

Evaluation of Antibacterial and Antifungal activities of *Helianthus annuus* Leaf Extracts Against Selected Fish Pathogens

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ABSTRACT

Medicinal plants serve as an important source of bioactive compounds that can be used to combat microbial infections, especially given the rise in antimicrobial resistance. This study investigated the phytochemical makeup and the *in vitro* antibacterial and antifungal properties of aqueous, ethanol, methanol, and petroleum ether extracts from the leaves of *Helianthus annuus* (sunflower) against certain fish and human pathogenic microorganisms. Initial qualitative phytochemical analysis identified a variety of secondary metabolites, such as alkaloids, flavonoids, phenols, steroids, terpenoids, saponins, tannins, proteins, carbohydrates, glycosides, oils, resins, and amino acids, with the methanolic extract showing the most diverse phytochemical content. The antibacterial effects were tested against *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella paratyphi A*, while the antifungal effects were assessed against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* using the agar well diffusion technique. All extracts exhibited antimicrobial activity that increased with dosage, with the methanol leaf extract proving to be

the most effective. Remarkably, the highest antibacterial activity was observed against *P. mirabilis* (46.67 ± 0.67 mm), and the most potent antifungal activity was against *A. niger* (31 ± 0.58 mm) at a concentration of 500 μ l. These results suggest that *H. annuus* leaves, particularly the methanolic extracts, have significant antibacterial and antifungal potential, which supports their traditional use and indicates their potential as a natural source for developing environmentally friendly antimicrobial agents.

Keywords: *Helianthus annuus*, Phytochemical screening, Antibacterial activity, Antifungal activity.

INTRODUCTION

Herbal medicines are crucial in healthcare systems worldwide, especially in developing nations like India, where medicinal plants and their derivatives are widely utilized for preventing and treating infectious diseases. These plant-based treatments are a fundamental part of primary healthcare and significantly enhance the socio-economic status of rural communities¹. Herbal extracts are abundant in biologically active compounds such as flavonoids, alkaloids, tannins, phenols, terpenoids, and essential oils, many of which have well-established antimicrobial, anti-inflammatory, and antioxidant effects².

Since ancient times, remedies derived from plants have been the cornerstone of traditional medical systems, particularly in rural areas with limited access to modern healthcare. In countries like Nigeria, reliance on herbal medicine has grown significantly, with numerous medicinal plants reported to have a wide range of biological and therapeutic properties^{3,4}. In recent years, the rapid rise and spread of antimicrobial-resistant pathogens affecting both humans and aquatic life have heightened the search for alternative and environmentally friendly antimicrobial agents. In this regard, medicinal plants are increasingly seen as promising sources of new bioactive compounds capable of fighting resistant microbial infections.

Medicinal plants are favoured for their affordability, accessibility, and perceived safety compared to synthetic drugs. They contain a wide array of secondary metabolites that boost therapeutic effectiveness and contribute to their increasing demand as natural alternatives to conventional antimicrobials⁵⁻⁸. Although plant-based formulations are generally linked with fewer side effects⁹, their medicinal claims need scientific validation through systematic phytochemical and pharmacological studies.

The practice of using plants for medicinal purposes can be traced back to the dawn of human civilization. Medicinal plants hold significant therapeutic and economic value worldwide, with around 75–80% of the global population, especially in developing countries, primarily depending on herbal medicines for basic healthcare needs¹⁰. This widespread dependence is mainly due to the perception that herbal remedies are safer, more affordable, and more environmental friendly than synthetic drugs¹¹.

Among many medicinal plants with acknowledged therapeutic potential, *Helianthus annuus* L. (family Asteraceae), commonly known as sunflower, has garnered significant interest due to its broad geographical distribution and varied pharmacological properties. This plant is widely cultivated in America, Africa, Asia, and Australia. Botanically, *H. annuus* is an annual herb distinguished by its large aromatic leaves and composite flower heads, which feature bright yellow ray florets and orange-brown disc florets¹².

The traditional and folkloric medicinal applications of *H. annuus* are well-documented¹³. The plant is reported to have a variety of pharmacological effects, including anti-inflammatory, analgesic, hypoglycemic, antioxidant, hepatoprotective, antifertility, and antimicrobial properties^{14–16}. Phytochemical studies have shown that different parts of the plant, especially the leaves, are rich in secondary metabolites like phenolics, flavonoids, alkaloids, terpenoids, saponins, tannins, and glycosides, which are closely linked to antimicrobial and antioxidant activities¹⁷.

Traditionally, the leaves of *H. annuus* are used to treat inflammatory conditions, fever, gastrointestinal issues, respiratory problems, microbial infections, and wounds¹⁸. Leaf decoctions and infusions are often used as diuretics, antidiabetic agents, expectorants, analgesics, and antimicrobials^{19,20}. Experimental research has further supported these traditional claims, showing that *H. annuus* leaves have significant anti-inflammatory, antioxidant, antifertility, antidiarrheal, antihistaminic, and antimicrobial activities, largely due to their rich phytochemical contents^{21–23}.

Microbial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, and fungal species like *Candida albicans* and *Aspergillus spp.* are responsible for serious infections in humans and aquatic organisms. The growing resistance of these pathogens to commonly used antimicrobial agents poses a significant public health challenge. Although previous studies have reported the antimicrobial potential of *H. annuus* against several bacterial and fungal pathogens²⁴,

comprehensive comparative studies evaluating the efficacy of different solvent extracts of *H. annuus* leaves against both human and fish pathogens remain limited.

Therefore, the present study was undertaken to evaluate the phytochemical compositions and *in vitro* antibacterial and antifungal activities of aqueous, ethanol, methanol, and petroleum ether extracts of *H. annuus* leaves against selected human and fish pathogenic microorganisms. This study aims to scientifically validate the traditional medicinal use of *H. annuus* leaves and to explore their potential as a natural, environmentally sustainable source of antimicrobial agents.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Leaves from *Helianthus annuus* were gathered in *Kannanur*, located in the Tiruchirappalli District of Tamil Nadu, India. The plant specimens were taxonomically verified at Rapinat Herbarium (RHT), Department of Botany, St. Joseph's College (Autonomous) in Tiruchirappalli, Tamil Nadu. After collection, the leaves were thoroughly cleaned, dried in the shade at ambient temperature, and then ground into powder. A total of 150 g of this powdered leaf material underwent Soxhlet extraction using solvents such as water, ethanol, methanol, and petroleum ether. The extraction process lasted between 24 and 48 hours with 800 ml of each solvent. The resulting extracts were filtered using Whatman No. 1 filter paper, concentrated, placed in airtight containers, labelled appropriately, and stored at 4°C until they were used for further analysis.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

A preliminary phytochemical screening was conducted on all four extracts to identify various phytochemical components, including alkaloids, phenols, flavonoids, steroids, terpenoids, saponins, tannins, proteins, carbohydrates, glycosides, gums, oils, resins, and amino acids, following the standard qualitative methods outlined by Wadood *et al*²⁵.

MICROORGANISMS USED

The microbial strains utilized in this research were sourced from K.A.P. Viswanathan Medical College in Tiruchirappalli, Tamil Nadu, India. The bacterial strains included *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella paratyphi* A. The fungal strains consisted of *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. Each microbial isolate was sub cultured and individually tested for antimicrobial activity against the leaf extracts. Chloramphenicol and

nystatin were employed as positive controls for antibacterial and antifungal activities, respectively.

***IN VITRO* ANTIBACTERIAL ACTIVITY**

The antibacterial properties of the aqueous, ethanol, methanol, and petroleum ether leaf extracts were assessed *in vitro* against selected pathogenic bacteria using the agar diffusion method. The assay was conducted on Muller–Hinton agar medium (Hi-Media). Bacterial suspensions were prepared and evenly distributed on the agar plates. Sterile discs soaked with varying concentrations of the extracts (100, 250, and 500 µl) were placed on the inoculated plates. Chloramphenicol was used as the reference standard. The plates were incubated at 37°C for 24 hours, after which the inhibition zones were measured and recorded in millimeters²⁶.

***IN VITRO* ANTIFUNGAL ACTIVITY**

The antifungal efficacy of the leaf extracts was evaluated *in vitro* against selected fungal pathogens using the agar diffusion method on Sabouraud's dextrose agar (Hi-Media). Fungal suspensions were prepared and uniformly spread on the agar plates. Sterile discs containing extract concentrations of 100, 250, and 500 µl were placed on the plates, with nystatin serving as the reference standard. The plates were incubated at 26°C for 72 hours. Following incubation, the inhibition zones were measured, and the minimum inhibitory concentration (MIC) was determined and recorded²⁷.

STATISTICAL ANALYSIS

All experiments were performed three times, and the findings are reported as the mean $\pm$ standard deviation (SD). The data are illustrated in figures. Heat maps were created using IBM SPSS Statistics software, where the mean zones of inhibition were initially calculated and then analyzed statistically. A one-way ANOVA followed by a post hoc comparison ($p < 0.05$) was conducted to identify significant differences among concentrations, and significance letters were assigned accordingly. The mean values were subsequently arranged into a matrix format, and a gradient colour scale was applied to produce the heat maps, with lighter shades indicating lower antimicrobial activity and darker shades signifying greater inhibition.

RESULTS AND DISCUSSION

Table 1: Qualitative phytochemical screening of leaf extracts of *Helianthus annuus* L.

S. No.	Phytochemical test	Aqueous	Ethanol	Methanol	Petroleum ether
1	Alkaloids	+	-	+	+

2	Phenols	+	+	+	+
3	Flavonoids	+	-	+	+
4	Steroids	+	+	+	+
5	Terpenoids	-	+	+	-
6	Saponins	+	-	+	-
7	Carbohydrates	+	+	+	-
8	Proteins	+	+	+	-
9	Tannins	+	+	+	-
10	Oils	+	+	+	-
11	Glycosides	+	-	-	-
12	Gums	-	+	+	-
13	Amino acids	+	+	+	-
14	Resins	+	+	+	+

(+) = Denotes presence of compound, (-) = Denotes absence of compound

The qualitative phytochemical examination of *Helianthus annuus* leaf extracts identified a range of bioactive compounds, with variations noted depending on the solvents employed (Table 1). Phenols, steroids, and resins were consistently found across all extracts. Alkaloids and flavonoids appeared in the aqueous, methanolic, and petroleum ether extracts but were missing from the ethanolic extract. Terpenoids were exclusive to the ethanol and methanol extracts, while saponins were present in both the aqueous and methanolic extracts.

Carbohydrates, proteins, tannins, oils, and amino acids were detected in the aqueous, ethanol, and methanol extracts but were absent in the petroleum ether extract. Glycosides were unique to the aqueous extract, whereas gums were found in the ethanol and methanol extracts. Overall, the methanolic extract exhibited the most varied phytochemical profile, followed by the aqueous and ethanolic extracts, with the petroleum ether extract containing relatively fewer components.

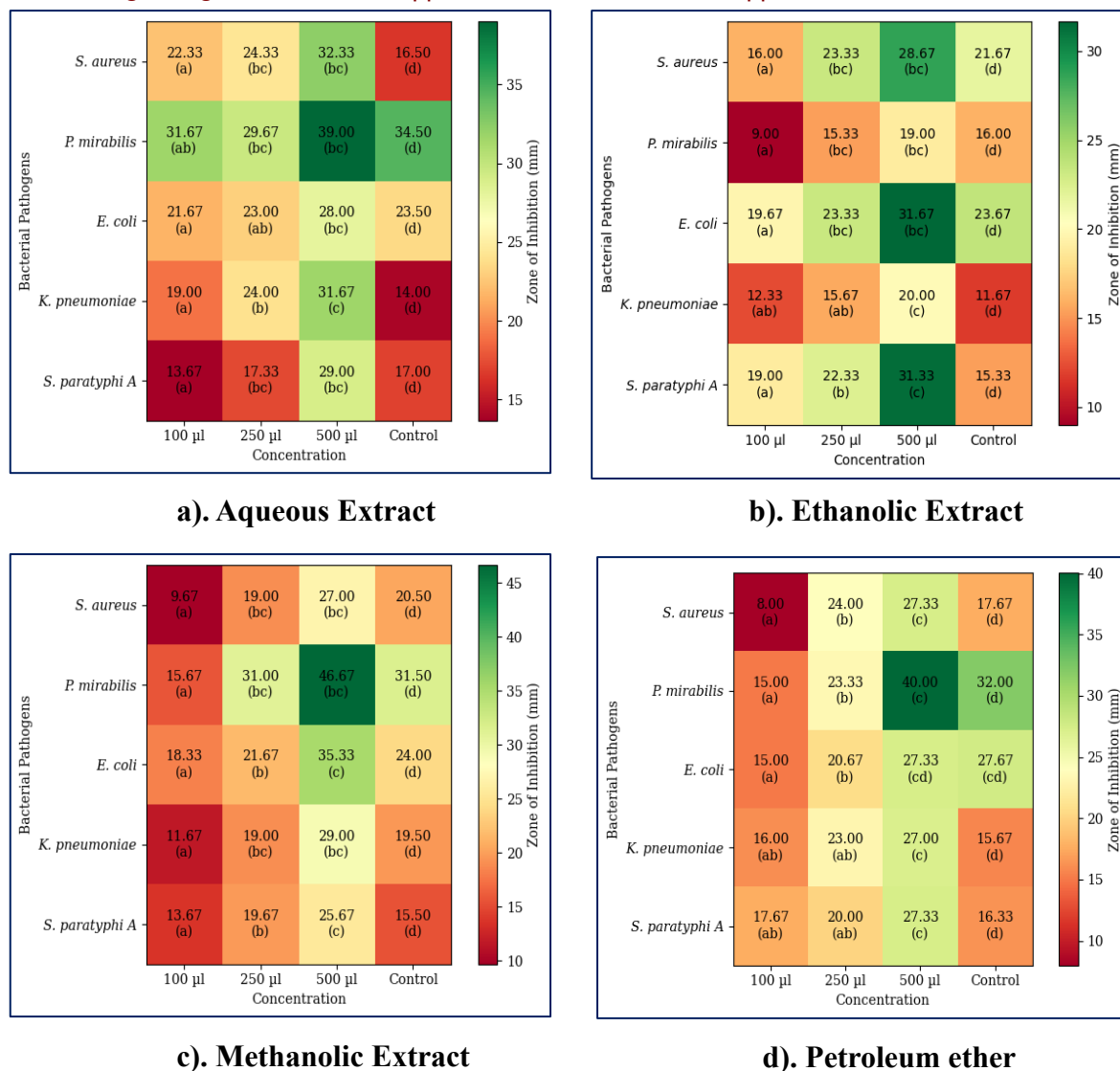


Fig. 1: Heat map showing antibacterial activity of the test samples against selected bacterial pathogens at different concentrations and control. Values inside each cell represent the mean zone of inhibition (mm). Color gradients indicate increasing antibacterial effectiveness, with green denoting higher inhibition. Lowercase letters associated with each value denote statistical significance within each row ($p < 0.05$).

The qualitative analysis of *Helianthus annuus* leaf extracts identified a wide array of bioactive substances, with the methanolic and aqueous extracts exhibiting the most abundant profiles. Phenols, steroids, and resins were detected in all solvent extracts, while other components varied according to the polarity of the solvent used. This aligns with previous research indicating that the polarity of solvents significantly affects the efficiency of phytochemical extraction, with polar solvents being more effective in extracting a broader spectrum of secondary metabolites^{28,29}.

The antimicrobial tests demonstrated a noticeable increase in inhibition zones that correlated with the concentration, as larger volumes of the extract led to more significant microbial inhibition. Such dose-response patterns are common in studies of plant antimicrobials because higher concentrations deliver greater amounts of active compounds³⁰.

The anti-bacterial and antifungal properties of extracts in aqueous, ethanolic, methanolic, and petroleum ether forms were evaluated against selected bacterial and fungal pathogens at different concentrations (Fig. 2). Generally, as the concentration increased, so did the inhibition, with statistically significant differences among concentrations for each organism denoted by different superscript letters ($p < 0.05$).

The aqueous extract demonstrated moderate antifungal effects, with inhibition increasing as the concentration rose. For *A. niger*, the inhibition zone expanded significantly from 12.00 mm at 100 μ l to 30.33 mm at 500 μ l ($p < 0.05$). A similar significant pattern was noted for *A. flavus*, where the 500 μ l concentration (25.33 mm) was markedly different from both 100 μ l and the control. In the case of *C. albicans*, the 500 μ l concentration (18.67 mm) showed a notable increase compared to 100 μ l. Conversely, *A. fumigatus* exhibited relatively low inhibition, with smaller yet statistically significant differences among the concentrations.

The ethanolic extract exhibited notably greater antifungal effects compared to the aqueous extract for most of the tested organisms. In the case of *A. niger*, inhibition increased significantly from 15.33 mm at 100 μ l to 28.67 mm at 500 μ l ($p < 0.05$). *A. flavus* also showed significant differences, with 500 μ l resulting in more inhibition than both 100 and 250 μ l. For *C. albicans*, the increase at 500 μ l was statistically significant when compared to lower concentrations. *A. fumigatus* experienced moderate inhibition, with 500 μ l showing a significant difference from the control, though not consistently from 250 μ l.

The methanolic extract exhibited the most potent antifungal properties overall. Specifically, for *A. niger*, the inhibition at 500 μ l (31.00 mm) was notably greater than at any other concentration ($p < 0.05$). Similarly, *C. albicans* and *A. flavus* demonstrated significant increases at 500 μ l compared to 100 μ l and the control. The inhibition of *A. fumigatus* rose markedly from 10.33 mm at 100 μ l to 23.00 mm at 500 μ l, indicating a pronounced concentration-dependent effect.

The petroleum ether extract showed the least antifungal effectiveness. While inhibition did increase with higher concentrations, the extent of this change was less pronounced than in polar extracts. For *A. niger*, a significant difference was observed between 500 μ l (20.67 mm)

and 100 μl (11.67 mm) ($p < 0.05$). *A. flavus* also exhibited notable differences at elevated concentrations. In contrast, *A. fumigatus* displayed minimal variation, with differences across concentrations being either minor or not statistically significant compared to other organisms.

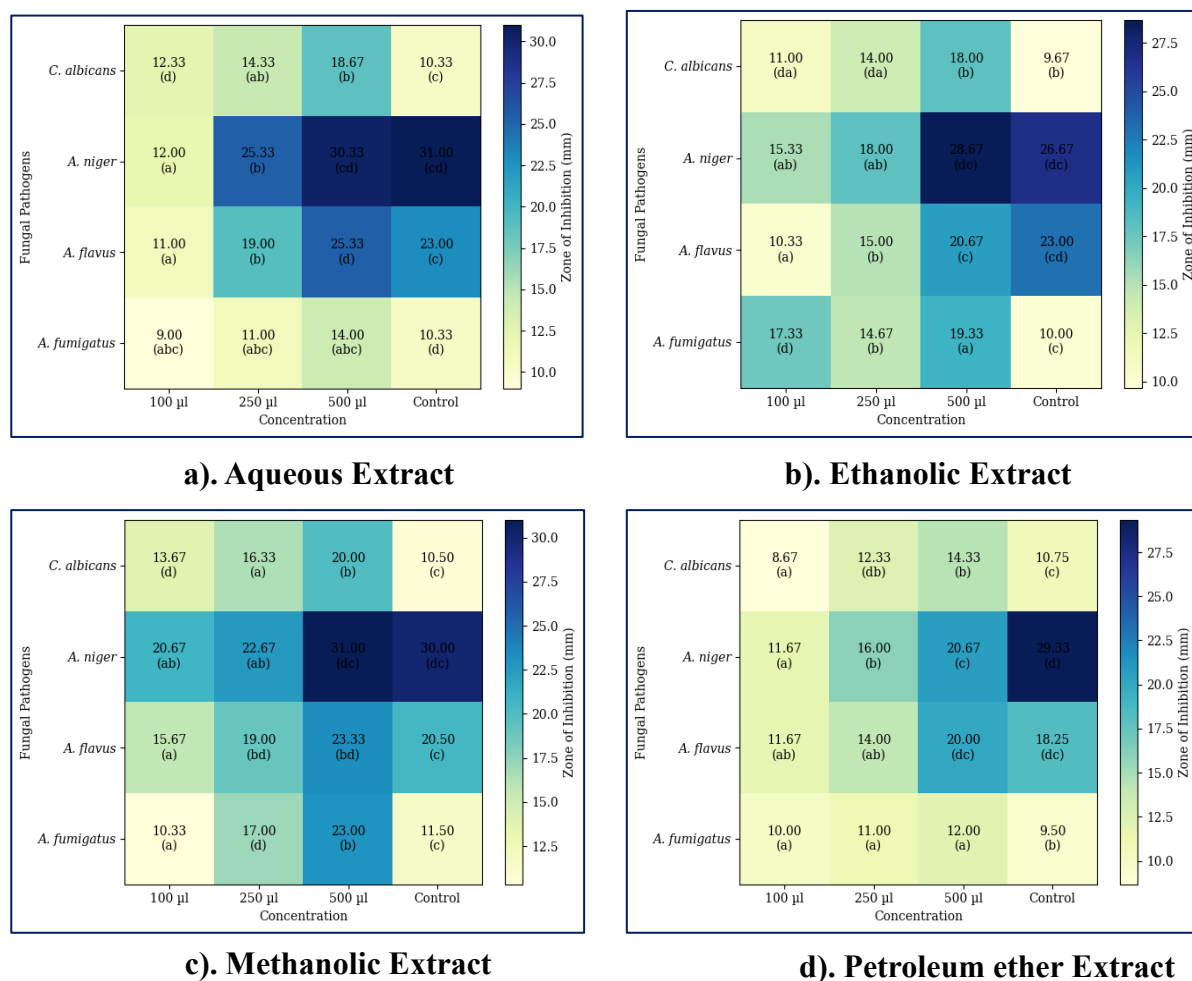


Fig. 2: Heat map representation of antifungal activity of the test samples against selected fungal pathogens at different concentrations compared with control. Values inside each cell indicate the mean zone of inhibition (mm). Colour intensity corresponds to the magnitude of antifungal effect, with darker blue shades representing higher inhibition. Different lowercase letters within each row indicate statistically significant differences among concentrations ($p < 0.05$).

The aqueous extract demonstrated moderate antibacterial effects. In *P. mirabilis*, the inhibition zone significantly expanded from 31.67 mm at 100 μl to 39.00 mm at 500 μl ($p < 0.05$). Both *S. aureus* and *K. pneumoniae* exhibited notable increases in inhibition at 500 μl compared to lower doses. *E. coli* showed a moderate yet statistically significant improvement at 500 μl , reaching 28.00 mm. *S. paratyphi* A had the least inhibition at 100 μl , with a marked increase at higher concentrations.

The ethanolic extract demonstrated more potent antibacterial effects compared to the aqueous extract against various pathogens. *E. coli* showed a notable increase in inhibition zones from 19.67 mm at 100 μ l to 31.67 mm at 500 μ l ($p < 0.05$). Both *S. paratyphi* A and *S. aureus* exhibited significant increases in a concentration-dependent manner. *K. pneumoniae* experienced moderate inhibition, with the 500 μ l concentration being significantly more effective than the 100 μ l. In contrast, *P. mirabilis* had relatively smaller inhibition zones, although the differences between concentrations were still statistically significant.

The methanolic extract demonstrated the most potent antibacterial effects overall. At a volume of 500 μ l, *P. mirabilis* experienced the greatest inhibition, measuring 46.67 mm, which was significantly more than at any other concentration ($p < 0.05$). *E. coli* and *K. pneumoniae* also showed notable and significant inhibition at 500 μ l, with measurements of 35.33 mm and 29.00 mm, respectively. *S. aureus* and *S. paratyphi* A exhibited a similar significant increase in inhibition with higher concentrations. This extract consistently outperformed other solvent extracts in terms of statistical activity.

The petroleum ether extract demonstrated lower antibacterial activity compared to polar solvents. Although inhibition increased significantly with concentration for *P. mirabilis* (40.00 mm at 500 μ l) and *S. aureus*, the zones were generally smaller for other organisms. *K. pneumoniae* and *S. paratyphi* A showed moderate but significant differences between concentrations. *E. coli* showed improvement at 500 μ l, though differences between 250 and 500 μ l were less pronounced.

Among the various solvents, the methanolic extract demonstrated the most potent antimicrobial activity against both bacterial and fungal strains. Methanol is particularly effective at extracting phenolics, flavonoids, and other polar bioactive compounds that contribute to antimicrobial properties. The significant inhibition observed against *E. coli*, *P. mirabilis*, *S. aureus*, and *A. niger* is consistent with earlier research highlighting the superior antimicrobial efficacy of methanolic plant extracts³¹⁻³³

The ethanolic extract ranked second in terms of activity. Ethanol's ability to dissolve both polar and moderately non-polar compounds accounts for its broad antimicrobial spectrum, although it is often slightly less effective than methanol^{34,35}. The aqueous extract showed moderate activity, which is anticipated since water primarily extracts hydrophilic components but may miss some potent antimicrobial phytochemicals³⁶⁻³⁸.

In contrast, the petroleum ether extract had the least activity, likely because non-polar solvents mainly extract lipids and terpenes, which may have weaker antimicrobial effects compared to phenolic compounds³⁹.

Bacterial strains were generally more vulnerable than fungal strains. This difference might be due to the complex structure of fungal cell walls, which contain chitin and glucans that can hinder the penetration of antimicrobial agents⁴⁰. Similar variations in susceptibility between bacteria and fungi have been noted in phytochemical antimicrobial studies⁴¹⁻⁴³.

CONCLUSION

The current research demonstrates that antimicrobial effectiveness significantly depended on both the type of solvent and its concentration. The methanolic extract at a concentration of 500 µl showed the strongest antibacterial and antifungal effects overall, with the ethanolic extract at 500 µl following closely. At 250 µl, a moderate level of inhibition was noted, while 100 µl resulted in the lowest activity against most test organisms. Aqueous extracts had moderate effects, whereas petroleum ether extracts exhibited relatively lower antimicrobial activity. Bacterial pathogens were generally more vulnerable than fungal species. Statistical analysis revealed significant differences among concentrations within each solvent treatment ($p < 0.05$), confirming a distinct dose-dependent response. In conclusion, the methanolic extract at 500 µl proved to be the most effective, underscoring the significance of polar solvents and higher concentrations in enhancing antimicrobial efficacy.

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CONFLICT OF INTEREST

All authors declared that there is no conflict of interest.

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