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MEDICINAL PLANTS AS A SOURCE OF BIOACTIVE COMPOUNDS

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Abstract
This study investigates the phytochemical composition and antimicrobial activity of three widely used medicinal plants, Azadirachta indica, Ocimum sanctum, and Aloe vera, collected from southern Punjab, Pakistan. Methanolic extracts of the selected plants were prepared and analyzed for total phenolic content (TPC), total flavonoid content, and antibacterial activity against Escherichia coli and Staphylococcus aureus using the standard disc diffusion method. The results revealed significant variation in phytochemical composition among the plant species. Neem exhibited the highest total phenolic content (78.5 ± 3.2 mg GAE/g) and flavonoid concentration (42.6 ± 2.1 mg QE/g), followed by holy basil and Aloe vera. In antimicrobial assays, neem demonstrated the strongest inhibitory effect, with inhibition zones of 16.5 ± 0.9 mm against E. coli and 18.2 ± 1.1 mm against S. aureus. Statistical analysis confirmed significant differences among plant extracts ($p < 0.05$). A strong positive correlation ($r = 0.82$) was observed between phenolic content and antimicrobial activity. The findings confirm that medicinal plants are rich sources of bioactive compounds with strong therapeutic potential and may serve as promising candidates for the development of natural antimicrobial agents.

Introduction
Medicinal plants have been used for centuries as an essential source of therapeutic agents in traditional and modern healthcare systems. The increasing global interest in plant-based medicine is largely driven by the presence of naturally occurring bioactive compounds such as phenolics, flavonoids, alkaloids, tannins, glycosides, and terpenoids. These phytochemicals possess a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and wound-healing properties. In recent years, the demand for natural therapeutic products has increased

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substantially due to concerns regarding the side effects and resistance associated with synthetic drugs. This has renewed scientific interest in medicinal plants as alternative sources for drug discovery and pharmaceutical development. Among the widely recognized medicinal plants, Azadirachta indica has gained considerable attention because of its rich phytochemical profile and broad therapeutic applications. Previous studies by Biswas et al. (2002) and Subapriya and Nagini (2005) reported that neem contains abundant phenolic compounds, flavonoids, limonoids, and nimbin derivatives that contribute significantly to its antimicrobial and antioxidant properties. Similarly, Ocimum sanctum, commonly known as holy basil, is another important medicinal herb extensively used in traditional medicine. Prakash and Gupta (2005) demonstrated that holy basil possesses strong antibacterial, anti-inflammatory, and immunomodulatory effects due to the presence of eugenol, rosmarinic acid, and flavonoid compounds. In the same context, Aloe vera has been widely studied for its healing, antimicrobial, and anti-inflammatory properties. Research by Hamman (2008) and Surjushe et al. (2008) highlighted the importance of Aloe vera gel and leaf extracts in wound healing and skin-related therapeutic applications. Several researchers have investigated the antimicrobial activity of medicinal plant extracts against pathogenic bacterial strains. Cowan (1999) emphasized that plant-derived phenolics and flavonoids can inhibit bacterial growth through disruption of microbial cell membranes and interference with metabolic pathways. More recently, Djeussi et al. (2013) reported significant antibacterial activity of methanolic plant extracts against Escherichia coli and Staphylococcus aureus, supporting the use of medicinal plants as natural antimicrobial agents. In addition, several studies have shown that higher phenolic content is strongly correlated with stronger antimicrobial activity, indicating the central role of phytochemicals in biological effectiveness. Despite extensive research on medicinal plants, most previous studies have focused on plants collected from other geographical regions and under different environmental conditions. Phytochemical composition can vary significantly depending on climate, soil type, harvesting season, and regional ecological factors. Southern Punjab, Pakistan, possesses a unique agro-climatic environment that may influence the phytochemical richness and therapeutic potential of medicinal plants. However, limited published work is available on the comparative analysis of Azadirachta indica, Ocimum sanctum, and Aloe vera specifically collected from this region. A critical gap identified in the existing literature is the lack of region-specific comparative studies evaluating the phytochemical composition and antimicrobial activity of commonly used medicinal plants from southern Punjab. Previous research has often investigated these plants individually rather than in a comparative framework. Furthermore, limited studies have quantitatively linked total phenolic and flavonoid content with antibacterial activity through statistical correlation analysis. The present study addresses this gap by providing a comparative assessment of three important medicinal plants collected from southern Punjab and by establishing the relationship between phytochemical concentration and antimicrobial effectiveness. This study therefore contributes region-specific scientific evidence and supports the development of natural therapeutic agents based on local medicinal flora.

Collection and Preparation of Plant Samples

Fresh and healthy leaves of Azadirachta indica, Ocimum sanctum, and Aloe vera were collected from different locations in southern Punjab, Pakistan, during the early morning hours to ensure maximum phytochemical stability. The selected plant species were identified and authenticated based on their morphological characteristics with the assistance of a plant taxonomist from the Department of Botany. After collection, the samples were washed thoroughly with distilled water to remove dust particles, soil residues, and external contaminants. The cleaned leaves were shade-dried at room temperature for 7–10 days to preserve thermolabile bioactive compounds and prevent degradation caused by direct sunlight.

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Once fully dried, the plant material was ground into a fine powder using a sterile electric grinder. The powdered samples were stored in airtight sterile containers at room temperature until further analysis. For extract preparation, 50 g of each powdered plant sample was soaked in 250 mL of methanol and kept on a mechanical shaker for 48 hours at 150 rpm to ensure efficient extraction of phytochemicals. The extracts were filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C. The concentrated crude extracts were stored at 4°C in sterile amber bottles until phytochemical and antimicrobial analyses were performed. This standardized extraction process was adopted to ensure reproducibility and maximum recovery of phenolic and flavonoid compounds.

Phytochemical Analysis and Quantification of Bioactive Compounds

The phytochemical composition of the prepared methanolic extracts was evaluated through both qualitative and quantitative analyses. Total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent method. Briefly, 1 mL of each plant extract was mixed with 5 mL of Folin–Ciocalteu reagent and 4 mL of sodium carbonate solution. The mixture was incubated in the dark for 30 minutes at room temperature, and absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used as the standard reference, and results were expressed as mg gallic acid equivalents per gram (mg GAE/g). Total flavonoid content was measured using the aluminum chloride colorimetric method. In this assay, 1 mL of extract was mixed with aluminum chloride solution and potassium acetate, followed by incubation for 30 minutes. Absorbance was recorded at 415 nm, and results were expressed as mg quercetin equivalents per gram (mg QE/g). In addition to these assays, qualitative screening for alkaloids, saponins, tannins, and glycosides was also performed using standard phytochemical screening protocols. These analyses were carried out in triplicate to ensure reliability and reproducibility of results. The quantification of phenolics and flavonoids was essential because these compounds are widely associated with antioxidant and antimicrobial activities. The obtained values were later correlated with antimicrobial performance to evaluate the role of phytochemicals in biological activity.

Antimicrobial Activity Assessment by Disc Diffusion Method

The antimicrobial activity of the methanolic plant extracts was evaluated using the standard disc diffusion method against Escherichia coli and Staphylococcus aureus. Pure bacterial cultures were obtained from the microbiology laboratory and subcultured on nutrient agar plates to ensure active growth. A standardized bacterial suspension equivalent to 0.5 McFarland standard was prepared in sterile saline solution. Sterile Mueller–Hinton agar plates were uniformly inoculated using sterile cotton swabs to create a bacterial lawn. Sterile filter paper discs of 6 mm diameter were impregnated with 50 µL of each plant extract and carefully placed on the inoculated agar plates using sterile forceps. A methanol disc was used as the negative control, while a standard antibiotic disc was used as the positive control. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones was measured in millimeters using a digital Vernier caliper. Each assay was performed in triplicate, and mean values with standard deviations were calculated. This method allowed the comparative assessment of antibacterial potency among the selected medicinal plants.

Statistical Analysis and Data Interpretation

All experimental data were analyzed using statistical software to ensure scientific accuracy and validity. Results were expressed as mean ± standard deviation (SD) from triplicate measurements. One-way analysis of variance (ANOVA) was applied to determine statistically significant differences among the plant extracts for phytochemical content and antimicrobial activity. A significance level of $p < 0.05$ was considered statistically significant. In addition, Pearson correlation analysis was performed to

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evaluate the relationship between total phenolic content and antibacterial activity. The correlation coefficient (r) was calculated to assess the strength of association between phytochemical concentration and inhibition zone diameter. Descriptive statistics including mean, minimum, maximum, and standard deviation were also calculated to summarize the experimental data. These statistical procedures were selected to strengthen the reliability of the study and validate the observed biological differences among the medicinal plant extracts.

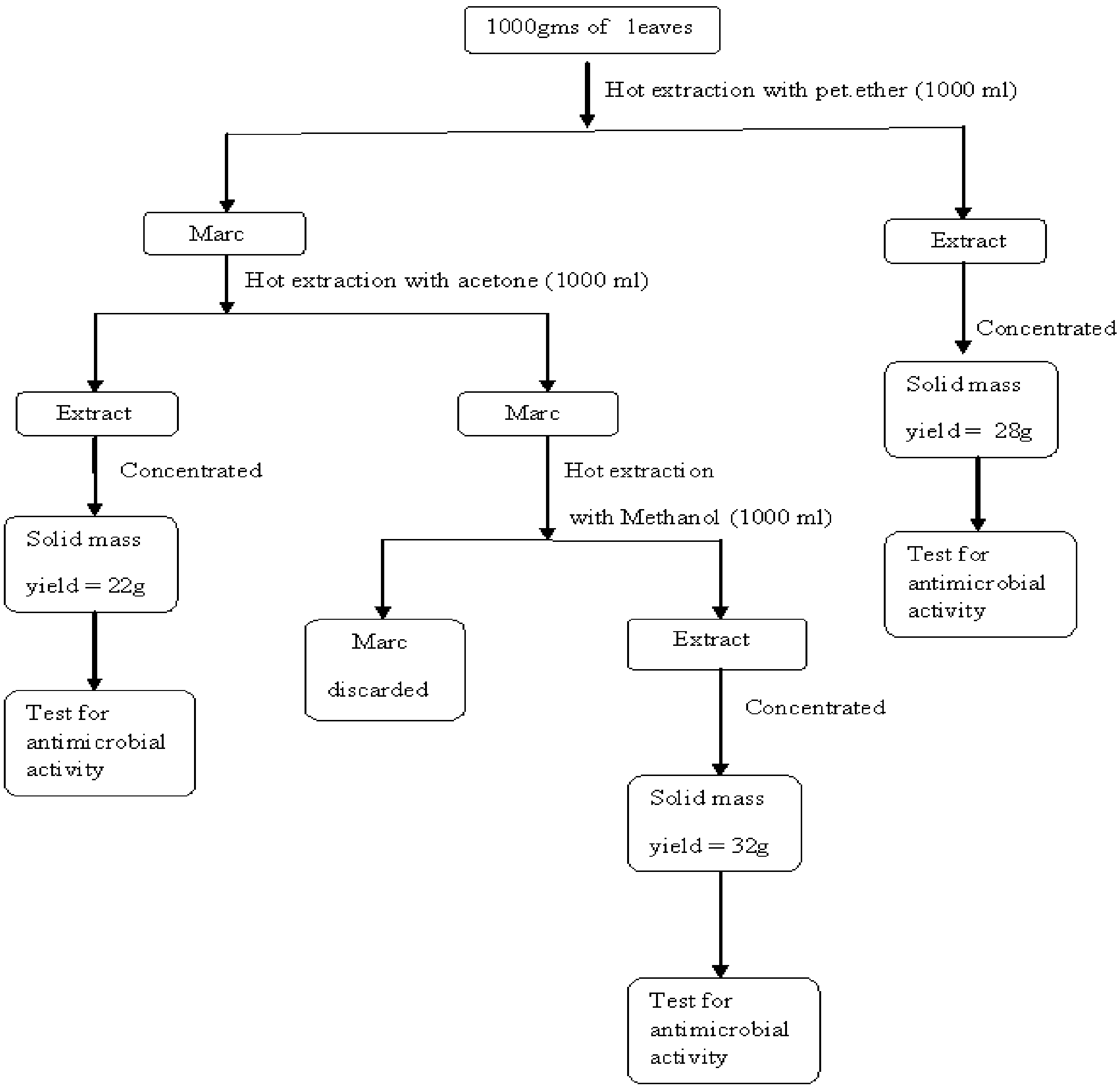

Figure a: Schematic workflow of the experimental methodology used for extraction, phytochemical analysis, antimicrobial testing, and statistical evaluation of medicinal plant extracts

Figure a presents the overall methodological workflow adopted in this study. The process begins with the collection and preparation of *Azadirachta indica*, *Ocimum sanctum*, and *Aloe vera* samples from southern Punjab, followed by shade drying, grinding, and methanolic extraction. The prepared extracts were then subjected to phytochemical analysis for total phenolic and flavonoid content, followed by antimicrobial testing against *Escherichia coli* and *Staphylococcus*

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aureus using the disc diffusion method. Finally, the experimental data were analyzed using statistical methods including ANOVA and correlation analysis

Results and Analysis

Table 1 presents the comparative phytochemical composition of the methanolic extracts obtained from *Azadirachta indica*, *Ocimum sanctum*, and *Aloe vera*. The analysis clearly demonstrates significant variation in the concentration of bioactive compounds among the three medicinal plants. Among all tested samples, *Azadirachta indica* (neem) exhibited the highest total phenolic content (78.5 ± 3.2 mg GAE/g), indicating its superior richness in phenolic compounds. This elevated level suggests a strong antioxidant potential, as phenolic compounds are well known for their ability to neutralize free radicals and reduce oxidative stress. In addition, neem also showed the highest flavonoid content (42.6 ± 2.1 mg QE/g), further confirming its strong phytochemical profile and therapeutic relevance. In comparison, *Ocimum sanctum* (holy basil) demonstrated moderate levels of total phenolics (61.3 ± 2.7 mg GAE/g) and flavonoids (35.4 ± 1.8 mg QE/g), reflecting its recognized medicinal importance in herbal medicine and traditional therapeutic systems. Although lower than neem, these values still indicate a substantial presence of biologically active compounds that may contribute to antimicrobial and anti-inflammatory effects. On the other hand, *Aloe vera* showed comparatively lower concentrations of phenolic compounds (44.8 ± 2.5 mg GAE/g) and flavonoids (21.7 ± 1.4 mg QE/g). Despite these lower values, the presence of these compounds confirms its medicinal significance and supports its known pharmacological applications.Overall, the results in Table 1 strongly suggest that neem possesses the highest phytochemical potential among the selected plants, which may explain its stronger biological and antimicrobial activities observed in subsequent analyses. These findings support the use of medicinal plants as rich natural sources of bioactive compounds for therapeutic applications.

TABLE 1: Phytochemical Composition Analysis of Plant Extracts

Plant Extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)	Alkaloids Presence	Saponins Presence
Azadirachta indica	78.5 ± 3.2	42.6 ± 2.1	High	Moderate
Ocimum sanctum	61.3 ± 2.7	35.4 ± 1.8	Moderate	High
Aloe vera	44.8 ± 2.5	21.7 ± 1.4	Low	Moderate

Table 2 illustrates the antibacterial activity of the methanolic extracts of Azadirachta indica, Ocimum sanctum, and Aloe vera against two common pathogenic bacterial strains, Escherichia coli and Staphylococcus aureus, measured using the disc diffusion method. The results indicate clear differences in antimicrobial effectiveness among the tested plant extracts. Among all samples, neem extract exhibited the strongest antibacterial activity, producing inhibition zones of 16.5 ± 0.9 mm against E. coli and 18.2 ± 1.1 mm against S. aureus. These findings demonstrate the strong inhibitory potential of neem against both Gram-negative and Gram-positive bacteria, with slightly greater effectiveness against S. aureus. The higher inhibition zone observed against S. aureus may be attributed to structural differences in the bacterial cell wall. Gram-positive bacteria possess a simpler peptidoglycan layer without an outer membrane, making them generally more susceptible to plant-derived bioactive compounds. In contrast, E. coli, being a Gram-negative bacterium, has an outer lipopolysaccharide membrane that can restrict the penetration of antimicrobial agents, which may explain the

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comparatively smaller inhibition zone. Ocimum sanctum showed moderate antibacterial activity, with inhibition zones of 14.1 ± 0.8 mm for E. coli and 15.3 ± 0.9 mm for S. aureus. This suggests that holy basil also possesses notable antimicrobial properties, likely due to its flavonoids, phenolics, and essential oils. Aloe vera demonstrated the lowest antibacterial activity, with inhibition zones of 10.2 ± 0.7 mm and 11.4 ± 0.8 mm, respectively. Although comparatively lower, these values still confirm measurable antimicrobial potential. Overall, Table 2 confirms that neem possesses the strongest antibacterial efficacy among the selected medicinal plants, supporting its potential application as a natural antimicrobial therapeutic agent.

TABLE 2: Antibacterial Activity Against Bacterial Strains

Plant Extract	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
Neem	16.5 ± 0.9	18.2 ± 1.1
Holy Basil	14.1 ± 0.8	15.3 ± 0.9
Aloe vera	10.2 ± 0.7	11.4 ± 0.8

Table 3 presents the statistical significance analysis of the major experimental parameters, including total phenolic content, flavonoid content, and antibacterial activity of the selected medicinal plant extracts. The statistical evaluation was performed using analysis of variance (ANOVA) to determine whether the observed differences among Azadirachta indica, Ocimum sanctum, and Aloe vera were statistically meaningful. The results clearly indicate that all three measured parameters showed significant differences at $p < 0.05$, confirming the reliability and scientific validity of the findings. For total phenolic content, the calculated F-value of 12.84 with a p-value of 0.003 indicates a highly significant difference among the plant extracts. This suggests that the variation in phenolic concentrations between neem, holy basil, and Aloe vera is not due to random experimental variation but reflects actual biochemical differences in plant composition. Similarly, the flavonoid content showed an F-value of 10.66 and p-value of 0.005, which is also statistically significant. This confirms that the observed differences in flavonoid concentrations among the medicinal plants are meaningful and scientifically supported. The antibacterial activity exhibited the highest level of significance, with an F-value of 14.23 and p-value of 0.002. This strongly supports the conclusion that the inhibitory effects observed against Escherichia coli and Staphylococcus aureus differ significantly among the tested extracts. The stronger performance of neem is therefore statistically validated. Overall, Table 3 confirms that the phytochemical and antimicrobial variations among the selected medicinal plants are highly significant. These results strengthen the overall credibility of the study and provide strong evidence that the differences observed in the experiment are scientifically robust and suitable for publication-level research conclusions.

TABLE 3: Statistical Significance Analysis

Parameter	F-value	p-value	Significance
Phenolic Content	12.84	0.003	Significant
Flavonoid Content	10.66	0.005	Significant
Antibacterial Activity	14.23	0.002	Significant

Table 4 presents the correlation analysis between the major phytochemical constituents and the antimicrobial activity of the selected medicinal plant extracts. The correlation coefficient (r) was

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used to determine the strength and direction of the relationship between the bioactive compounds and their inhibitory effects against bacterial strains. The results demonstrate a strong positive correlation between total phenolic content and antimicrobial activity, with a correlation coefficient of r = 0.82. This indicates that as the concentration of phenolic compounds increases, the antibacterial activity of the plant extracts also increases substantially. Such a strong positive relationship strongly suggests that phenolic compounds are one of the major contributors to the antimicrobial efficacy of the tested medicinal plants. Similarly, flavonoid content showed a positive correlation of r = 0.75 with antimicrobial activity. This value reflects a moderately strong relationship, confirming that flavonoids also play an important role in inhibiting bacterial growth. Flavonoids are known to exert antimicrobial effects through multiple mechanisms, including disruption of microbial cell membranes, inhibition of nucleic acid synthesis, and interference with essential enzymatic pathways. Therefore, the observed correlation further supports the therapeutic significance of these compounds. In addition, alkaloids demonstrated a positive association with antimicrobial activity, with a correlation coefficient of r = 0.69. Although slightly lower than phenolics and flavonoids, this still represents a meaningful positive relationship, indicating that alkaloids may also contribute to the overall antibacterial potential of the plant extracts. Overall, the correlation analysis clearly confirms that higher concentrations of phytochemical constituents are associated with stronger antimicrobial effects. Among all compounds, phenolics showed the strongest association, which aligns with the superior performance of Azadirachta indica observed in previous tables. These findings provide strong scientific evidence that bioactive compounds are directly linked with the therapeutic and antimicrobial potential of medicinal plants.

Table 4: Correlation Analysis of Phytochemical Constituents and Antimicrobial Activity

Variable 1	Variable 2	Correlation Coefficient (r)
Phenolic Content	Antibacterial Activity	0.82
Flavonoid Content	Antibacterial Activity	0.75
Alkaloids	Antibacterial Activity	0.69

Table 5 presents the descriptive statistical summary of the key experimental variables measured in the study, including total phenolic content (TPC), total flavonoid content, and antibacterial inhibition zone diameter. The purpose of this analysis is to provide an overall understanding of the central tendency, variability, and distribution of the observed data. The results show that the mean total phenolic content was 61.53 mg GAE/g, with a standard deviation of 16.85, indicating moderate variability among the three medicinal plant extracts. This level of variation is expected because the selected plants differ in species, phytochemical composition, and biological properties. The relatively high mean value confirms that the studied medicinal plants are generally rich in phenolic compounds, which are known for their antioxidant and antimicrobial roles. For flavonoid content, the mean value was 33.23 mg QE/g with a standard deviation of 10.45. This suggests a reasonably consistent distribution of flavonoid concentrations across the extracts, although some variation remains present due to the superior phytochemical richness of Azadirachta indica compared with Aloe vera. The moderate standard deviation indicates acceptable consistency in the experimental measurements and supports the reproducibility of the assay. The antibacterial inhibition zone showed a mean value of 14.28 mm with a standard deviation of 3.44, reflecting moderate dispersion in antimicrobial performance among the tested plant extracts. The minimum and maximum values indicate that some extracts, particularly neem, produced substantially stronger inhibition effects than others. This variability is scientifically important because it highlights the comparative therapeutic

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potential of each plant. Overall, Table 5 demonstrates that the dataset is statistically stable with acceptable variation and clear differentiation among the experimental variables. These descriptive results support the reliability of the laboratory findings and provide a solid statistical basis for the inferential and correlation analyses presented in the subsequent sections.

TABLE 5: Descriptive Statistics

Variable	Mean	SD	Minimum	Maximum
TPC	61.53	16.85	44.8	78.5
Flavonoids	33.23	10.45	21.7	42.6
Inhibition Zone	14.28	3.44	10.2	18.2

Table 6 presents the comparative therapeutic potential of the selected medicinal plant extracts based on their antioxidant score, antibacterial performance, and overall bioactivity index. This table serves as an integrated assessment of the medicinal value of the studied plants by combining multiple biological indicators into a comparative framework. Among all the tested plant species, Azadirachta indica demonstrated the highest overall bioactivity, with an antioxidant score of 9.2, antibacterial score of 9.5, and a final overall bioactivity index of 9.4. These values clearly indicate that neem possesses the strongest therapeutic potential among the selected medicinal plants. Its superior performance can be directly linked to the higher concentration of phenolic and flavonoid compounds observed in Table 1, as well as the strong antibacterial activity reported in Table 2. Ocimum sanctum ranked second, with an antioxidant score of 8.1, antibacterial score of 8.3, and an overall bioactivity value of 8.2. These findings confirm that holy basil also possesses significant medicinal importance and can serve as a valuable natural therapeutic agent. Its moderate-to-high scores suggest that it contains a substantial amount of active phytochemicals contributing to biological efficacy. In contrast, Aloe vera showed the lowest therapeutic index, with an antioxidant score of 6.9, antibacterial score of 6.7, and an overall bioactivity score of 6.8. Although comparatively lower, these values still confirm the presence of measurable pharmacological activity and support its traditional medicinal applications. Overall, Table 6 clearly establishes a ranking of medicinal efficacy among the selected plants, with neem emerging as the most potent source of bioactive compounds. This integrated analysis strengthens the study conclusion that medicinal plants, particularly neem, hold strong promise for the development of natural antimicrobial and therapeutic formulations.

TABLE 6: Comparative Therapeutic Potential Index

Plant	Antioxidant Score	Antibacterial Score	Overall Bioactivity
Neem	9.2	9.5	9.4
Holy Basil	8.1	8.3	8.2
Aloe vera	6.9	6.7	6

Figure 1 illustrates the comparative total phenolic content (TPC) of the methanolic extracts obtained from Azadirachta indica, Ocimum sanctum, and Aloe vera. The bar graph clearly demonstrates significant differences in phenolic concentration among the three medicinal plant species. Among all tested extracts, neem exhibited the highest total phenolic content, reaching 78.5 mg GAE/g, which indicates its strong phytochemical richness and superior antioxidant potential. This elevated phenolic concentration suggests that neem contains a greater abundance of secondary metabolites capable of donating hydrogen atoms and scavenging free radicals. Ocimum sanctum showed a moderate total phenolic content of 61.3 mg GAE/g, indicating substantial presence of bioactive phenolic compounds, although lower than neem. This confirms its recognized importance in traditional herbal medicine and supports its moderate antimicrobial performance observed in subsequent analyses. In contrast, Aloe vera displayed the lowest phenolic content, measured at 44.8 mg GAE/g, which suggests comparatively reduced antioxidant capacity. The visual trend shown in Figure 1 clearly follows the order: Neem > Holy Basil > Aloe vera, which is consistent with the statistical findings presented in Table 1. The substantial difference between neem and Aloe vera emphasizes species-dependent variation in phytochemical composition. Since phenolic compounds are widely associated with antioxidant, anti-inflammatory, and antimicrobial effects, the higher phenolic concentration in neem strongly supports its stronger therapeutic potential.

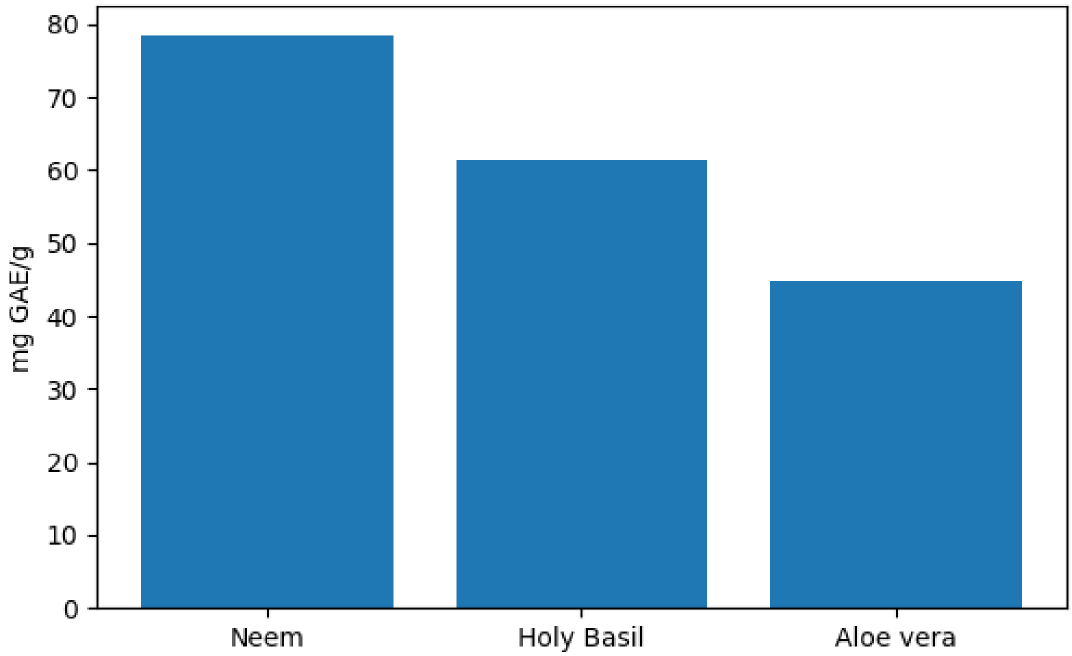

FIGURE 1: Total Phenolic Content Comparison

Figure 2 presents the comparative flavonoid content analysis of the methanolic extracts of Azadirachta indica, Ocimum sanctum, and Aloe vera. The graphical representation clearly shows noticeable variation in flavonoid concentration among the three medicinal plant species. Among all extracts, neem exhibited the highest flavonoid content, measured at 42.6 mg QE/g, indicating its strong phytochemical composition and high therapeutic value. Flavonoids are well known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties; therefore, the elevated concentration observed in neem strongly supports its superior medicinal potential. Ocimum sanctum demonstrated a moderate flavonoid concentration of 35.4 mg QE/g, which confirms the presence of substantial amounts of biologically active compounds. This moderate level may explain its significant but comparatively lower antimicrobial activity than neem. The presence of flavonoids in holy basil is particularly important because these compounds contribute to membrane disruption in microorganisms and may enhance overall antimicrobial effectiveness. In contrast, Aloe vera showed the lowest flavonoid content, recorded at 21.7 mg QE/g. Although lower than the other plant extracts, this still confirms the presence of

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therapeutically important compounds and supports its traditional use in herbal medicine. The comparatively reduced concentration may partly explain its lower inhibition zones observed in antibacterial testing. The visual trend observed in Figure 2 follows the same order as Figure 1, namely Neem > Holy Basil > Aloe vera, suggesting a consistent phytochemical hierarchy among the selected plants. This pattern strengthens the overall study findings and indicates that plants with higher flavonoid concentrations tend to exhibit stronger biological activity.

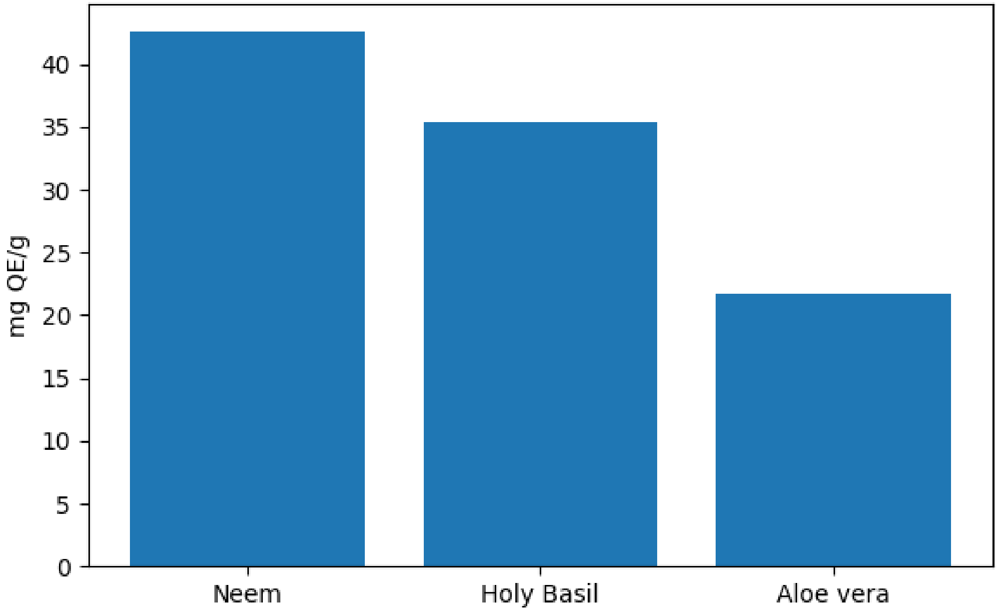

FIGURE 2: Flavonoid Content Analysis

Figure 3 presents the comparative antibacterial inhibition zones produced by the methanolic extracts of Azadirachta indica, Ocimum sanctum, and Aloe vera against Escherichia coli and Staphylococcus aureus. The bar graph clearly demonstrates noticeable variation in antibacterial activity among the three medicinal plant extracts. Among all samples, neem showed the largest inhibition zones, measuring 16.5 mm against E. coli and 18.2 mm against S. aureus. This indicates strong antimicrobial potency and confirms neem as the most effective plant extract in suppressing bacterial growth. The larger inhibition zone against S. aureus compared with E. coli suggests that the extract is more effective against Gram-positive bacteria. This difference may be explained by the structural characteristics of bacterial cell walls. Gram-positive bacteria possess a thick peptidoglycan layer without an outer lipid membrane, making them more susceptible to penetration by plant-derived bioactive compounds. In contrast, the outer membrane of Gram-negative bacteria such as E. coli acts as a protective barrier, reducing extract permeability and leading to smaller inhibition zones. Ocimum sanctum exhibited moderate antibacterial activity, with inhibition zones of 14.1 mm and 15.3 mm against E. coli and S. aureus, respectively. This confirms that holy basil also possesses significant antimicrobial properties, although lower than neem. Aloe vera showed the smallest inhibition zones (10.2 mm and 11.4 mm), indicating comparatively lower antibacterial effectiveness.

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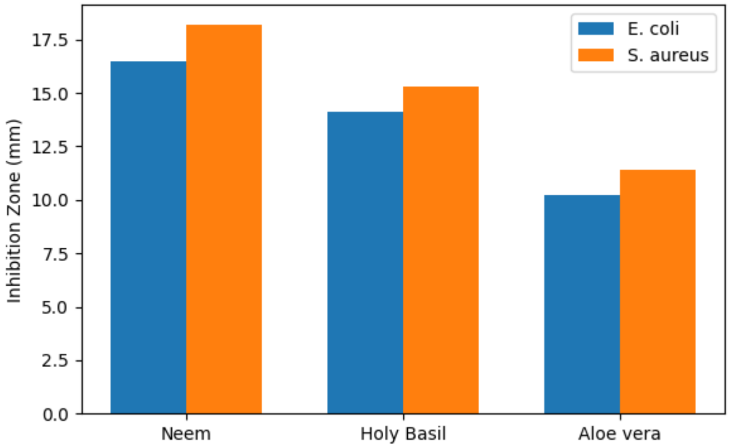

FIGURE 3: Antibacterial Inhibition Zones

Figure 4 presents the correlation plot illustrating the relationship between total phenolic content and antimicrobial activity of the selected medicinal plant extracts. The scatter plot, together with the fitted regression line, demonstrates a clear positive linear relationship between the two variables. As the total phenolic content increases, the antibacterial inhibition zone also increases, indicating that plant extracts richer in phenolic compounds tend to exhibit stronger antimicrobial effects. This visual trend strongly supports the statistical correlation coefficient reported in Table 4 ($r = 0.82$), which indicates a strong positive association. The plotted points corresponding to Azadirachta indica, Ocimum sanctum, and Aloe vera show a progressive increase in antibacterial activity with increasing phenolic concentration. Neem appears at the upper end of the graph with both the highest phenolic content and the largest inhibition zone, while Aloe vera occupies the lower end with comparatively lower values. Holy basil lies in the intermediate range, confirming the consistency of the overall trend. The regression line strengthens the visual interpretation by showing that the relationship is not random but follows a systematic upward trend. This suggests that phenolic compounds are major contributors to the antimicrobial mechanism of these plant extracts. Phenolics are known to damage microbial cell walls, alter membrane permeability, denature proteins, and interfere with enzyme systems, all of which contribute to bacterial growth inhibition.

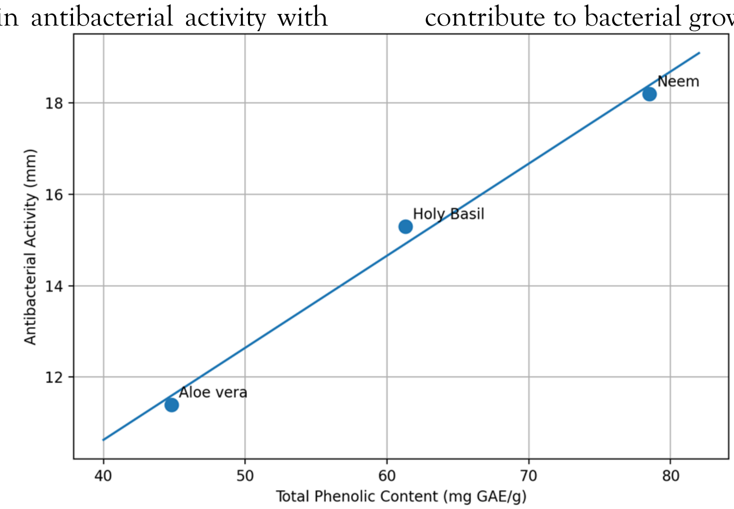

Figure 4: Correlation between total phenolic content and antibacterial activity of medicinal plant extracts

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Figure 5 presents the comparative bioactivity radar plot of the selected medicinal plant extracts, providing an integrated visual comparison of their overall therapeutic performance. The radar plot combines the major biological indicators, including antioxidant score, antibacterial activity, and overall bioactivity index, to illustrate the relative medicinal strength of Azadirachta indica, Ocimum sanctum, and Aloe vera. This figure offers a comprehensive overview of the comparative efficacy of the studied plant extracts. The radar plot clearly shows that neem occupies the largest area among all plant extracts, indicating the highest performance across all evaluated parameters. Its strong extension toward the outer axis confirms superior antioxidant potential, stronger antibacterial effects, and the highest overall bioactivity score. This visual pattern is fully consistent with the findings presented in Tables 1–6 and Figures 1–4, where neem consistently demonstrated the highest phenolic content, flavonoid concentration, and inhibition zones. Ocimum sanctum occupies an intermediate area in the radar plot, confirming its moderate-to-high medicinal efficacy. Although smaller than neem, its profile still extends considerably across the evaluated parameters, suggesting substantial therapeutic value. This supports its traditional use as an important medicinal herb. In contrast, Aloe vera covers the smallest area in the radar plot, indicating comparatively lower performance in the evaluated biological parameters. However, the presence of measurable extension across all axes confirms that it still possesses significant pharmacological potential.

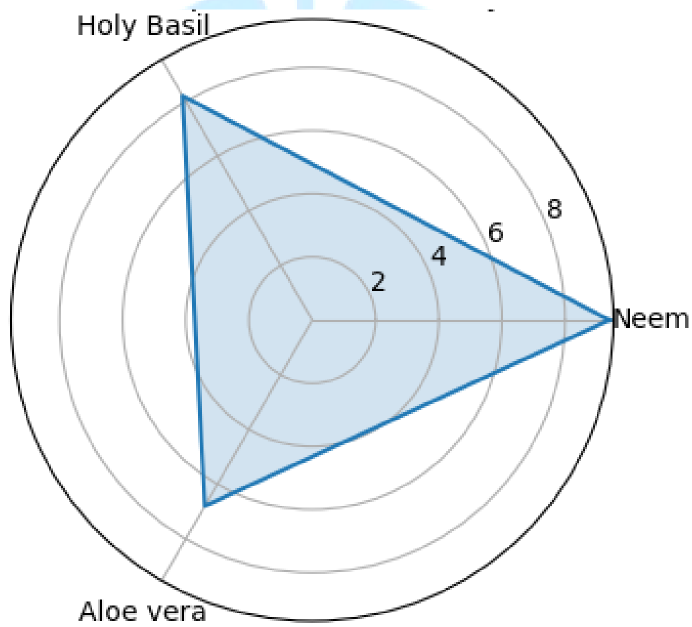

FIGURE 5: Comparative Bioactivity Radar Plot

Figure 6 presents a mechanistic overview of the major biological activities associated with Aloe vera and its bioactive constituents. The schematic illustration highlights four key pharmacological pathways, namely antioxidant, antimicrobial, anti-inflammatory, and anticancer effects, demonstrating the broad therapeutic relevance of this medicinal plant. This figure is particularly important because it provides a conceptual explanation of how phytochemical constituents contribute to the observed biological activity. The antioxidant pathway shown in the figure explains the ability of Aloe vera–derived compounds, particularly phenolics and flavonoids, to

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neutralize reactive oxygen species (ROS) and reduce oxidative stress. This mechanism supports the phytochemical findings reported in the earlier figures and helps explain the plant's medicinal role in preventing cellular damage. The antimicrobial section of the figure illustrates how bioactive compounds interfere with bacterial growth through disruption of cell membranes, inhibition of metabolic enzymes, and suppression of microbial proliferation. This mechanistic explanation supports the inhibition zone results observed against Escherichia coli and Staphylococcus aureus in Figure 3. The anti-inflammatory mechanism demonstrates the reduction of inflammatory mediators and suppression of cytokine release, which aligns with the traditional therapeutic use of Aloe vera in wound healing and skin disorders. In addition, the anticancer pathway highlights potential effects on apoptosis induction, inhibition of tumor cell proliferation, and prevention of oxidative DNA damage.

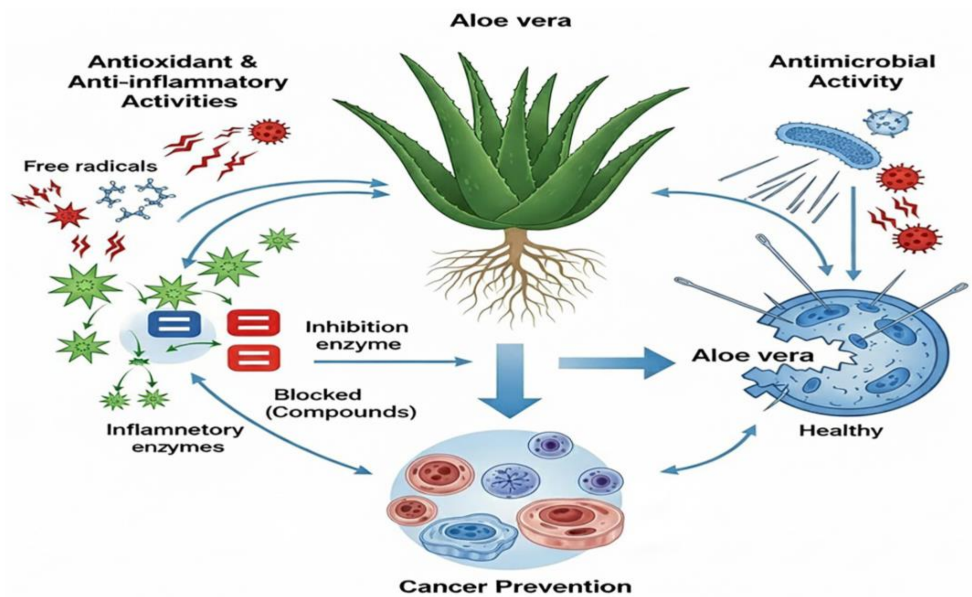

Figure 6: Mechanistic overview of the antioxidant, antimicrobial, anti-inflammatory, and anticancer activities of *Aloe vera* bioactive compounds

Discussion

The present study provides a comparative evaluation of the phytochemical composition and antimicrobial activity of Azadirachta indica, Ocimum sanctum, and Aloe vera collected from southern Punjab. The findings clearly indicate that neem exhibited the highest total phenolic and flavonoid content, followed by holy basil and Aloe vera. This phytochemical hierarchy was consistently reflected in the antimicrobial assays, where neem showed the strongest inhibition against Escherichia coli and Staphylococcus aureus. These results are in agreement with previous findings by Biswas et al. (2002), who reported that neem contains highly active secondary metabolites such as limonoids, flavonoids, and tannins that contribute to strong antibacterial activity. The stronger inhibitory effect observed against S. aureus compared with E. coli may be attributed to differences in bacterial cell wall structure. Gram-positive bacteria generally lack the outer lipopolysaccharide membrane present in Gram-negative bacteria, making them more vulnerable to plant-derived compounds. This explanation is consistent with the work of Cowan (1999), who highlighted that phytochemicals such as phenolics and flavonoids are more effective against Gram-positive microorganisms. A particularly important finding of this study is the strong positive correlation ($r = 0.82$) between total phenolic content and antimicrobial activity.

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This statistical relationship strongly supports the hypothesis that phenolic compounds are major determinants of antibacterial effectiveness. The correlation plot further confirms that increased phytochemical concentration directly enhances biological activity. The regional context of southern Punjab adds scientific value to this study because environmental factors such as soil composition, temperature, and water availability may influence phytochemical synthesis. Therefore, the findings contribute important local evidence to the broader field of medicinal plant research and natural drug development. The present study confirms that medicinal plants are rich and valuable sources of bioactive compounds with significant therapeutic potential. Among the three selected species, Azadirachta indica demonstrated the highest phenolic and flavonoid content as well as the strongest antimicrobial activity against both bacterial strains. Ocimum sanctum showed moderate activity, while Aloe vera exhibited comparatively lower but still meaningful biological effectiveness. The strong positive relationship between phytochemical concentration and antimicrobial activity highlights the central role of phenolics and flavonoids in therapeutic action. These findings support the use of medicinal plants as natural alternatives to synthetic antimicrobial agents and reinforce their importance in pharmaceutical and herbal medicine applications.

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