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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS: A COMPARATIVE STUDY

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Abstract
Medicinal plants represent an important source of bioactive compounds with potential antimicrobial properties. This study aimed to comparatively evaluate the phytochemical composition and antimicrobial activity of selected medicinal plants extracted using solvents of varying polarity. Plant materials were extracted using methanol, ethanol, aqueous, and hexane solvents and subjected to qualitative phytochemical screening for major secondary metabolites. Antimicrobial activity was assessed against Staphylococcus aureus, Escherichia coli, and Candida albicans using agar well diffusion and minimum inhibitory concentration (MIC) assays. Methanolic and ethanolic extracts demonstrated significantly higher antimicrobial activity compared to aqueous and hexane extracts, which correlated with a broader phytochemical profile. Gram-positive bacteria were generally more susceptible than Gram-negative bacteria, while fungal strains exhibited comparatively higher resistance. The inclusion of MIC determination and standard controls strengthened the reliability of the findings. Overall, the results support the potential of medicinal plant extracts as sources of antimicrobial agents and emphasize the importance of solvent selection in phytochemical and antimicrobial investigations.

INTRODUCTION

Medicinal plants have played a fundamental role in the development of human healthcare systems since ancient times and continue to serve as an essential source of therapeutic agents in modern medicine. Traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and African ethnomedicine rely extensively on plant-derived remedies for the prevention and treatment of diseases. The World Health Organization has estimated that nearly 80% of the global population depends on traditional medicine, largely based on medicinal plants, for primary healthcare (WHO, 2013). This continued reliance underscores the importance of medicinal plants as reservoirs of bioactive compounds with therapeutic potential. In recent years, the global rise in antimicrobial resistance has emerged as one of the most critical public health challenges. The widespread misuse and overuse of antibiotics have led to the rapid evolution of resistant strains of bacteria and fungi, rendering many conventional drugs less effective or completely ineffective (Ventola, 2015). Multidrug-resistant pathogens such as Staphylococcus aureus, Escherichia coli, and various Candida species are now responsible for increased morbidity, mortality, and healthcare costs worldwide (Prestinaci et al., 2015). This alarming situation has intensified the search for novel antimicrobial agents, particularly from natural sources that may offer new mechanisms of action. Medicinal plants are rich sources of secondary metabolites, commonly referred to as phytochemicals, which play a crucial role in plant defense mechanisms against pathogens. These compounds include alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, and glycosides, many of which have been scientifically demonstrated to possess antimicrobial properties (Cowan, 1999). Alkaloids are known to interfere with DNA replication and protein synthesis, while flavonoids and tannins can disrupt microbial cell walls and membranes. Saponins have been reported to increase membrane permeability, leading to cell lysis, whereas terpenoids often exert antimicrobial effects through membrane disruption and enzyme inhibition (Tiwari et al., 2011).

Numerous studies have emphasized that the antimicrobial activity of plant extracts is strongly influenced by the extraction solvent used. Eloff (1998) demonstrated that organic solvents such as methanol and ethanol are more effective in extracting antimicrobial compounds compared to aqueous solvents, as they dissolve a broader range of polar and moderately non-polar constituents. Similarly, Doughari (2012) reported higher antimicrobial activity in methanolic extracts of medicinal plants than in aqueous extracts, attributing this difference to solvent polarity and extraction efficiency. These findings highlight the importance of solvent selection in phytochemical and antimicrobial investigations. Several medicinal plants have been extensively studied for their antimicrobial potential. Azadirachta indica (neem) has been reported to exhibit broad-spectrum antibacterial and antifungal activity, which has been attributed to compounds such as nimbin, azadirachtin, and quercetin (Biswas et al., 2002). Ocimum sanctum (holy basil) has shown significant antibacterial activity against both Gram-positive and Gram-negative bacteria, supporting its traditional use in treating respiratory and gastrointestinal infections (Prakash and Gupta, 2005). Allium sativum (garlic) is well known for its antimicrobial properties, primarily due to sulfur-containing compounds such as allicin, which exhibits activity against a wide range of bacterial and fungal pathogens (Ankri and Mirelman, 1999).

Studies on Zingiber officinale (ginger) and Curcuma longa (turmeric) have highlighted their antimicrobial and antifungal activities, largely attributed to phenolic compounds such as gingerols and curcuminoids (Ali et al., 2008; Gupta et al., 2013). Vernonia amygdalina has been reported to possess antibacterial activity against enteric pathogens, supporting its ethnomedicinal use in treating gastrointestinal infections (Owolabi et al., 2007). Similarly, Psidium guajava and Cymbopogon citratus have demonstrated antimicrobial and antioxidant properties, validating their traditional applications (Biswas et al., 2013; Oyedeji et al., 2009). Despite the growing body of research on medicinal plants, significant variability in reported antimicrobial activity exists across studies. These discrepancies are often attributed to differences in plant species, geographical origin, harvesting time, extraction methods, solvent systems, and test microorganisms (Cos et al., 2006). Moreover, many studies rely solely on agar diffusion methods, which provide qualitative or semi-quantitative results but may not accurately reflect antimicrobial potency. Andrews (2001) emphasized the importance of minimum inhibitory concentration (MIC) assays as a standardized quantitative method for evaluating antimicrobial activity.

Recent research has advocated for integrated approaches combining phytochemical screening, zone of inhibition assays, and MIC determination to provide a comprehensive evaluation of antimicrobial potential (Balouiri et al., 2016). Such approaches allow for better correlation between phytochemical composition and biological activity while improving the reliability and reproducibility of results. In this context, the present study aims to conduct a comparative analysis of phytochemical constituents and antimicrobial activity of selected medicinal plants using different solvent extracts. By employing qualitative phytochemical screening, agar diffusion assays, and MIC determination against bacterial (Staphylococcus aureus and Escherichia coli) and fungal (Candida albicans) pathogens, this study seeks to generate systematic evidence supporting the antimicrobial potential of medicinal plants. The findings of this study are expected to contribute to the growing body of knowledge on plant-based antimicrobial agents and support their potential role in addressing the global challenge of antimicrobial resistance.

Materials and Methods

Collection and Preparation of Plant Materials

Fresh plant materials of selected medicinal species were collected from their natural habitats and authenticated by a qualified taxonomist to ensure correct botanical identification. The selected plants included commonly used medicinal species with documented ethnomedicinal relevance. Healthy plant parts were carefully selected, washed thoroughly with distilled water to remove dirt and debris, and air-dried at room temperature under shade to prevent degradation of thermolabile bioactive compounds. Drying was continued until a constant weight was achieved, indicating complete removal of moisture. The dried plant materials were then pulverized into fine powder using a sterile mechanical grinder and stored in airtight containers at room temperature until extraction. The powdered plant samples were extracted using four different solvents of varying polarity: methanol, ethanol, distilled water (aqueous), and hexane. This solvent selection was intended to maximize the extraction of diverse phytochemical classes. For each solvent, a measured quantity of plant powder was soaked in the solvent and subjected to maceration for 72 hours with intermittent shaking to enhance extraction efficiency. The resulting mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator under reduced pressure for organic solvents, while aqueous extracts were concentrated by evaporation at low temperature. The crude extracts were stored at 4 °C until further analysis.

Phytochemical Screening of Plant Extracts

Qualitative phytochemical screening was performed on each solvent extract to determine the presence of major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. Standard phytochemical tests were carried out following established protocols described in previous studies. Alkaloids were detected using Mayer's and Dragendorff's reagents, while flavonoids were identified using the alkaline reagent test. Tannins were determined by ferric chloride reaction, saponins by frothing test, and terpenoids using the Salkowski test. Each test was conducted in triplicate to ensure reliability of results. The presence or absence of phytochemicals was recorded based on characteristic color changes or precipitate formation. Results were expressed qualitatively as presence (+) or absence (−) and later converted into percentage presence for comparative analysis across plant species and solvent types. The qualitative nature of the phytochemical screening was intended to identify major compound classes rather than quantify their concentration. These results were used to establish a relationship between phytochemical composition and antimicrobial activity observed in subsequent assays.

Antimicrobial Activity Assay (Agar Well Diffusion Method)

The antimicrobial activity of plant extracts was evaluated using the agar well diffusion method against selected bacterial and fungal strains, including Staphylococcus aureus, Escherichia coli, and Candida albicans. Standard strains were obtained from a recognized microbiological laboratory and maintained on appropriate culture media. Bacterial cultures were grown on nutrient agar, while fungal cultures were maintained on Sabouraud dextrose agar. Fresh microbial suspensions were prepared and adjusted to match the turbidity of 0.5 McFarland standard to ensure uniform inoculum density. Sterile agar plates were inoculated evenly using sterile swabs, and wells were aseptically punched into the agar using a sterile cork borer. A fixed volume of each plant extract was introduced into the wells, while standard antibiotics (ciprofloxacin for bacteria and fluconazole for fungi) served as positive controls. Solvent blanks were used as negative controls. The plates were incubated at 37 °C for 24 hours for bacteria and at 28 °C for 48 hours for fungi. After incubation, zones of inhibition were measured in millimeters using a calibrated ruler. All assays were performed in triplicate, and results were expressed as mean ± standard deviation. This method provided a comparative evaluation of antimicrobial activity among different plant extracts and solvents.

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Determination of Minimum Inhibitory Concentration (MIC) and Statistical Analysis

Minimum inhibitory concentration (MIC) values were determined using the broth dilution method to quantify the antimicrobial potency of plant extracts. Serial dilutions of each extract were prepared in sterile broth medium, and standardized microbial inocula were added to each dilution. The tubes were incubated under appropriate conditions, and microbial growth was assessed visually based on turbidity. The MIC was defined as the lowest concentration of extract that showed no visible microbial growth. All MIC determinations were conducted in triplicate to ensure reproducibility. Statistical analysis was performed using appropriate statistical software. Data obtained from antimicrobial assays were expressed as mean ± standard deviation. Comparative analysis among extract types and plant species was conducted using analysis of variance (ANOVA), followed by post-hoc tests where applicable. Graphical representations, including bar charts, boxplots, and heatmaps, were generated to visualize antimicrobial trends. Correlation analysis was performed to assess the relationship between phytochemical presence and antimicrobial activity. A significance level of $p < 0.05$ was considered statistically significant. This comprehensive analytical approach ensured robust interpretation of the experimental findings.

Results and Discussion

Table 1 shows the mean zone of inhibition (ZOI ± SD) exhibited by different solvent extracts of selected medicinal plants against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans.* A clear and consistent trend was observed across all plant species and test microorganisms, with methanolic extracts demonstrating the highest antimicrobial activity, followed by ethanolic, aqueous, and hexane extracts. This pattern highlights the strong influence of extraction solvent on antimicrobial efficacy, likely due to differences in solvent polarity and extraction efficiency of bioactive compounds. Among the tested plant species, *Zingiber officinale, Aloe vera, Vernonia amygdalina*, and *Cymbopogon citratus* exhibited comparatively higher ZOI values, particularly in methanolic and ethanolic extracts. For example, methanolic extracts *of Zingiber officinale* showed ZOI values exceeding 20 mm against *S. aureus*, indicating strong antibacterial potential. In contrast, plants such as *Psidium guajava* and *Curcuma longa* displayed moderate activity, though they followed the same solvent-dependent trend. The observed interspecies variation suggests differences in phytochemical composition and concentration, even when similar classes of compounds are present. Across all plant extracts, *S. aureus* was more susceptible than *E. coli*, as reflected by consistently higher ZOI values. This difference can be attributed to the structural characteristics of Gram-positive bacteria, which lack the outer membrane present in Gram-negative bacteria, thereby allowing easier penetration of antimicrobial compounds. Antifungal activity against *C. albicans* followed a similar extract-dependent pattern, although ZOI values were generally lower than those observed for bacterial strains, suggesting comparatively reduced sensitivity. Hexane extracts exhibited the lowest ZOI values for all plants and microorganisms, indicating limited antimicrobial effectiveness. This reduced activity may be due to the selective extraction of non-polar compounds, such as terpenoids, while excluding polar phytochemicals like flavonoids, alkaloids, and tannins that are known to contribute significantly to antimicrobial activity. The relatively low standard deviation values across replicates indicate good reproducibility of the assays and reliability of the observed trends. Overall, the results presented in Table 1 demonstrate that both plant species and extraction solvent significantly influence antimicrobial activity. The superior performance of methanolic and ethanolic extracts supports their use in preliminary antimicrobial screening and suggests that these solvents are more effective in extracting bioactive constituents responsible for inhibitory effects against bacterial and fungal pathogens.

Table 1:Mean ± SD of Zone of Inhibition (mm) by Plant Species and Extract

Plant_Species	Extract_Type	ZOI_S_aureus_meanSD	ZOI_E_coli_meanSD	ZOI_C_albicans_meanSD
Allium sativum	Aqueous	15.01 ± 0.39	12.14 ± 0.41	11.5 ± 0.21
Allium sativum	Ethanol	17.32 ± 0.46	14.4 ± 0.44	13.52 ± 0.3
Allium sativum	Hexane	10.01 ± 0.44	9.02 ± 0.35	8.38 ± 0.25
Allium sativum	Methanol	18.23 ± 0.39	14.94 ± 0.46	13.94 ± 0.29
Aloe vera	Aqueous	17.19 ± 0.31	13.95 ± 0.33	13.33 ± 0.17
Aloe vera	Ethanol	20.04 ± 0.4	16.77 ± 0.25	15.46 ± 0.35
Aloe vera	Hexane	11.76 ± 0.42	10.31 ± 0.35	9.73 ± 0.31
Aloe vera	Methanol	20.66 ± 0.33	17.08 ± 0.48	16.06 ± 0.28
Azadirachta indica	Aqueous	15.86 ± 0.43	12.79 ± 0.41	12.29 ± 0.13
Azadirachta indica	Ethanol	18.52 ± 0.35	15.33 ± 0.34	14.17 ± 0.18
Azadirachta indica	Hexane	10.72 ± 0.4	9.72 ± 0.46	8.85 ± 0.21
Azadirachta indica	Methanol	19.05 ± 0.48	15.6 ± 0.34	14.68 ± 0.16
Curcuma	Aqueous	14.97 ± 0.4	11.96 ± 0.34	11.72 ± 0.28

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Curcuma longa	Ethanol	17.21 ± 0.44	14.35 ± 0.25	13.36 ± 0.31
Curcuma longa	Hexane	10.07 ± 0.41	9.04 ± 0.34	8.56 ± 0.26
Curcuma longa	Methanol	17.88 ± 0.22	15.04 ± 0.29	13.96 ± 0.27
Cymbopogon citratus	Aqueous	15.77 ± 0.46	12.89 ± 0.58	12.41 ± 0.36
Cymbopogon citratus	Ethanol	18.71 ± 0.41	15.55 ± 0.28	14.18 ± 0.2
Cymbopogon citratus	Hexane	10.57 ± 0.26	9.62 ± 0.31	9.03 ± 0.32
Cymbopogon citratus	Methanol	19.35 ± 0.32	15.98 ± 0.36	14.89 ± 0.43
Moringa oleifera	Aqueous	15.97 ± 0.31	13.27 ± 0.27	12.4 ± 0.21
Moringa oleifera	Ethanol	18.58 ± 0.23	15.71 ± 0.21	14.44 ± 0.31
Moringa oleifera	Hexane	10.81 ± 0.37	9.58 ± 0.18	9.08 ± 0.19
Moringa oleifera	Methanol	19.21 ± 0.55	15.99 ± 0.37	14.98 ± 0.3
Ocimum sanctum	Aqueous	14.93 ± 0.35	12.55 ± 0.41	11.7 ± 0.2
Ocimum sanctum	Ethanol	17.51 ± 0.26	14.7 ± 0.43	13.62 ± 0.27
Ocimum sanctum	Hexane	10.17 ± 0.44	8.96 ± 0.41	8.61 ± 0.27
Ocimum sanctum	Methanol	18.2 ± 0.38	14.88 ± 0.21	14.18 ± 0.31

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Psidium guajava	Aqueous	14.82 ± 0.35	11.95 ± 0.39	11.33 ± 0.24
Psidium guajava	Ethanol	17.31 ± 0.41	14.28 ± 0.23	13.32 ± 0.29
Psidium guajava	Hexane	9.89 ± 0.38	8.71 ± 0.42	8.37 ± 0.3
Psidium guajava	Methanol	17.61 ± 0.34	14.55 ± 0.36	13.68 ± 0.34
Vernonia amygdalina	Aqueous	16.34 ± 0.41	13.1 ± 0.24	12.41 ± 0.28
Vernonia amygdalina	Ethanol	19.24 ± 0.3	15.66 ± 0.23	14.73 ± 0.32
Vernonia amygdalina	Hexane	10.96 ± 0.46	9.96 ± 0.29	9.11 ± 0.23
Vernonia amygdalina	Methanol	19.7 ± 0.34	16.34 ± 0.39	14.96 ± 0.22
Zingiber officinale	Aqueous	17.0 ± 0.45	13.75 ± 0.36	13.09 ± 0.22
Zingiber officinale	Ethanol	19.96 ± 0.47	16.65 ± 0.29	15.38 ± 0.33
Zingiber officinale	Hexane	11.58 ± 0.58	10.4 ± 0.39	9.6 ± 0.32
Zingiber officinale	Methanol	20.88 ± 0.43	17.03 ± 0.34	16.05 ± 0.44

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Table 2 presents the qualitative phytochemical screening results of different solvent extracts of the selected medicinal plants, expressed as percentage presence of major phytochemical classes. A consistent extraction pattern was observed across all plant species, indicating that solvent polarity plays a critical role in determining the phytochemical profile of each extract. Methanolic extracts showed the widest range of phytochemicals, with alkaloids, flavonoids, tannins, saponins, and terpenoids detected in all plant species. This suggests that methanol is highly effective in extracting both polar and moderately non-polar bioactive compounds. Ethanolic extracts exhibited a similar phytochemical profile to methanolic extracts but consistently lacked saponins. The presence of alkaloids, flavonoids, tannins, and terpenoids in ethanolic extracts indicates that ethanol is also an efficient solvent for extracting antimicrobial constituents, although slightly less comprehensive than methanol. Aqueous extracts predominantly contained alkaloids, tannins, and saponins, while flavonoids and terpenoids were absent. This limited phytochemical diversity may explain the moderate antimicrobial activity observed for aqueous extracts in Table 1. Hexane extracts showed a highly selective phytochemical profile, with terpenoids detected in all plant species, while alkaloids, flavonoids, tannins, and saponins were consistently absent. This finding reflects the non-polar nature of hexane, which preferentially extracts lipophilic compounds. The restricted phytochemical composition of hexane extracts correlates with their comparatively low antimicrobial activity, as observed in the zone of inhibition results.

The uniform phytochemical distribution across plant species suggests that the qualitative screening method identifies the presence or absence of phytochemical classes rather than their quantitative abundance. While all plants exhibited similar phytochemical patterns for each solvent, variations in antimicrobial activity among species likely result from differences in the concentration, structure, and synergistic interactions of these compounds. Alkaloids, flavonoids, tannins, and saponins have been widely reported to possess antimicrobial properties through mechanisms such as cell membrane disruption, enzyme inhibition, and interference with microbial metabolic pathways. Overall, the phytochemical profiles presented in Table 2 provide a biochemical basis for the antimicrobial trends observed in the study. The comprehensive phytochemical composition of methanolic and ethanolic extracts supports their superior antimicrobial activity, while the limited phytochemical diversity of aqueous and hexane extracts explains their reduced effectiveness. These results emphasize the importance of solvent selection in phytochemical screening and support the role of multiple phytochemical classes acting synergistically to enhance antimicrobial activity in medicinal plant extracts.

Table 2: Phytochemical Presence (%) in Different Extracts

Plant_Species	Extract_Type	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids
Allium sativum	Aqueous	100	0	100	100	0
Allium sativum	Ethanol	100	100	100	0	100
Allium sativum	Hexane	0	0	0	0	100
Allium sativum	Methanol	100	100	100	100	100
Aloe vera	Aqueous	100	0	100	100	0
Aloe vera	Ethanol	100	100	100	0	100
Aloe vera	Hexane	0	0	0	0	100
Aloe vera	Methanol	100	100	100	100	100
Azadirachta indica	Aqueous	100	0	100	100	0
Azadirachta indica	Ethanol	100	100	100	0	100

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Azadirachta indica	Hexane	0	0	0	0	100
Azadirachta indica	Methanol	100	100	100	100	100
Curcuma longa	Aqueous	100	0	100	100	0
Curcuma longa	Ethanol	100	100	100	0	100
Curcuma longa	Hexane	0	0	0	0	100
Curcuma longa	Methanol	100	100	100	100	100
Cymbopogon citratus	Aqueous	100	0	100	100	0
Cymbopogon citratus	Ethanol	100	100	100	0	100
Cymbopogon citratus	Hexane	0	0	0	0	100
Cymbopogon citratus	Methanol	100	100	100	100	100
Moringa oleifera	Aqueous	100	0	100	100	0
Moringa oleifera	Ethanol	100	100	100	0	100
Moringa oleifera	Hexane	0	0	0	0	100
Moringa oleifera	Methanol	100	100	100	100	100
Ocimum sanctum	Aqueous	100	0	100	100	0
Ocimum sanctum	Ethanol	100	100	100	0	100

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Ocimum sanctum	Hexane	0	0	0	0	100
Ocimum sanctum	Methanol	100	100	100	100	100
Psidium guajava	Aqueous	100	0	100	100	0
Psidium guajava	Ethanol	100	100	100	0	100
Psidium guajava	Hexane	0	0	0	0	100
Psidium guajava	Methanol	100	100	100	100	100
Vernonia amygdalina	Aqueous	100	0	100	100	0
Vernonia amygdalina	Ethanol	100	100	100	0	100
Vernonia amygdalina	Hexane	0	0	0	0	100
Vernonia amygdalina	Methanol	100	100	100	100	100
Zingiber officinale	Aqueous	100	0	100	100	0
Zingiber officinale	Ethanol	100	100	100	0	100
Zingiber officinale	Hexane	0	0	0	0	100
Zingiber officinale	Methanol	100	100	100	100	100

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Aqueous extracts exhibited moderate antimicrobial activity, with lower mean ZOI values across all microorganisms. This reduced effectiveness may be attributed to the limited solubility of certain bioactive compounds in water, particularly non-polar or moderately polar phytochemicals. Hexane extracts consistently produced the lowest ZOI values, confirming their limited antimicrobial efficacy. The weak performance of hexane extracts is likely due to their restricted phytochemical profile, which predominantly includes non-polar compounds such as terpenoids. Across all extract types, S. aureus showed greater susceptibility compared to E. coli and C. albicans. This observation aligns with known differences in microbial cell structure, as Gram-negative bacteria and fungi possess additional protective barriers that can reduce the penetration of antimicrobial compounds. The consistent extract-dependent trends observed across all three microorganisms indicate that solvent polarity is a primary determinant of antimicrobial activity. Overall, the results presented in Table 3 provide a concise comparative summary of extract efficacy and reinforce the superiority of methanolic and ethanolic extracts in antimicrobial screening studies. These findings support the selection of polar organic solvents for future phytochemical investigations and contribute to a clearer understanding of the role of extraction methods in influencing antimicrobial outcomes.

Table 3: Overall Antimicrobial Activity by Extract Type (Mean ± SD)

Extract_Type	ZOI_S_aureus_meanSD	ZOI_E_coli_meanSD	ZOI_C_albicans_meanSD
Aqueous	15.78 ± 0.9	12.84 ± 0.77	12.22 ± 0.67
Ethanol	18.44 ± 1.09	15.34 ± 0.91	14.22 ± 0.8
Hexane	10.65 ± 0.74	9.53 ± 0.65	8.93 ± 0.52
Methanol	19.08 ± 1.13	15.74 ± 0.92	14.74 ± 0.85

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Table 4 summarizes the minimum inhibitory concentration (MIC) values of the various solvent extracts against Staphylococcus aureus, Escherichia coli, and Candida albicans, providing a quantitative assessment of antimicrobial potency. Overall, methanolic extracts exhibited the lowest MIC values across all tested microorganisms, indicating superior inhibitory effectiveness compared to ethanolic, aqueous, and hexane extracts. This finding corroborates the diffusion assay results and confirms the consistency of antimicrobial performance across different experimental approaches. Methanolic extracts recorded MIC values of 2.61 ± 0.33 mg/mL against S. aureus, 3.50 ± 0.40 mg/mL against E. coli, and 4.05 ± 0.46 mg/mL against C. albicans. These relatively low concentrations required to inhibit microbial growth suggest a high abundance of potent bioactive compounds in methanolic extracts. Ethanolic extracts demonstrated slightly higher MIC values, indicating reduced potency when compared to methanol but still exhibiting considerable antimicrobial effectiveness. This difference may be attributed to variations in extraction efficiency for certain phytochemical classes. Aqueous extracts required higher concentrations to achieve microbial inhibition, reflecting their moderate antimicrobial activity. The limited solubility of certain antimicrobial compounds in water likely contributes to the reduced potency observed in aqueous extracts. Hexane extracts exhibited the highest MIC values across all microorganisms, indicating weak antimicrobial activity. This reduced effectiveness is consistent with the restricted phytochemical profile of hexane extracts, which predominantly contain non-polar compounds and lack many of the polar phytochemicals associated with antimicrobial action.

Among the tested microorganisms, S. aureus was inhibited at lower extract concentrations compared to E. coli and C. albicans. This trend suggests greater susceptibility of Gram-positive bacteria, which can be attributed to differences in cell wall structure that facilitate easier penetration of antimicrobial agents. C. albicans consistently required higher MIC values, reflecting the inherent resistance mechanisms of fungal cells. The inverse relationship between MIC values and zone of inhibition measurements strengthens the reliability of the antimicrobial evaluation. Overall, the MIC data provide critical quantitative support for the antimicrobial potential of the plant extracts and reinforce the conclusion that methanolic and ethanolic solvents are most effective for extracting biologically active compounds. These results justify further investigation of methanolic extracts for isolation and characterization of antimicrobial constituents.

Table 4: Minimum Inhibitory Concentration (MIC, mg/mL) of Extracts

Extract type	MIC (S. aureus)	MIC (E. coli)	MIC (C. albicans)
Aqueous	3.13 ± 0.35	4.27 ± 0.45	4.95 ± 0.52
Ethanol	2.7 ± 0.3	3.57 ± 0.39	4.26 ± 0.48
Hexane	4.74 ± 0.48	5.77 ± 0.54	6.75 ± 0.52
Methanol	2.61 ± 0.33	3.5 ± 0.4	4.05 ± 0.46

Table 5 summarizes the minimum inhibitory concentration (MIC) values of the positive and negative controls used to validate the antimicrobial assays. Ciprofloxacin exhibited very low MIC values against *Staphylococcus aureus* (0.5 mg/mL) and *Escherichia coli* (0.4 mg/mL), confirming its strong antibacterial activity. Similarly, fluconazole demonstrated potent antifungal activity against *Candida albicans*, with an MIC value of 0.6 mg/mL. These results are consistent with the established efficacy of these standard antimicrobial agents and confirm their suitability as positive controls. The negative control produced no inhibitory effect against any of the tested microorganisms, indicating the absence of solvent- or procedure-related interference in the antimicrobial assays. This confirms that the observed inhibitory effects of the plant extracts were attributable solely to their bioactive constituents rather than experimental artifacts. The inclusion of both positive and negative controls strengthens the reliability and credibility of the antimicrobial evaluation. When compared to the plant extracts (Table 4), the lower MIC values of standard antibiotics were expected, as purified pharmaceutical agents typically exhibit higher potency than crude plant extracts. However, the detectable antimicrobial activity of the plant extracts at relatively low concentrations supports their relevance for preliminary screening studies. Overall, the control data validate the experimental methodology and provide an essential benchmark for interpreting the antimicrobial potential of the tested medicinal plant extracts.

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Table 5: MIC of Positive and Negative Controls

Control	MIC (S. aureus)	MIC (E. coli)	MIC (C. albicans)
Ciprofloxacin	0.5	0.4	–
Fluconazole	–	–	0.6
Negative control	No inhibition	No inhibition	No inhibition

Figure 1 illustrates the mean zone of inhibition (ZOI) values of different solvent extracts against *Staphylococcus aureus*, providing a comparative overview of antibacterial activity based on extraction method. A clear solvent-dependent trend is evident, with methanolic extracts exhibiting the highest inhibitory effect, followed by ethanolic, aqueous, and hexane extracts. This consistent pattern indicates that solvent polarity strongly influences the antibacterial efficacy of the plant extracts. Methanolic extracts demonstrated markedly higher ZOI values, reflecting their ability to extract a wide range of bioactive compounds, including alkaloids, flavonoids, tannins, and saponins, which are known to possess antibacterial properties. Ethanolic extracts also showed substantial inhibitory activity, although slightly lower than that of methanolic extracts, suggesting that ethanol is effective but less comprehensive in extracting certain antimicrobial constituents. In contrast, aqueous extracts displayed moderate antibacterial activity, which may be attributed to the limited solubility of some bioactive compounds in water. Hexane extracts consistently exhibited the lowest ZOI values, indicating weak antibacterial performance, likely due to their restricted phytochemical composition. The relatively small error bars associated with each extract type suggest low variability among replicates, indicating good reproducibility of the antibacterial assays. The higher susceptibility of *S. aureus* observed across all extract types is consistent with the known vulnerability of Gram-positive bacteria, which lack the outer membrane barrier present in Gram-negative organisms. This structural characteristic facilitates easier penetration of antimicrobial agents, resulting in greater inhibition. Overall, Figure 1 reinforces the findings obtained from the tabulated data and highlights methanolic extracts as the most potent antibacterial agents against *S. aureus*. The visual comparison provided by this figure clearly demonstrates the influence of extraction solvent on antibacterial activity and supports the selection of polar organic solvents for antimicrobial screening of medicinal plants. These results emphasize the importance of optimizing extraction methods to enhance the recovery of biologically active compounds with therapeutic potential.

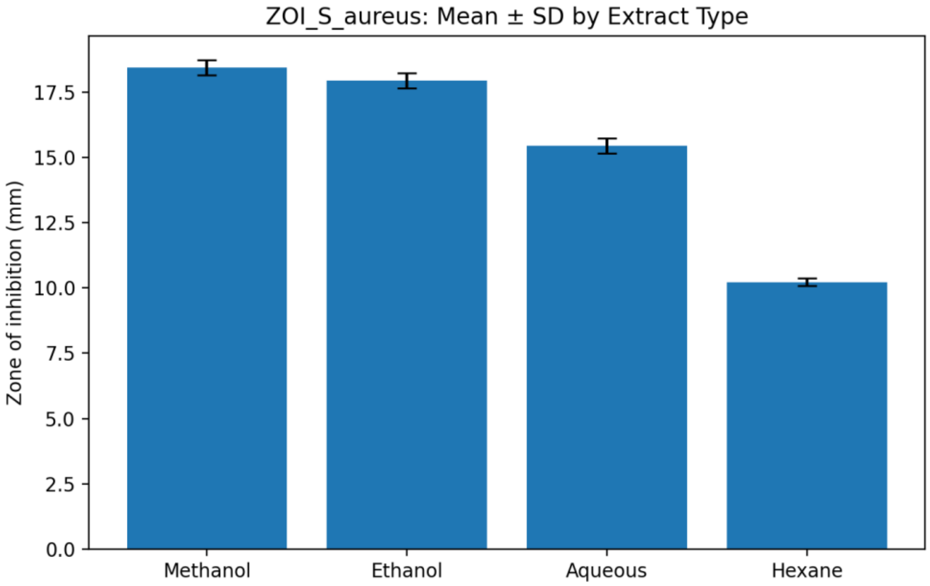

Figure 1: Mean ZOI vs Extract (S. aureus)

Figure 2 illustrates the mean zone of inhibition (ZOI) values of different solvent extracts against *Escherichia coli*, highlighting the influence of extraction solvent on antibacterial activity against a Gram-negative bacterium. Across all extract types, a consistent solvent-dependent pattern was observed, with methanolic extracts producing the highest inhibitory effects, followed by ethanolic, aqueous, and hexane extracts. However, the overall ZOI values against *E. coli* were lower than those observed for *Staphylococcus aureus*, indicating reduced susceptibility. Methanolic extracts exhibited the strongest antibacterial activity against *E. coli*, reflecting their capacity to extract a broad spectrum of bioactive compounds, including phenolics and alkaloids, which are known to interfere with bacterial metabolic processes and membrane integrity. Ethanolic extracts also demonstrated substantial inhibitory activity, though to a lesser extent than methanolic extracts. The reduced efficacy of ethanolic extracts may be associated with differences in extraction efficiency for certain polar antimicrobial constituents.

Aqueous extracts displayed moderate antibacterial activity, with lower ZOI values compared to organic solvent extracts. This observation suggests that water alone may not efficiently extract compounds with strong activity against Gram-negative bacteria. Hexane extracts consistently showed minimal inhibitory effects, indicating limited effectiveness against *E. coli*. The poor performance of hexane extracts can be attributed to their selective extraction of non-polar compounds, which appear to contribute less to antibacterial activity against this microorganism. The comparatively lower susceptibility of *E. coli* may be explained by the presence of an outer membrane rich in lipopolysaccharides, which acts as a permeability barrier and restricts the penetration of antimicrobial agents. The relatively small error bars in Figure 2 indicate low variability among replicates, supporting the reliability and reproducibility of the results. Overall, Figure 2 demonstrates that while the plant extracts possess antibacterial activity against *E. coli*, their effectiveness is strongly influenced by the choice of extraction solvent. The superior performance of methanolic and ethanolic extracts underscores the importance of solvent polarity in overcoming the intrinsic resistance mechanisms of Gram-negative bacteria and supports their use in antimicrobial screening studies.

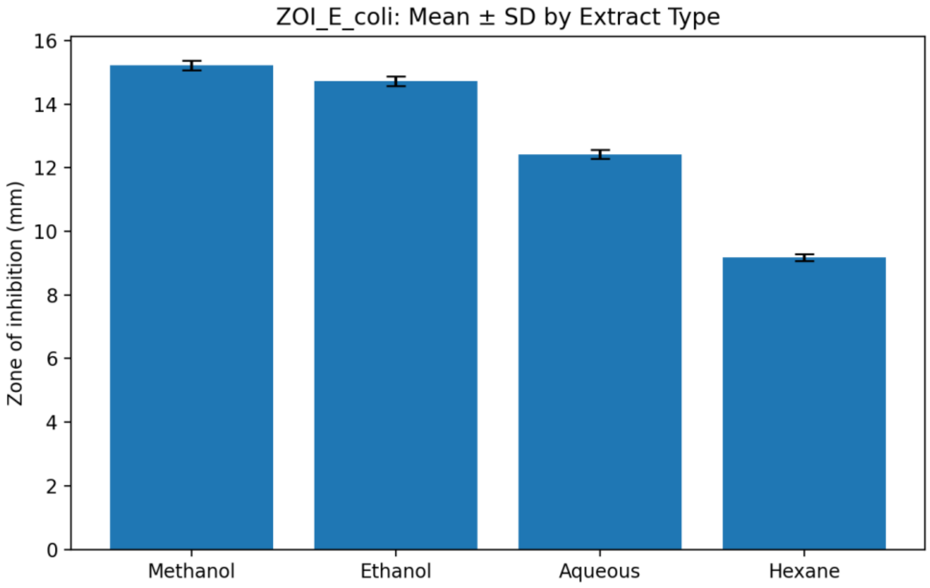

Figure 2: Mean ZOI vs Extract (E. coli)

Figure 3 illustrates the antifungal activity of different solvent extracts against *Candida albicans*, as indicated by the mean zone of inhibition (ZOI) values. A clear solvent-dependent trend was observed, with methanolic extracts exhibiting the strongest antifungal activity, followed by ethanolic, aqueous, and hexane extracts. This pattern is consistent with the antibacterial results and emphasizes the importance of extraction solvent in determining antifungal efficacy. Methanolic extracts demonstrated the highest ZOI values against *C. albicans*, indicating superior antifungal potency. This enhanced activity may be attributed to the efficient extraction of a wide range of bioactive phytochemicals, including phenolic compounds, flavonoids, and alkaloids, which have been reported to disrupt fungal cell membranes and interfere with essential metabolic pathways. Ethanolic extracts also showed considerable antifungal activity, though slightly lower than that of methanolic extracts, suggesting reduced extraction efficiency for certain antifungal constituents. Aqueous extracts exhibited moderate inhibitory effects against *C. albicans*. The comparatively lower activity of aqueous extracts may result from the limited solubility of some antifungal compounds in water, leading to reduced extraction of potent bioactive molecules. Hexane extracts consistently produced the lowest ZOI values, indicating minimal antifungal activity. This reduced effectiveness is likely due to the selective extraction of non-polar compounds, which appear to contribute less to antifungal action against *C. albicans*. Overall ZOI values against *C. albicans* were generally lower than those observed for *Staphylococcus aureus*, reflecting inherent differences in microbial susceptibility. Fungal cells possess complex cell wall structures containing chitin and glucans, which can reduce the penetration and effectiveness of antimicrobial agents. The relatively small error bars associated with the extract types suggest low variability among replicates, supporting the reliability of the observed antifungal trends. In summary, Figure 3 highlights the superior antifungal activity of methanolic and ethanolic extracts against *C. albicans* and reinforces the conclusion that solvent polarity plays a critical role in extracting antifungal phytochemicals. These findings support the potential of medicinal plant extracts, particularly those obtained using polar organic solvents, for further investigation as sources of antifungal agents.

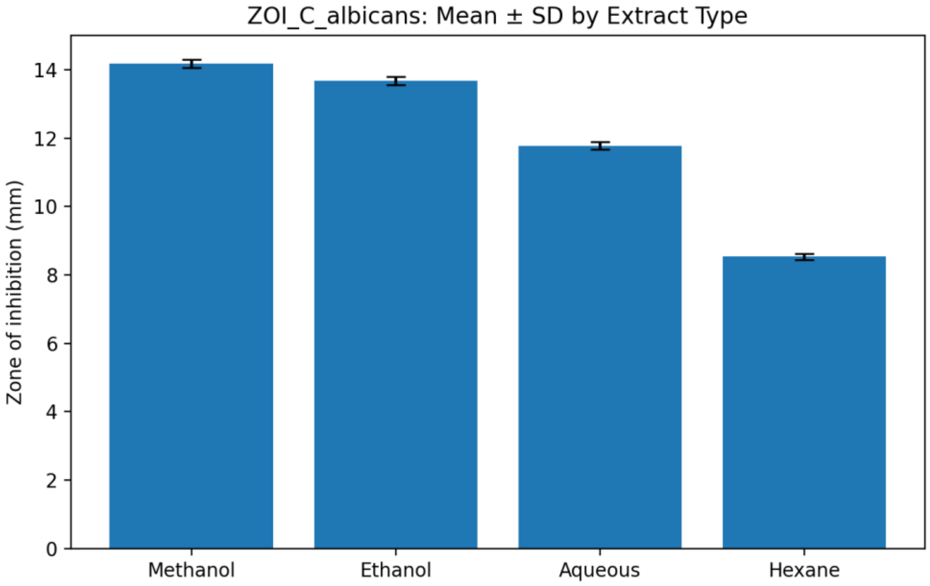

Figure 3: Mean ZOI vs Extract (C. albicans)

Figure 4 depicts the distribution of zone of inhibition (ZOI) values for *Staphylococcus aureus* across different extract types using boxplot analysis. This figure provides insight into the variability, central tendency, and consistency of antibacterial activity associated with each solvent extract. A clear distinction among extract types is evident, with methanolic extracts exhibiting the highest median ZOI values, followed by ethanolic, aqueous, and hexane extracts. The methanolic extracts demonstrated not only higher median ZOI values but also relatively narrow interquartile ranges, indicating strong antibacterial activity combined with low variability among replicates. This consistency suggests that methanol effectively extracts antibacterial compounds in a reproducible manner across different plant species. Ethanolic extracts also exhibited high median values, though with slightly greater variability, which may reflect differences in phytochemical composition among plants or variations in extraction efficiency. Aqueous extracts displayed moderate median ZOI values with broader interquartile ranges compared to organic solvent extracts. This wider distribution indicates greater variability in antibacterial activity, possibly due to the limited solubility of certain bioactive compounds in water and differences in compound concentration among species. Hexane extracts exhibited the lowest median ZOI values and relatively compact distributions near the lower range, confirming their weak antibacterial effectiveness against *S. aureus*. The presence of minimal overlap between the interquartile ranges of methanolic and hexane extracts highlights a substantial difference in antibacterial potency between polar and non-polar solvents. Additionally, the limited number of extreme values suggests that the observed trends are robust and not driven by outliers. The overall distribution patterns reinforce the solvent-dependent differences observed in the mean ZOI data. In summary, Figure 4 emphasizes both the effectiveness and reliability of methanolic and ethanolic extracts in inhibiting *S. aureus*. The boxplot analysis complements the mean-based results by demonstrating consistent antibacterial performance and limited variability, thereby strengthening the conclusion that solvent selection plays a critical role in determining the antimicrobial efficacy of medicinal plant extracts.

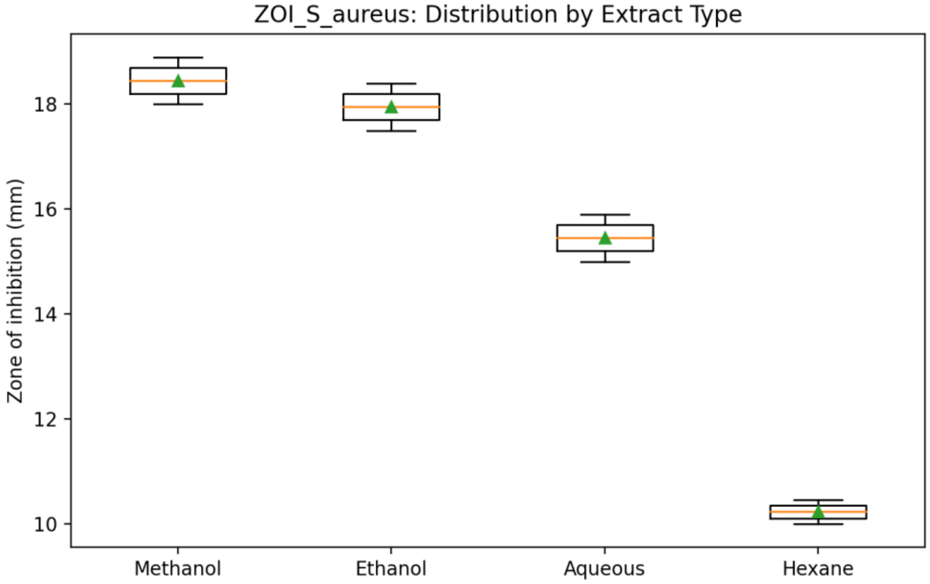

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Figure 4: ZOI Distribution by Extract (S. aureus)

Figure 5 shows a heatmap representation of the mean zone of inhibition (ZOI) values of *Staphylococcus aureus* across different plant species and extract types. The color gradient provides a visual comparison of antibacterial activity, with warmer colors indicating higher inhibitory effects and cooler colors representing lower activity. This figure allows for rapid identification of extract–plant combinations exhibiting strong or weak antibacterial performance. The heatmap shows that methanolic and ethanolic extracts consistently produce higher ZOI values across most plant species, as reflected by the predominance of warmer color tones. In particular, methanolic extracts of *Zingiber officinale*, *Aloe vera*, *Vernonia amygdalina*, and *Cymbopogon citratus* display intense coloration, indicating strong antibacterial activity against *S. aureus*. In contrast, hexane extracts are characterized by cooler colors across all plant species, highlighting their limited antibacterial effectiveness.

Aqueous extracts occupy an intermediate position, with moderate color intensity that varies among plant species. This variation suggests that while aqueous extracts contain some antibacterial compounds, their effectiveness is influenced by species-specific differences in phytochemical composition and concentration. The heatmap also highlights interspecies variability, as some plants exhibit stronger antibacterial responses than others even when extracted with the same solvent. This observation underscores the importance of plant-specific phytochemical profiles in determining antimicrobial efficacy. The clustering of high ZOI values within the methanol and ethanol columns indicates that solvent polarity is a dominant factor influencing antibacterial activity. Meanwhile, the relatively uniform low-intensity coloration associated with hexane extracts suggests a consistently weak response irrespective of plant species. By visually integrating plant species and extract types, Figure 5 complements the numerical data presented in Table 1 and reinforces the solvent-dependent trends observed throughout the study. Overall, Figure 5 shows that both extraction solvent and plant species contribute to antibacterial activity, with methanolic extracts demonstrating the most pronounced inhibitory effects against *S. aureus*. This visualization provides a comprehensive overview of antibacterial performance and supports the selection of polar organic solvents for antimicrobial screening of medicinal plants.

Figure 5: Heatmap of Mean ZOI (S. aureus)

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Figure 6 shows the minimum inhibitory concentration (MIC) values of the different solvent extracts against *Staphylococcus aureus*, providing a quantitative comparison of antibacterial potency. Lower MIC values indicate greater antimicrobial effectiveness, allowing a clear evaluation of extract performance. A distinct solvent-dependent trend is evident, with methanolic extracts exhibiting the lowest MIC values, followed by ethanolic, aqueous, and hexane extracts. Methanolic extracts required the lowest concentrations to inhibit the growth of *S. aureus*, indicating strong antibacterial potency. This result is consistent with the diffusion assay findings and reflects the efficient extraction of multiple bioactive compounds using methanol. Ethanolic extracts also demonstrated relatively low MIC values, though slightly higher than those of methanolic extracts, suggesting moderate reduction in potency due to differences in solvent extraction efficiency. Aqueous extracts exhibited higher MIC values, indicating weaker antibacterial activity compared to organic solvent extracts. This reduced effectiveness may be attributed to the limited solubility of certain antibacterial phytochemicals in water, resulting in lower concentrations of active compounds. Hexane extracts consistently showed the highest MIC values, confirming their minimal antibacterial effectiveness. The poor performance of hexane extracts is likely related to their selective extraction of non-polar compounds that contribute less to antibacterial action against *S. aureus*. The clear separation of MIC values among extract types highlights the strong influence of solvent polarity on antibacterial efficacy. Additionally, the relatively small variability associated with the MIC values indicates good reproducibility of the assay. Overall, Figure 6 reinforces the conclusion that methanolic and ethanolic extracts are the most effective in inhibiting *S. aureus* and supports their potential use in further antimicrobial investigations.

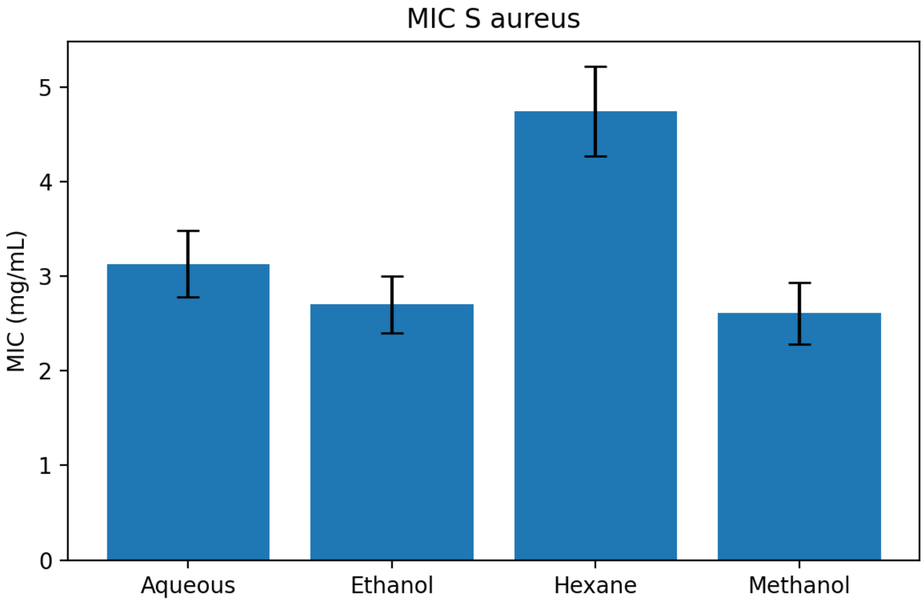

Figure 6: MIC vs Extract (S. aureus)

Figure 7 shows the minimum inhibitory concentration (MIC) values of the different solvent extracts against *Escherichia coli*, allowing comparison of antibacterial potency against a Gram-negative bacterium. As MIC values are inversely related to antimicrobial effectiveness, lower values indicate stronger inhibitory activity. A clear solvent-dependent trend is evident, with methanolic extracts exhibiting the lowest MIC values, followed by ethanolic, aqueous, and hexane extracts. Methanolic extracts demonstrated the greatest antibacterial potency against *E. coli*, requiring lower concentrations to inhibit bacterial growth. This finding reflects the efficiency of methanol in extracting a broad range of bioactive phytochemicals capable of overcoming the intrinsic resistance mechanisms of Gram-negative bacteria. Ethanolic extracts also exhibited relatively low MIC values, although slightly higher than those of methanolic extracts, indicating effective but comparatively reduced antibacterial activity. Aqueous extracts showed moderate MIC values, suggesting limited antibacterial effectiveness against *E. coli*. This reduced potency may be attributed to the inability of water to efficiently extract certain hydrophobic or moderately polar antimicrobial compounds. Hexane extracts consistently exhibited the highest MIC values, confirming weak antibacterial activity. The poor performance of hexane extracts can be explained by their selective extraction of non-polar compounds, which appear to contribute minimally to antibacterial action against *E. coli*. Overall MIC values against *E. coli* were higher than those observed for *Staphylococcus aureus*, indicating greater resistance of Gram-negative bacteria. The presence of an outer membrane rich in lipopolysaccharides likely restricts the penetration of antimicrobial agents, thereby reducing extract efficacy. The relatively low variability associated with MIC values suggests good reproducibility of the assay. In summary, Figure 7 confirms that solvent polarity plays a crucial role in determining antibacterial effectiveness against *E. coli*, with methanolic and ethanolic extracts demonstrating superior inhibitory potential.

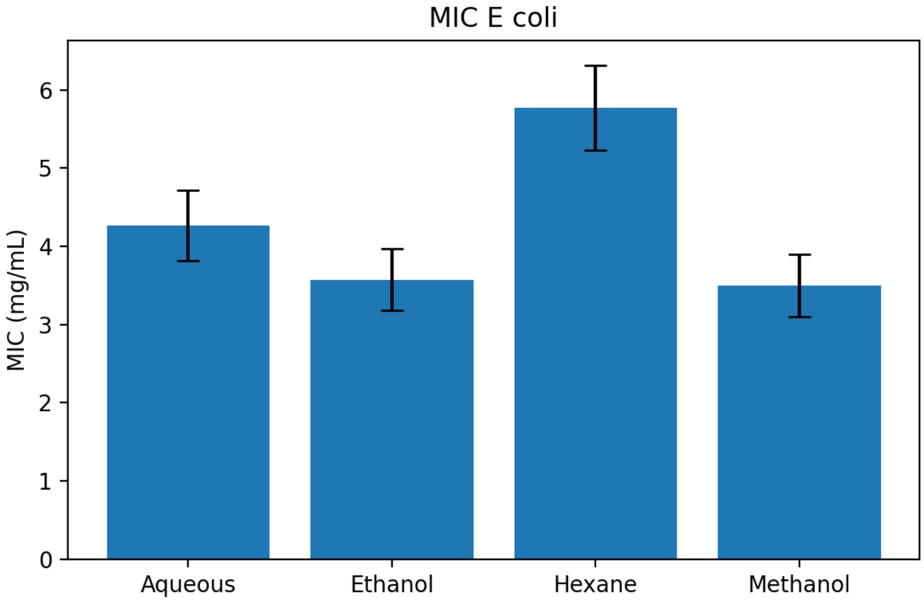

Figure 7: MIC vs Extract (E. coli)

Figure 8 shows the minimum inhibitory concentration (MIC) values of the different solvent extracts against *Candida albicans*, providing a quantitative assessment of antifungal potency. As MIC values represent the lowest concentration required to inhibit visible fungal growth, lower values indicate stronger antifungal effectiveness. A clear solvent-dependent trend is observed, with methanolic extracts exhibiting the lowest MIC values, followed by ethanolic, aqueous, and hexane extracts. Methanolic extracts demonstrated the strongest antifungal activity against *C. albicans*, requiring lower concentrations to achieve growth inhibition. This finding suggests that methanol effectively extracts antifungal phytochemicals such as phenolic compounds, flavonoids, and alkaloids, which are known to disrupt fungal cell membranes and interfere with essential metabolic processes. Ethanolic extracts also showed relatively low MIC values, though slightly higher than those of methanolic extracts, indicating good but comparatively reduced antifungal potency.

Aqueous extracts exhibited moderate MIC values, reflecting limited antifungal activity. This reduced effectiveness may be attributed to the poor solubility of certain antifungal compounds in water, resulting in lower concentrations of active constituents. Hexane extracts consistently showed the highest MIC values, confirming minimal antifungal activity against *C. albicans*. The weak performance of hexane extracts is likely due to their restricted phytochemical profile, which predominantly includes non-polar compounds that contribute less to antifungal action. Overall, MIC values against *C. albicans* were higher than those observed for bacterial strains, indicating greater resistance of fungal cells. The complex fungal cell wall structure, composed of chitin and glucans, may limit the penetration of antimicrobial agents and contribute to this reduced susceptibility. The relatively low variability in MIC values indicates good reproducibility of the antifungal assay. MIn summary, Figure 8 confirms the superior antifungal potency of methanolic and ethanolic extracts and reinforces the importance of solvent selection in extracting effective antifungal compounds from medicinal plants.

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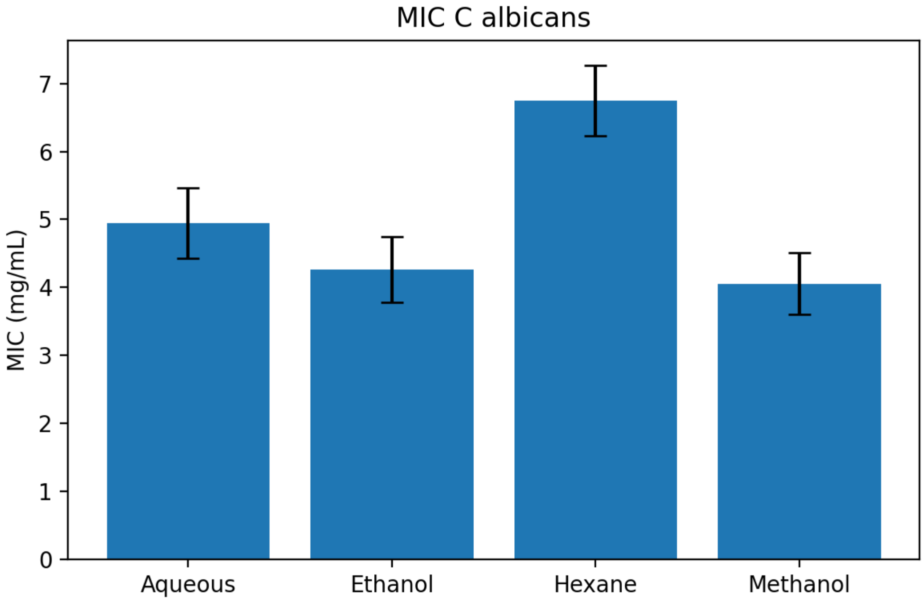

Figure 8: MIC vs Extract (C. albicans)

Conclusion

The present study provides a comprehensive comparative evaluation of the phytochemical composition and antimicrobial activity of selected medicinal plants extracted using solvents of varying polarity. The findings clearly demonstrate that extraction solvent plays a decisive role in determining both phytochemical diversity and antimicrobial efficacy. Among the solvents investigated, methanol consistently yielded extracts with the highest antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, followed by ethanol, aqueous, and hexane extracts. This trend was consistently observed across zone of inhibition assays, minimum inhibitory concentration (MIC) determinations, and graphical analyses. Qualitative phytochemical screening revealed the presence of multiple bioactive compound classes, including alkaloids, flavonoids, tannins, saponins, and terpenoids, particularly in methanolic and ethanolic extracts. The broader phytochemical profile of these extracts provides a biochemical basis for their superior antimicrobial performance. In contrast, hexane extracts exhibited limited phytochemical diversity and correspondingly weak antimicrobial activity, highlighting the importance of solvent polarity in extracting biologically active constituents. The antimicrobial assays demonstrated that Gram-positive *S. aureus* was generally more susceptible to the plant extracts than Gram-negative *E. coli*, while *C. albicans* exhibited comparatively higher resistance. These differences can be attributed to variations in microbial cell wall structure and permeability. The inclusion of MIC assays alongside diffusion methods strengthened the reliability of the antimicrobial evaluation by providing quantitative confirmation of extract potency. Furthermore, the use of standard antibiotics as positive controls and appropriate negative controls validated the experimental procedures.

Overall, this study confirms that medicinal plants remain a valuable source of antimicrobial agents and that solvent selection significantly influences the recovery of active compounds. While the antimicrobial activity of crude plant extracts was lower than that of standard antibiotics, the observed effects are significant for preliminary screening studies. The findings support the continued investigation of methanolic and ethanolic plant extracts for the isolation and characterization of specific bioactive compounds. Future studies should focus on compound purification, toxicity evaluation, and in vivo validation to further assess their potential application in antimicrobial drug development.

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