. Plants produce a vast array of secondary metabolites with diverse biological activities, including antibacterial and antifungal properties [4]. *P. indica*, a plant belonging to the Rubiaceae family, has been used in traditional medicine for various ailments, including infectious diseases [5]. Some studies have explored its potential for managing different conditions, such as diabetes and inflammation [6]. However, limited scientific research exists to validate its potential as an antimicrobial agent. Previous studies have hinted at the potential of Pavetta species for antimicrobial activity. Extracts from Pavetta crassipes have demonstrated effectiveness against various bacterial strains, including Staphylococcus aureus and Klebsiella pneumoniae [7,8]. Furthermore, research suggests Pavetta crassipes leaf extracts exhibit activity against Mycobacterium tuberculosis, the causative agent of tuberculosis [9]. Building upon this initial research, this study investigates the antibacterial and antifungal properties of *P. indica* leaves using an ethanolic extract (PI). The agar well diffusion method will be employed to assess the inhibitory effects of PI against a panel of clinically relevant bacterial and fungal strains. This research contributes to the exploration of *P. indica* as a potential source of broad-spectrum antimicrobial agents, potentially leading to the development of novel therapeutic strategies to combat infectious diseases. However, limited scientific data exists to validate its potential as an antimicrobial agent. This study aimed to investigate the antibacterial and antifungal properties of *P. indica* leaves using an ethanolic extract (PI). The agar well diffusion method was employed to assess the inhibitory effects of PI against a panel of clinically relevant bacterial and fungal strains. This research contributes to the exploration of natural products with potential for developing novel broad-spectrum antimicrobial therapies.

2. MATERIALS AND METHODS

This section details the materials and methods employed to investigate the antibacterial and antifungal properties of *P. indica* leaves.

2.1 Plant Material and Extract Preparation

Fresh leaves of *P. indica* were collected from Kolli Hills, Namakkal District, Tamil Nadu, India. The plant was authenticated by comparison with authentic specimens housed at the Rapinat Herbarium, St. Joseph College (Autonomous), Tiruchirappalli, Tamil Nadu, India. A voucher specimen (No. T.B.003) has been deposited at the herbarium for future reference.

2.2 Extraction

The collected leaves were shade-dried at ambient temperature for 15 days. Subsequently, the dried leaves were pulverized into a fine powder using a grinder. A known weight (50 grams) of the powdered material was macerated in 500 ml of absolute ethanol for 48 h with occasional agitation. The mixture was then filtered using Whatman filter paper No. 1. The filtrate was concentrated under reduced pressure using a rotary evaporator to yield a crude ethanolic extract (PI). The extract was weighed, and the yield (%) was calculated. The concentrated extract was stored at 4^{o}C until further use.

2.3 Microorganisms

2.3.1 Bacterial strains

The study employed the following bacterial strains: *Escherichia coli* (ATCC 25922), *Streptococcus faecalis (*ATCC 29212), *Bacillus subtilis (*ATCC 6633), and *Pseudomonas aeruginosa (*ATCC 27853). obtained from a source of American Type Culture Collection (ATCC).

2.3.2 Fungal strains

The following fungal strains were used in the study: *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (ATCC 90237), *Cryptococcus neoformans* (ATCC 20924), *Sporothrix schenckii* (ATCC 52457). These strains were also obtained from a source of American Type Culture Collection (ATCC).

2.4 Media and Culture Conditions

2.4.1 Bacterial Culture

Mueller-Hinton agar (MHA) media plates were used for bacterial growth and susceptibility testing. The bacterial strains were revived in Mueller-Hinton broth (MHB) and incubated at 37°C for 24 hours.

2.4.2 Fungal Culture

Sabouraud Dextrose Agar (SDA) media plates were used for fungal growth and susceptibility testing. The fungal strains were revived in Sabouraud Dextrose Broth (SDB) and incubated at 25°C for 48 hours.

2.5 Agar Well Diffusion Assay

Preparation of Inoculum: Broth cultures of bacterial strains were adjusted to a turbidity comparable to a 0.5 McFarland standard using sterile saline solution. Similarly, fungal cultures were adjusted to a turbidity matching a 0.5 McFarland standard using sterile saline solution.

Inoculum Seeding: Sterile swabs were dipped into the standardized inoculum suspensions and used to spread the inoculum evenly onto the surfaces of MHA and SDA plates, ensuring a confluent lawn of growth.

2.6 Well Creation and Sample Application

Using a sterile cork borer, wells with a diameter of 6 mm were created in the solidified agar media on the plates. Different concentrations (e.g., 500 µg/mL, 250 µg/mL, 100 µg/mL, and 50 µg/mL) of the *P. indica* ethanolic extract (PI) were prepared in sterile distilled water. A volume of 100µL of each extract concentration was pipetted into designated wells on the inoculated plates. Sterile distilled water served as a negative control. Gentamicin and Amphotericin B were used as positive controls for antibacterial and antifungal activity, respectively. The plates were allowed to stand for 2 h to facilitate diffusion of the test compounds into the agar.

2.7 Incubation and Zone Measurement

The inoculated plates were incubated at 37°C for 24 hours for bacterial strains and at 25°C for 48 hours for fungal strains. After incubation, the diameters of the inhibition zones surrounding the wells were measured in millimeters using a calibrated ruler. Each experiment was performed in triplicate, and the average zone of inhibition for each concentration was calculated.

2.8 Data Analysis

The data obtained from the agar well diffusion assay, including the diameters of the inhibition zones for each extract concentration and control, were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by a post-hoc test (e.g., Tukey's multiple comparison test) to determine significant differences between groups. Statistical significance was set at $p < 0.05$. The data was presented as mean ± standard deviation (SD) of the zone of inhibition diameters.

3. RESULTS AND DISCUSSION

Antibacterial and Antifungal Activity of *P. indica* Extract. This section explores the potential of *P. indica* leaves as a source of natural antimicrobials. The agar well diffusion assay evaluated the inhibitory activity of an ethanolic extract (PI) against various bacterial and fungal strains.

3.1 Antibacterial Activity

The results revealed promising antibacterial activity of PI against all tested bacterial strains: *Escherichia coli, Streptococcus faecalis, Bacillus subtilis, and Pseudomonas aeruginosa*. A dose-dependent response was observed, with increasing inhibition zone diameters corresponding to higher PI concentrations (Fig. 1). Table 1 summarizes the mean zone of inhibition (mm) and standard deviation (SD) for each bacterial strain treated with different PI concentrations and the positive control antibiotic. Streptococcus faecalis displayed the highest susceptibility, with a remarkable inhibition zone of 11 mm at the highest PI concentration (500 µg/mL). Similarly, *E. coli* exhibited significant susceptibility, reaching an inhibition zone of 12 mm at the same concentration. Bacillus subtilis and Pseudomonas aeruginosa showed moderate susceptibility, with inhibition zones ranging from 3.2 mm to 10 mm depending on the PI concentration.

3.2 Antifungal Activity

PI also demonstrated antifungal activity against the tested fungal strains: Candida albicans, Aspergillus fumigatus, Cryptococcus

neoformans, and *Sporothrix schenckii* (Fig. 2). Similar to the bacterial results, a dose-dependent trend was observed, with increasing inhibition zones at higher PI concentrations. Table 2 showcases the mean zone of inhibition (mm) and standard deviation (SD) for each fungal strain treated with different PI concentrations and the positive control antifungal agent. Interestingly, *Cryptococcus neoformans* displayed the highest susceptibility among the fungi, with an inhibition zone of 13 mm at the highest PI concentration. Candida albicans also exhibited notable susceptibility, reaching an inhibition zone of 11 mm at 500 µg/mL PI. *Aspergillus fumigatus* showed moderate susceptibility, while *Sporothrix schenckii* displayed the least susceptibility among the tested fungi. Unveiling the Antimicrobial Potential of *P. indica*. This study delves into the exciting potential of *P. indica* leaves as a natural source of broad-spectrum antimicrobial agents. The findings, employing the agar well diffusion assay, paint a promising picture of the ethanolic extract (PI)'s activity against various bacterial and fungal strains.

3.3 Antibacterial Arsenal of *P. indica*

The results unveil a potent antibacterial arsenal within PI. Notably, all tested bacterial strains, encompassing Escherichia coli, Streptococcus faecalis, Bacillus subtilis, and Pseudomonas aeruginosa, exhibited dose-dependent susceptibility. This fascinating observation suggests the presence of a diverse range of antibacterial compounds within the *P. indica* leaves [10]. Streptococcus faecalis emerged as particularly vulnerable, with an impressive inhibition zone at the highest PI concentration. Similarly, *E. coli* displayed significant susceptibility, highlighting the potential of PI against these common bacterial culprits [10]. Bacillus subtilis and Pseudomonas aeruginosa, while showing moderate susceptibility, warrant further investigation for a more comprehensive understanding of PI's antibacterial spectrum.

Table 1. Antibacterial Activity of ethanolic leaves extract of *P. indica*

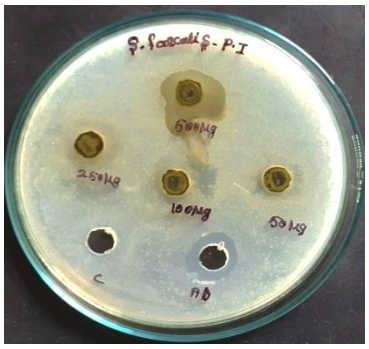
S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	Antibiotic (Ab)Gentamycin
1.	*Streptococcus faecalis*	PI	11±2.0	8±1.0	6.5±0.5	3.3±0.3	6±1.0
2.	*Bacillus subtilis*		10±1.0	7.5±0.5	4.4±0.4	3.2±0.2	6±1.0
3.	*E.Coli*		12±1.0	9.3±0.3	6.2±0.2	5.1±0.1	10±1.0
4.	*Pseudomonas aeruginosa*		10±1.0	7.5±0.5	4.4±0.4	3.1±0.1	12±1.0

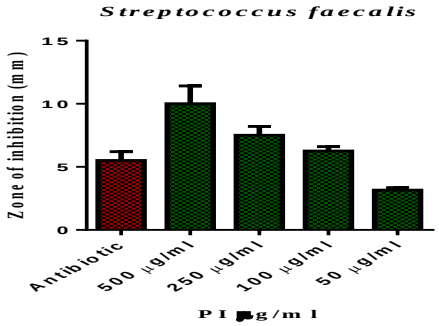
SD – Standard Deviation, Significance - p< 0.05; AB = Antibiotic (Ab) Gentamycin

Table 2. Antifungal Activity of ethanolic leaves extract of *P. indica*

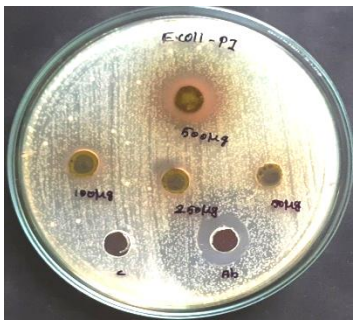
S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	AM (Antimycotic drug-Amphotericin B)
1.	*Candida albicans*	PI	11±2.0	7.5±0.5	3.2±0.2	2.1±0.1	7±1.0
2.	*Aspergillus fumigatus*		7±1.0	3.4±0.4	2.3±0.3	0	7±1.0
3.	*Cryptococcus neoformans*		13±2.0	9.5±0.5	4.4±0.4	3.1±0.1	11±0.1
4.	*Sporothrix schenckii*		7±1.0	5.5±0.5	3.3±0.3	2.2±0.2	23±1.0

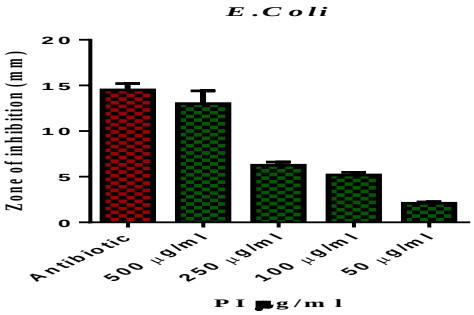
SD – Standard Deviation, Significance - p< 0.05; AM (Antimycotic drug-Amphotericin B)



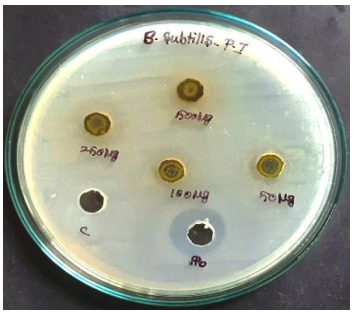


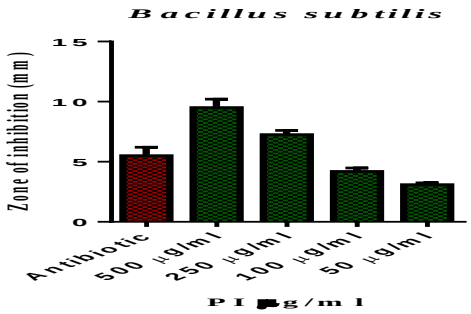
Streptococcus faecalis



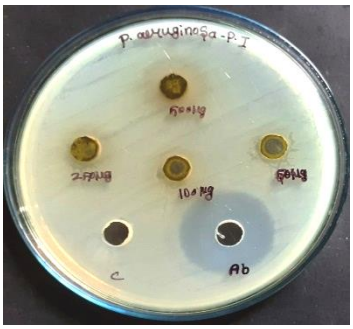


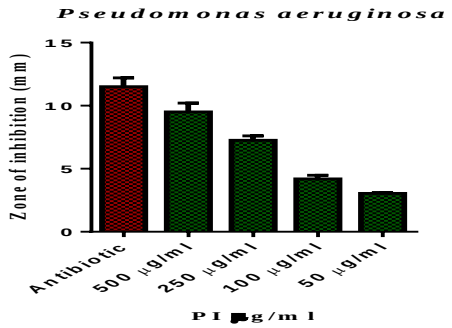
Bacillus subtilis





E.Coli





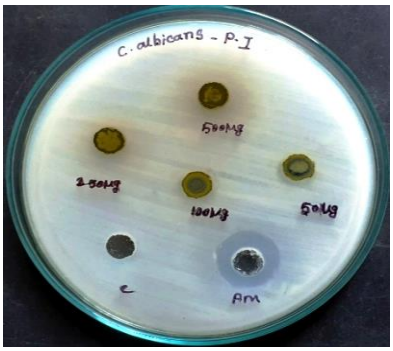
Pseudomonas aeruginosa

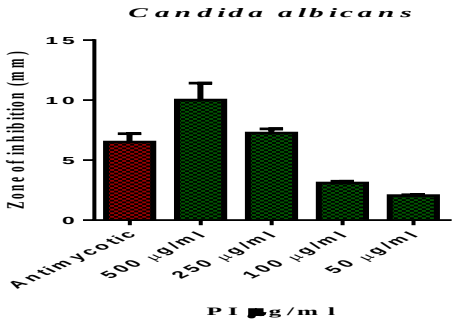
Fig. 1. Antibacterial Activity of ethanolic leaves extract of P. indica

3.4 Antifungal Activity: A Promising Frontier

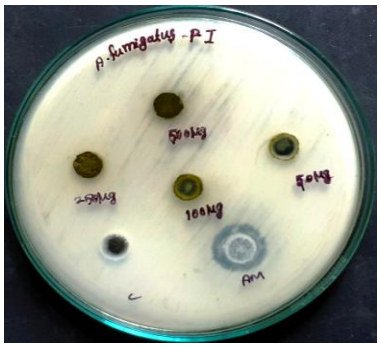
Beyond its antibacterial prowess, PI also exhibited antifungal activity against a panel of fungal strains. This dual-pronged approach against both bacterial and fungal pathogens adds another layer of intrigue to the potential applications of *P. indica*. Similar to the bacterial results, a dose-dependent trend was observed,

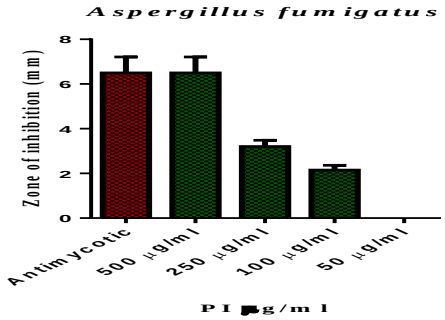
with increasing inhibition zones at higher PI concentrations. Cryptococcus neoformans, a potentially life-threatening fungus, displayed the highest susceptibility among the tested strains. This finding is particularly encouraging, suggesting PI may hold promise in combating this serious pathogen. Candida albicans, another common fungal adversary, also exhibited notable susceptibility, indicating PI's potential broad-spectrum antifungal activity [11]. Although Aspergillus fumigatus showed moderate susceptibility, and *Sporothrix schenckii* displayed the least, further exploration is necessary to understand the specific mechanisms of action against these fungi.



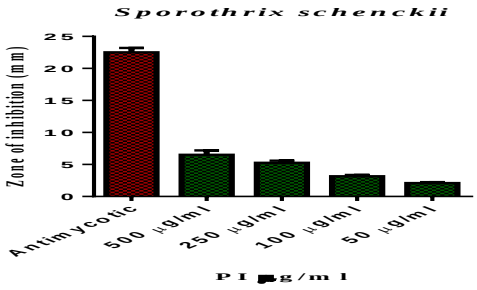


Candida albicans

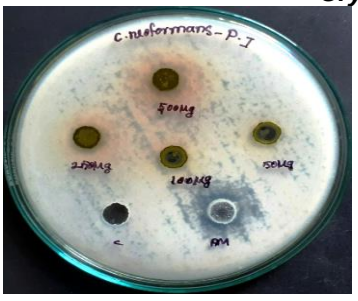


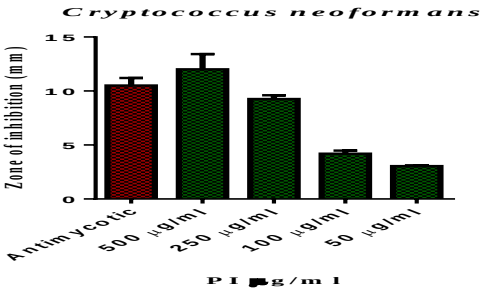


Aspergillus fumigatus



Cryptococcus neoformans





Sporothrix schenckii

Fig. 2. Antifungal Activity of ethanolic leaves extract of P. indica

3.5 Looking Forward: Unveiling the Secrets Within

These findings provide compelling evidence for the potential of *P. indica* as a source of natural antimicrobials. The observed broad-spectrum activity against both bacteria and fungi warrant further investigation. Future research should focus on identifying the specific bioactive compounds responsible for this remarkable activity. Elucidating the mechanisms of action of these compounds will be crucial for developing novel therapeutic strategies to combat infectious diseases [12].

3.6 Limitations and the Road Ahead

The agar well diffusion assay provides a valuable initial assessment of antimicrobial activity. However, future studies should employ broth microdilution methods to determine the minimum inhibitory concentration (MIC) of PI against the tested microorganisms. This quantitative measure will provide a clearer picture of the extract's potency [13]. Additionally, isolation and identification of the bioactive compounds within PI are critical steps in harnessing their full potential for drug development.

5. CONCLUSION

This study unveils the exciting potential of *P. indica* leaves as a natural source of broad-spectrum antimicrobial agents. The observed activity against various bacterial and fungal strains paves the way for further exploration towards developing novel therapeutic strategies to combat infectious diseases. As we continue to unravel the secrets within *P. indica*, the future of natural antimicrobial solutions may be closer than ever.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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