

## Assessing Antimicrobial Potential of *Physalis minima* L. Aerial Vegetative Parts

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### ABSTRACT

Plants, being a repository of countless bioactive molecules, have long been used as antimicrobial agents for combating a wide range of infections. The current research was aimed at assessing *in vitro* antibacterial and antifungal potential of *P. minima*. Plant extracts were prepared in polar (methanol, aqueous) and non-polar (chloroform, benzene) solvent from aerial vegetative parts using Soxhlet apparatus. Antimicrobial activity of the extracts was examined against seven bacterial and four fungal strains using disc diffusion and micro broth dilution assay. Highest antibacterial efficacy was found in methanolic extract against *M. smegmatis* ( $16.9 \pm 0.07$ ) followed by *E. coli* ( $14.8 \pm 0.15$ ) and *P. aeruginosa* ( $14.7 \pm 0.15$ ). *P. minima* extracts were also found to be effective against *C. violaceum*, *M. smegmatis* and *P. aeruginosa*, all of which showed resistance to standard antibiotic (ampicillin). Methanolic extract also exhibited highest antifungal activity, whereas aqueous extract was nearly inactive against the tested fungal strains. Chloroform extract was most active against *F. oxysporum* ( $12.5 \pm 0.29$ ) and least inhibition was recorded for *R. solani* ( $11.2 \pm 0.44$ ). Benzene extract was found to be potent for all the fungal strains except *R. solani*. Among the tested fungal strains, *C. albicans* was found to be the most susceptible as all the extracts exhibited ZOI against *C. albicans*. Interestingly, the fungal strain was found to be resistant to the broad spectrum antifungal i.e. fluconazole. The findings of current study showed *P. minima* to exhibit significant antimicrobial efficacy suggesting it to be a source for novel antimicrobial compounds.

**Key words:** Antibacterial, antifungal, disc diffusion assay, MIC, *Physalis minima*

### INTRODUCTION

Mother nature has bestowed abundant botanical wealth on us through plants, and herbal treatment against various health ailments is known since ancient times (Audah, 2019; Siddiqui *et al.*, 2022). Traditional healers have documented the curative properties of a number of plants like neem, tulsi, aloe vera, ginger, garlic, etc. (Ahmed, 2016). Plants serve as a substantial source of drugs not only in conventional medical treatments but also in modern medicines (Süntar, 2020; Bruce, 2022). As per WHO, globally 80% people depend on plant based medicines for their primary medical care entails (Sen and Chakraborty, 2017; Khan and Ahmad, 2019).

Microbes are cosmopolitan in nature and essential for the well-being of human beings. However, a small percentage of microbes induce various infectious ailments and are the main causes of morbidity and mortality across the globe. Antimicrobial drugs such as  $\beta$ -lactams, aminoglycosides, tetracyclines,

polyenes, azoles and thiocarbamates have been extensively used to inhibit the growth of microbes over the past few decades. But, microbes are expected to acquire resistance to currently available antibiotics. In a study, it has been shown that after seven to eight years of continuous use, microbes become resilient to the synthetic drugs and develop resistance (Martens and Demain, 2017; Uddin *et al.*, 2021). Finding of new antimicrobials thus becomes more pertinent especially from natural compounds as the pipeline of new synthetic antibiotics has been dry since the 1970's (Butler *et al.*, 2017; Lewis, 2020).

Plants are of particular interest as they contain a broad spectrum of bioactive compounds with inherent antimicrobial potential, have fewer adverse effects, and are less likely to cause drug resistance over synthetic antibiotics (Ody, 2017; Ruddaraju *et al.*, 2020). Plant-based remedies were the sole method for treating microbial infections until the discovery of first antibiotic i.e. penicillin (Mubeen *et al.*, 2021). Some of the plants attributed towards antibacterial and antifungal

potential were *Zingiber officinale*, *Helichrysum cymosum*, *Andrographis paniculata*, *Curcuma longa*, *Datura metel* and *Psidium gujava* (Morais-Braga *et al.*, 2017; Álvarez-Martínez *et al.*, 2021). Therefore, there is a pressing need to look into more and more plants for their potential to prevent microbial infections.

*Physalis minima* L., commonly known as ‘pilpotan’ is a widespread, fast-growing, pantropical annual herb growing profusely on field edges, waste ground, rice and cotton fields, grassy localities and roadsides, etc. (Jain *et al.*, 2000). *Physalis* is widespread in tropics, sub-tropics, and warm temperate zones including, Afghanistan, Australia, Singapore, Malaysia, Baluchistan, Ceylon, Asia and Tropical Africa. The plant is well known for its remedial value and is used as tonic, appetizer, diuretic and laxative (Sasikala and Meena, 2016; Singh *et al.*, 2020). Crushed leaves are used against snake bite and as a poultice for ulcer. Fruit paste and ash of aerial plant parts are used to heal burns, cuts and other dermatological problems (Thakur and Sidhu, 2017; Godara *et al.*, 2019). Whole plant juice is used to cure earache, gout, colic and urinary diseases and also acts as febrifuge and purgative (Ganesan and Xu, 2017; Gupta and Malhotra, 2020). The plant is a huge repository of chemical compounds viz., physalins, withaferin A and physaminilides, etc. which attribute various pharmacological properties to the plant (Daud *et al.*, 2016; Zhang *et al.*, 2020; Rohilla *et al.*, 2022).

Looking at the vast therapeutic importance of plant, the current research was aimed at assessing *in vitro* antimicrobial efficacy of *P.*

*minima* extracts as a way to broaden the range of botanical compounds effective against various microbial strains.

## MATERIALS AND METHODS

Basic chemicals and solvents were procured from Himedia, CDH and SRL India and were of analytical quality. Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), sterile discs, antibiotic and antifungal discs were obtained by HiMedia. Aerial vegetative parts of *P. minima* were collected from Sunaria village, Rohtak province, Haryana, India between coordinates 28.8590° N, 76.5705° E (Fig. 1). Freshly collected plant parts were first washed using tap water and then twice with distilled water (DW) to remove foreign particles. After drying in the shade for 20-25 days, the plant material was ground into a coarse powder. Plant extracts were prepared in four solvents (1:5 w/v) viz., aqueous (10.2), methanol (5.1), chloroform (4.1) and benzene (0.1) using Soxhlet apparatus. Extraction was carried out till the solvent became colourless. Plant extract was passed through Whatman Filter Paper No. 1 and excess solvent was evaporated with rotary vacuum evaporator (Buchi Type, Gallen). The concentrated crude extract was kept at 4°C for future use.

Percentage yield (% PY) of plant extract was evaluated as:

$$\text{PY} = (\text{Weight of crude plant extract} / \text{Weight of dry plant material}) \times 100$$

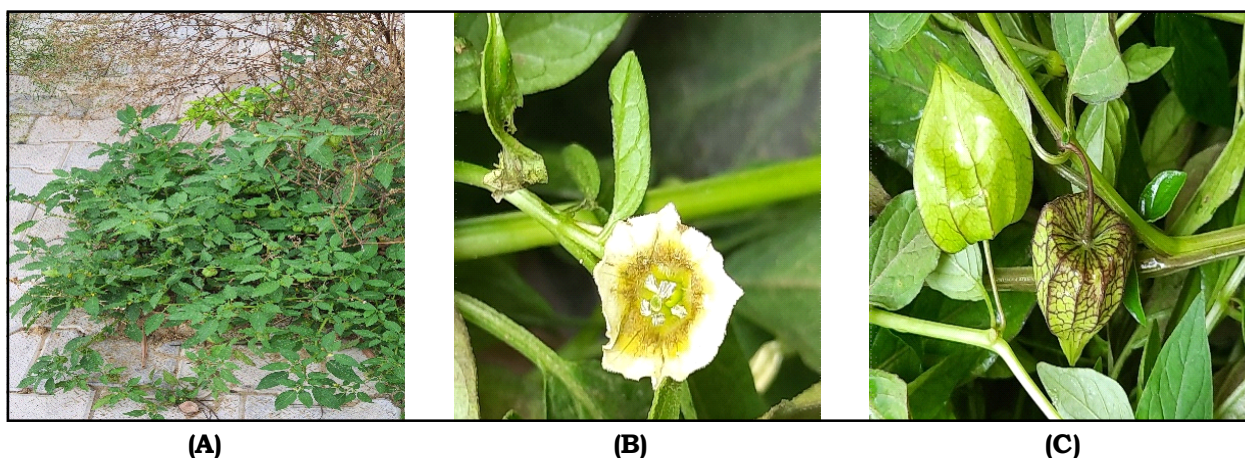


Fig. 1. *Physalis minima* L.: Growing in vacant area (A), Flower with brown spots on corolla (B) and Ten-veined fruit with persistent calyx (C).

Seven bacterial strains viz., *Escherichia coli* (MTCC-41), *Chromobacterium violaceum* (MTCC-2656), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-2453), *Bacillus subtilis* (MTCC-2057), *Mycobacterium smegmatis* (MTCC-992), *Staphylococcus aureus* (MTCC-96) and four fungal strains viz. *Candida albicans* (MTCC-183), *Fusarium oxysporum* (MTCC-7392), *Penicillium expansum* (MTCC-2818) and *Rhizoctonia solani* (MTCC-4633) acquired from CSIR-IMTECH Microbial Type Culture Collection (MTCC) Chandigarh, India were used to assess the antimicrobial potential of plant extracts. Antimicrobial efficacy of plant extracts was checked by disc diffusion and micro broth dilution assay. Four conc. (concentrations) of plant extract i.e. 100, 50, 25 and 12.5 mg/ml were prepared by re-constituting the plant extracts in DMSO.

NB and PDB were dissolved in DW and autoclaved for 25 min at 121°C to prepare the bacterial and fungal inoculums, respectively. A colony of bacteria and fungi was picked up and added to the sterilized culture tube having 15 ml each of their respective broth. Culture tubes were kept in shaker cum B.O.D. incubator at 37°C for 16 h and at 28°C for 72 h for bacterial and fungal cultures respectively. Bacterial and fungal cultures were adjusted to 0.5 McFarland ( $1.5 \times 10^8$  CFU/ml) and used for further experiments.

Antibacterial and antifungal efficacy of plant extracts was assessed using disc diffusion assay. In brief, NA plates were impregnated with sterile discs after being inoculated with 100 µl of bacterial inoculum. In contrast, PDA plates inoculated with 20 µl of fungal inoculum were used for antifungal assay. Discs were loaded with varying conc. of plant extract (100, 50, 25 and 12.5 mg/ml). Ampicillin (0.1 mg/ml) and fluconazole (0.1 mg/ml) were used as positive controls for antibacterial and antifungal assay, respectively. DMSO was used as negative control. NA plates were kept at 37°C for 24 h, while PDA plates were kept in a B.O.D. incubator at 28°C for 72 h. ZOI (zone of inhibition) obtained was recorded with HiMedia Antibiotic ZoneScale-C. The experiment was performed in triplicates and mean of diameter of ZOI (mm) was taken as final value.

Minimum inhibitory conc. (MIC) of plant extracts that inhibits the growth of bacterial and fungal strains was obtained by micro broth dilution assay through two-fold serial dilutions

(Sarker *et al.*, 2007). 100 µl of NB was added to each well of 96-microtiter plate (12x8 size; Tarson) up to 12 wells. 100 µl of plant extract was added in first well to each row and serially diluted up to 12 wells. 10 µl each of bacterial inoculum ( $1.5 \times 10^8$  CFU/ml) and resazurin dye (0.04% w/v in DW) were added into each well. Petri plates were covered with parafilm to prevent media evaporation and bacterial dehydration and set aside in a B.O.D incubator at 37°C for 24 h. The conc. of plant extract at which no change in colour was observed (blue to pink) was noted as MIC value for a given bacteria. For antifungal micro broth dilution assay, 100 µl of PDB was added to each well of 96-microtiter plate. 100 µl of plant extract was added in first well to each row and serially diluted up to 12 wells. After adding 10 µl of fungal inoculum ( $1.5 \times 10^8$  CFU/ml) in each well, plates were covered with parafilm to prevent media evaporation and kept in a B.O.D incubator at 28°C for 72 h. The conc. of plant extract at which no visible growth of fungal strains could be seen was documented as MIC value for a given fungus.

Graphical analysis was done using Origin Pro-2021. All the experimentation was done in triplicates to ensure the reproducibility of results and data were presented as mean value  $\pm$ SE and p value <0.05 was taken as statistically significant.

## RESULTS AND DISCUSSION

*P. minima* is a traditionally important plant known for its medicinal value and maintaining human health. The current study was aimed at assessing antimicrobial efficacy of *P. minima* aerial vegetative parts. Extraction was carried out in polar (Methanol, Aqueous) and non-polar (Chloroform, Benzene) solvents using Soxhlet extraction and % yield obtained with different solvents are represented in Table 1. ME (methanolic extract) showed highest yield (25.48%), followed by AqE (aqueous extract;

**Table 1.** Percentage yield of *P. minima* extracts prepared in different solvents

S. No.	Plant extract	Weight of crude plant extract (g)	Yield (%)
1.	ME	12.74	25.48
2.	AqE	4.74	9.48
3.	CE	5.12	10.24
4.	BE	2.40	4.80



9.48%) and CE (chloroform extract; 10.24%). Least percentage yield i.e. 4.80% was obtained in BE (benzene extract).

Soxhlet extraction (solid-liquid or hot continuous extraction) was preferred as extraction method due to its numerous advantages. It is a very simple method with clear design that aids in visual oversight of the extraction process. It is economically viable because only a small amount of solvent is required, as well as the solvent is reusable after the process of distillation. In addition, a small quantity of raw material makes it possible to extract a large amount of metabolites (Ingle *et al.*, 2017; Fagbemi *et al.*, 2021).

Seven bacterial strains were used in the present study to assess the antibacterial efficacy of plant extracts by disc diffusion assay. This is the method of choice because it is simple, reliable, versatile, affordable and the results are easy to interpret (Coorevits *et al.*, 2015; Khan *et al.*, 2019). ZOI (mm) obtained against different bacterial strains with plant extracts is shown in Figs. 2 and 3. The results revealed that ME exhibited highest antibacterial activity against *M. smegmatis* ( $16.9 \pm 0.07$ ) followed by *E. coli* ( $14.8 \pm 0.15$ ) and

*P. aeruginosa* ( $14.7 \pm 0.15$ ). ME was found to be least effective against *K. pneumoniae* ( $11.7 \pm 0.17$ ). AqE, was more effective against *C. violaceum* ( $14.1 \pm 0.16$ ) followed by *E. coli* ( $10.8 \pm 0.14$ ) and *B. subtilis* ( $10.1 \pm 0.16$ ). AqE exhibited least inhibitory activity against *S. aureus* ( $7.8 \pm 0.11$ ). CE showed highest ZOI against *B. subtilis* ( $14.3 \pm 0.14$ ) followed by *S. aureus* ( $12.9 \pm 0.10$ ) and *K. pneumoniae* ( $12.4 \pm 0.23$ ). Least inhibitory potential was recorded against *M. smegmatis* ( $10.8 \pm 0.16$ ). BE was highly active against *B. subtilis* ( $17.7 \pm 0.14$ ) and *M. smegmatis* ( $15.5 \pm 0.28$ ) and least effective against *P. aeruginosa* ( $11.1 \pm 0.34$ ). Other research groups also carried out the antibacterial studies on *P. minima* extracts against different bacterial strains and their results are in line with present study findings (Banothu *et al.*, 2017; Pradeepkumar *et al.*, 2022). Interestingly, all the plant extracts were found to be effective even against *C. violaceum*, *M. smegmatis* and *P. aeruginosa*, all of which exhibited resistance to the positive control (ampicillin). The antibacterial activity was due to the presence of secondary metabolites like alkaloids, tannins, phenols and flavonoids, etc. These phytochemicals pass through cell wall of microorganism and

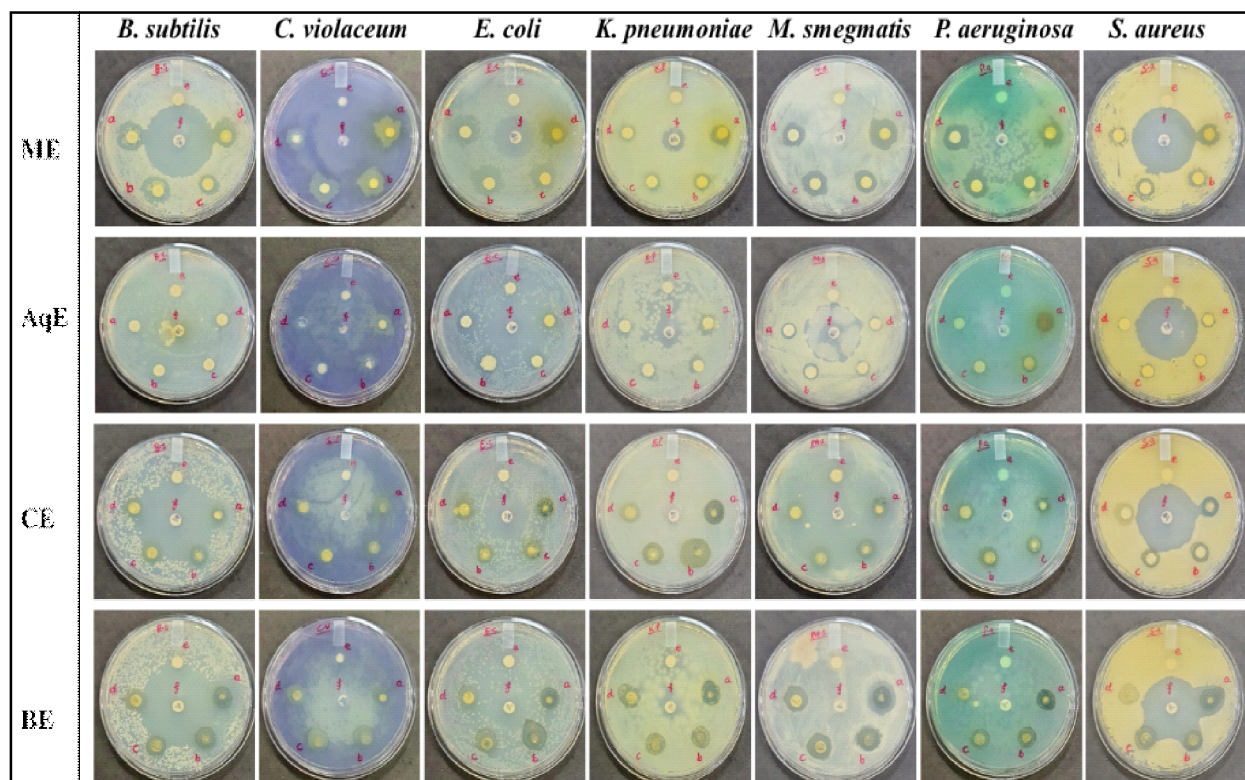


Fig. 2. Disc diffusion assay of *P. minima* extracts against seven bacterial strains.

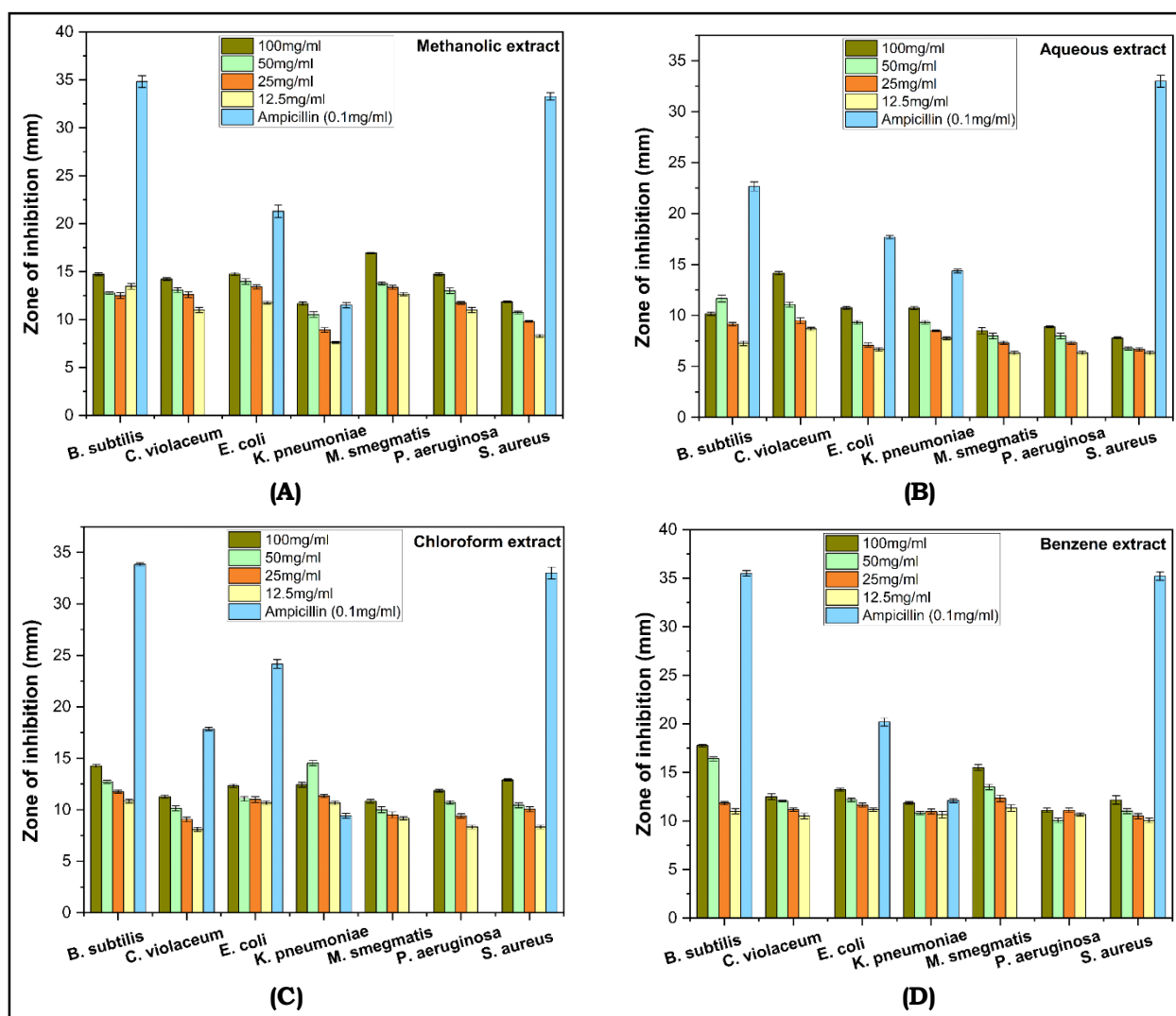


Fig. 3. ZOI of *P. minima* extracts against seven bacterial strains: Methanolic extract (A), Aqueous extract (B); Chloroform extract (C) and Benzene extract (D).

interfere with their ability to survive (Bhatla *et al.*, 2018).

MIC of plant extracts and standard compound (ampicillin) was evaluated with 96-well microbroth dilution assay and the results are represented in Table 2. For ME, least MIC (3.125 mg/ml) was observed against five bacterial strains i.e. *B. subtilis*, *C. violaceum*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. For

AqE, MIC value obtained was 12.5 mg/ml against all the tested bacterial strains. CE and BE exhibited lowest MIC (1.562 mg/ml) for *S. aureus*. For ampicillin, least MIC was recorded in case of *B. subtilis* (0.00625 mg/ml) followed by *E. coli* (0.0125 mg/ml) and *K. pneumoniae* and *S. aureus* (0.025 mg/ml). No inhibitory activity was observed against *C. violaceum* which was in line with the disc diffusion assay

**Table 2.** MIC of *P. minima* extracts (mg/ml) and positive control against seven bacterial strains

<i>P. minima</i> extracts and positive control	<i>B. subtilis</i>	<i>C. violaceum</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>M. smegmatis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
ME	3.125±0.0	3.125±0.0	12.5±0.0	3.125±0.0	6.25±0.0	3.125±0.0	3.125±0.0
AqE	12.5±0.0	12.5±0.0	12.5±0.0	12.5±0.0	12.5±0.0	12.5±0.0	12.5±0.0
CE	3.125±0.0	3.125±0.0	3.125±0.0	6.25±0.0	6.25±0.0	3.125±0.0	1.562±0.0
BE	3.125±0.0	3.125±0.0	3.125±0.0	6.25±0.0	6.25±0.0	3.125±0.0	1.562±0.0
Ampicillin	0.00625±0.0	0.0±0.0	0.0125±0.0	0.025±0.0	0.1±0.0	0.1±0.0	0.025±0.0



outcomes. Although various researchers used disc diffusion assay to evaluate antibacterial activity of *P. minima*, but only few studies have been carried out on MIC assay. For instance, Banothu *et al.* (2017) tested antibacterial activity of different extracts of *P. minima* and found that MIC value lied between 0.125 to 4.0 mg/ml. However, in the current study, MIC value ranged from 0.00625 to 1.562 mg/ml, suggesting plant extracts to possess significant antibacterial potential. This could be probably due to different methods of plant extraction adopted in the present study.

Antifungal potential of plant extracts was assessed against four fungal strains using disc diffusion assay, and the ZOI (mm) obtained is shown in Figs. 4 and 5. The results revealed that ME exhibited highest antifungal activity against *C. albicans* ( $16.5 \pm 0.29$ ) followed by *R. solani* ( $15.1 \pm 0.35$ ) and *F. oxysporum* ( $11.8 \pm 0.50$ ). ME was found to be least effective against *P. expansum* ( $11.1 \pm 0.49$ ). AqE was effective only against *C. albicans* ( $9.0 \pm 0.29$ ) and was found to be inactive against rest three fungal strains. CE showed highest ZOI against *F. oxysporum* ( $12.5 \pm 0.29$ ) followed by *P. expansum* ( $11.8 \pm 0.44$ ) and *C. albicans* ( $11.5 \pm 0.29$ ). Least inhibitory

potential was recorded against *R. solani* ( $11.2 \pm 0.44$ ). BE is active against *C. albicans* ( $11.5 \pm 0.29$ ), *F. oxysporum* ( $11.3 \pm 0.35$ ) and *P. expansum* ( $11.0 \pm 0.29$ ). In contrast, no ZOI was observed in BE against *R. solani*. Interestingly, all the plant extracts were found to be effective against *C. albicans*, which exhibited resistance to the broad-spectrum antifungal drug (fluconazole). Although a number of studies documented the antibacterial activity of *P. minima*, but very few studies were carried out on its antifungal potential (Banothu *et al.*, 2017; Pradeepkumar *et al.*, 2022). Angamuthu *et al.* (2014) did antimicrobial analysis of leaf and fruit extracts of *P. minima* and concluded that methanolic extract exhibited higher antimicrobial potential. The findings of current research suggested that *P. minima* had significant antifungal efficacy, and could be used to combat fungal infections.

MIC of plant extracts and standard compound was evaluated with 96-well microbroth dilution assay and the results are represented in Table 3. For ME, least MIC (3.125 mg/ml) was observed against all the fungal strains excepted *C. albicans*. AqE was found to be inactive against all the fungal strains tested except *C.*

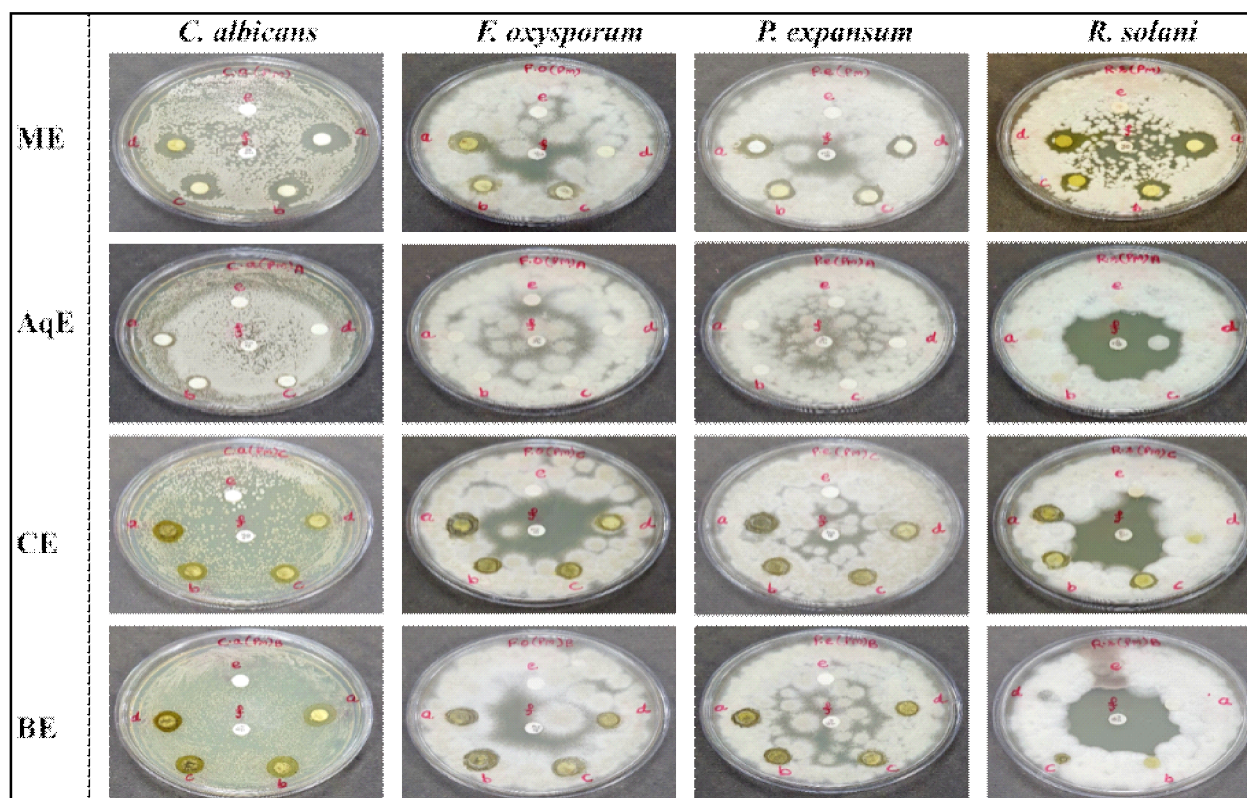


Fig. 4. Disc diffusion assay of *P. minima* extracts against four fungal strains.

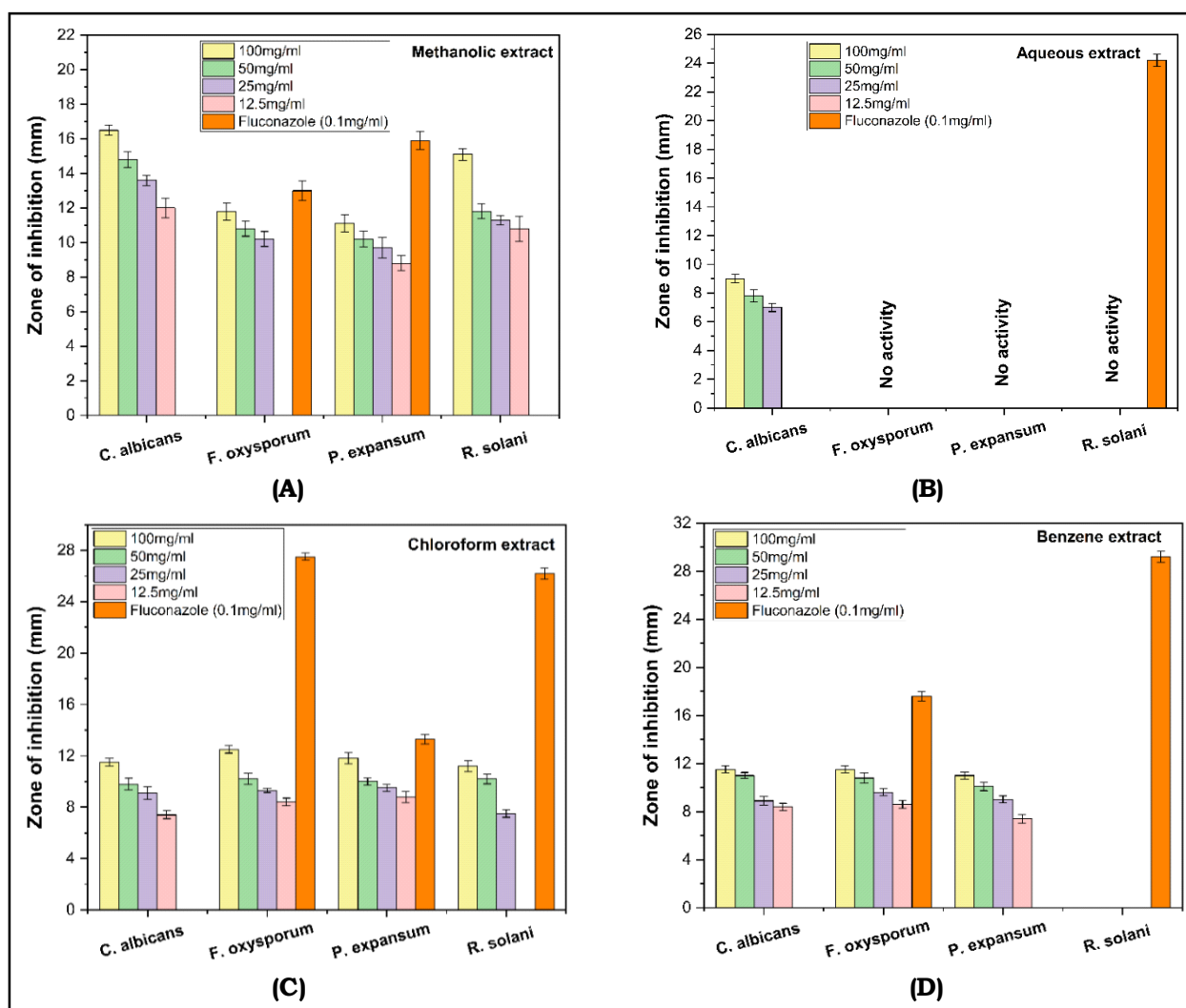


Fig. 5. ZOI of *P. minima* extracts against four fungal strains: Methanolic extract (A), Aqueous extract (B), Chloroform extract (C) and Benzene extract (D).

**Table 3.** MIC of *P. minima* extracts (mg/ml) and positive control against four fungal strains

<i>P. minima</i> extracts and positive control	<i>C. albicans</i>	<i>F. oxysporum</i>	<i>P. expansum</i>	<i>R. solani</i>
ME	6.25±0.0	3.125±0.0	3.125±0.0	3.125±0.0
AqE	12.5±0.0	12.5±0.0	12.5±0.0	12.5±0.0
CE	1.562±0.0	3.125±0.0	3.125±0.0	3.125±0.0
BE	1.562±0.0	3.125±0.0	3.125±0.0	3.125±0.0
Fluconazole	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0

*albicans*. CE and BE exhibited lowest MIC (1.562 mg/ml) for *C. albicans*. For fluconazole, MIC was 0.1 mg/ml for *C. albicans* and *P. expansum*. No inhibitory activity was observed against *F. oxysporum* and *R. solani*. Although various researchers used disc diffusion assay to evaluate antimicrobial activity of *P. minima* but, only few studies have carried out MIC assay.

## CONCLUSION

In the present research work, four extracts of *P. minima* were tested for *in vitro* antimicrobial activity against seven bacterial and four fungal strains. According to the current findings, ME exhibited highest percentage yield as compared to other solvents indicating that methanol is the most suitable solvent for phyto-

compounds extraction. Since the solvent influenced the extraction percentage, it also affected *in vitro* biological activities. It was supported by current findings that ME had the highest antimicrobial activity among all the extracts. All plant extracts were found to be effective against *C. violaceum*, *M. smegmatis* and *P. aeruginosa*, which showed resistance to the broad-spectrum antibiotic (ampicillin). Among the tested fungal strains, *C. albicans* was found to be resistant to standard drug i.e. fluconazole, but was most sensitive to the plant extracts. Therefore, it indicates that *P. minima* extracts may be further explored for its antimicrobial properties against more microbial strains and especially against drug resistant strains. The present findings also provide a platform for researchers to isolate and purify new botanical compounds and opening up novel possibilities of treating bacterial and fungal infections with herbal remedies.

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