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PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS WITH ANTIBACTERIAL PROPERTIES

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Abstract

The worldwide increase of antibiotic-resistant bacterial strains requires immediate development of alternative antimicrobial medicines. Medical plants possess therapeutic properties because of their bioactive phytochemicals which demonstrate antimicrobial action. Secondary metabolites produced by plants including alkaloids and flavonoids and tannins and saponins and terpenoids demonstrate inhibitory properties against different harmful microorganisms. The analysis of antibacterial medicinal plants in this study evaluates their potential to combat Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli bacterial infections. The conventional qualitative and quantitative techniques were used for phytochemical screening to detect important secondary metabolites. Scientists utilized the disc diffusion method to measure inhibition zone widths for evaluating antibacterial activities of these plant extracts. They conducted the minimum inhibitory concentration (MIC) assay to find the lowest effective concentration which halted bacterial growth. Plants containing elevated levels of alkaloids together with phenolic chemicals and flavonoids demonstrated the strongest antibacterial properties. Two of the selected plants demonstrated significant inhibitory properties against S. aureus and E. coli through their active compounds in Ocimum sanctum (holy basil) and Curcuma longa (turmeric).

These findings demonstrate the potential of medicinal plants strong in phytochemicals as a viable natural antibacterial agent source. These bioactive substances work by disrupting bacterial membranes, inhibiting enzymes, and interfering with the creation of proteins. These plant-derived chemicals may be utilized as supplemental or alternative antimicrobial agents to treat bacterial infections, especially those brought on by drug-resistant strains, due to their natural origin and broad-spectrum activity. In order to create plant-based antibacterial medicines, more study on the separation, purification, and structural characterisation of these phytochemicals as well as their therapeutic uses would be essential.

Keywords: *Phytochemicals, Antibacterial activity, Medicinal plants, Bioactive compounds, Drug resistance, Natural antibiotics.*

1. Introduction.

The worldwide health crisis due to multidrug-resistant (MDR) bacteria requires new antibacterial medicines (*Ventola, C. L. (2015*). Research into bioactive chemical therapeutic benefits of medicinal plants grows as numerous civilizations have traditionally used these plants to treat infectious diseases (*Newman, D. J., & Cragg, G. M. (2020*). Phytochemicals including alkaloids and flavonoids and tannins and terpenoids and phenolic compounds display antibacterial properties through their ability to disrupt bacterial cell walls and block enzyme activity and disrupt protein synthesis (*Cowan, M. M. (1999*). Scientific research demonstrates that plant-derived compounds successfully fight against Gram-positive and Gram-negative bacterial strains (*Goyal, S., Samsher, & Ramawat, K. G. (2021*). The identification of specific bioactive substances responsible for antibacterial effects requires complete phytochemical analysis. The research evaluates antibacterial properties of selected medicinal plants through in vitro testing of their phytochemical content.

1.1 Background and Significance of Antibacterial Research.

A. The growing incidence of microorganisms resistant to antibiotics and its effects on world health.
B. The drawbacks of synthetic antibiotics, including as side effects and the emergence of resistance.
C. The requirement for substitute antibacterial substances, especially those derived from natural sources.

World health faces an ongoing serious threat from bacterial infections because of antibiotic-resistant strains that have emerged. The development of multidrug-resistant (MDR) pathogens such as Methicillin-resistant Staphylococcus aureus (MRSA) and Enterobacteriaceae that produce Extended-spectrum β-lactamase (ESBL) occurs faster because of excessive and improper antibiotic usage in medical facilities and agricultural settings (Ventola, 2015). Alternative treatment options must be pursued because antimicrobial resistance (AMR) stands as a top public health threat according to the World Health Organization.(*World Health Organization (WHO). (2021*).

Synthetic antibiotics might produce severe side effects which include allergic reactions and gastrointestinal complications and gut microbiota dysbiosis.(*Huttner, A., Harbarth, S., Hope, W. W., Lipman, J., & Roberts, J. A. (2020*). The development of new antibiotics has slowed substantially during the last few decades while the medical field requires antibacterial agents that produce minimal side effects and resistances. Reported that medicinal plants have become significant because they might serve as alternative sources of bioactive substances which potentially show antibacterial properties.

1.2 Role of Medicinal Plants in Antibacterial Therapy.

For numerous generations traditional medicine included the use of medicinal plants as treatments for infectious diseases throughout different cultures. Plants contain bioactive phytochemicals that defend organisms against microbial diseases. Scientists have thoroughly studied the antiviral and antifungal and antibacterial properties of these substances. The complex plant-derived structures serve as obstacles to microbial resistance development thus resulting in low reported bacterial resistance rates.

A wide range of medicinal herbs demonstrates powerful antibacterial properties. The antibacterial qualities of Azadirachta indica (neem) are attributed to its limonoids and flavonoids compounds.(*Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002*).The abundant curcumin compound in Curcuma longa (turmeric) demonstrates its ability to prevent bacterial growth through its damaging effect on bacterial membranes.(*Moghadamtousi, S. Z., Kadir, H. A., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014*). Scientific evidence about antibacterial properties of therapeutic plants requires detailed phytochemical research and in vitro testing for their valid confirmation.

In ethnopharmacology, medicinal plants have been used historically and traditionally. Bioactive secondary metabolites with antibacterial properties can be found in medicinal plants. Examples of typical medicinal plants with antibacterial properties and their uses.

1.3 Phytochemicals and Their Antibacterial Mechanisms.

The overview will explore primary phytochemical groups which consist of phenolics, alkaloids, flavonoids and both tannins and saponins and terpenoids along with their methods of action against bacterial cells. Plants produce antimicrobials that differ from conventional antibiotic medications.

Secondary metabolites known as phytochemicals serve as the source of antimicrobial properties in plants. The antibacterial substances found in plants operate through different mechanisms including.

A. **Alkaloids:** Disrupt the translation of proteins and the production of DNA in bacteria.(*Cushnie, T. P., & Lamb, A. J. (2011*).
B. **Flavonoids:** Break down the membranes of bacteria to prevent the production of biofilms.(*Daglia, M. (2012*).
C. **Tannins:** Cause cellular damage by binding to bacterial proteins and enzymes.(*Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009*).
D. **Saponins:** Create holes in the membranes of bacteria that allow the cytoplasm to flow out.(*Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019*).
E. **Terpenoid:** Serve as oxidative stress inducers and inhibitors of bacterial enzymes.(*Saleem, M., Nazir, M., Ali, M. S., Hussain, H., Lee, Y. S., Riaz, N., & Jabbar, A. (2019*).

The complex antibacterial mechanisms of phytochemicals reduce the formation of antibiotic resistance compared to synthetic antibiotics. The search for new antimicrobial substances has focused on plant-derived compounds because they show promise as alternative antibiotics in medical research.

1.4 Rationale for the Study.

The scientific community lacks complete evidence regarding therapeutic plant phytochemicals and their mechanisms despite accumulating data about their antibacterial properties. Traditional medicinal plants contain phytochemical components which scientists have only partially identified and fully studied. The studies aim to fill existing knowledge gaps through phytochemical analysis and in vitro antimicrobial assessments of several plants that have proven ethnopharmacological importance.

The analysis of plant-derived antimicrobials opens new therapeutic possibilities because of the worldwide rise in antibiotic resistance. Research shows that phytochemicals may develop into natural antibacterial agents which can be used in pharmaceutical formulations and food preservation and healthcare applications.

1.5 Objectives of the Study.
A selection of medicinal plants will be tested for phytochemical identification through qualitative and quantitative methods to evaluate their antimicrobial properties against P. aeruginosa, S. aureus, and E. coli bacteria to understand phytochemical mechanisms of bacterial inhibition.

2. Materials and Methods.

A detailed description of the methods for phytochemical analysis and antibacterial activity evaluation of selected medicinal plants appears in this section. The laboratory work followed established procedures for guaranteed precision running two times in a controlled environment.

2.1 Selection of Medicinal Plants.
The selection of medicinal plants for this study was based on their documented antimicrobial usage and supporting research regarding their phytochemical composition. The plants under investigation were:
A. **Azadirachta indica** (Neem)
B. **Ocimum sanctum** (Holy Basil)
C. **Curcuma longa** (Turmeric)
D. **Aloe vera**
A skilled botanist verified the botanical origin of fresh leaves and rhizomes which came from trusted sources. The plant specimens received storage in the herbarium of a respected university.

2.2 Preparation of Plant Extracts.
2.2.1 Collection and Drying.
The plant components (leaves and rhizomes) received distilled water treatment for cleaning purposes. The plant materials underwent shade-drying for seven to ten days at room temperature (25 ± 2°C) until they reached complete dehydration.

2.2.2 Grinding and Powdering.
The dried plant materials underwent grinding with an electric grinder until they became a fine powder before sieving. The powdered samples were stored at 4°C under sealed conditions until their next use.

2.2.3 Extraction Procedure.
The cold maceration technique was used to extract the bioactive components utilizing aqueous (distilled water) solvents and 95% ethanol.
A. The solvent ratio stands at 1:10 (w/v) because 1 g of powdered material mixes with 10 mL of solvent.
B. The mixture remained at 120 rpm in a shaker incubator while being kept at room temperature for 48 hours.
C. Whatman No. 1 filter paper served to filter the extracts while a rotary evaporator at 40°C and low pressure concentrated them.
D. The concentrated extracts received storage in sterile amber bottles at 4°C until additional examination occurred.

2.3 Phytochemical Screening.
2.3.1 Qualitative Phytochemical Analysis.
Standard biochemical assays were used to identify the phytochemical elements in the plant extracts.(*Harborne, J. B. (1998*).
A. **Alkaloids** – *Mayer's and Wagner's reagent tests*
B. **Flavonoids** – *Lead acetate and alkaline reagent tests*
C. **Tannins** – *Ferric chloride and gelatin tests*
D. **Saponins** – *Foam test*
E. **Terpenoids** – *Salkowski test*
2.3.2 Quantitative Phytochemical Analysis.
Key phytochemical concentrations were measured using UV-Vis spectrophotometric techniques:
A. **Total Phenolic Content (TPC)** – Measured using **Folin-Ciocalteu's reagent** at **765 nm**.(*Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999*).
B. **Total Flavonoid Content (TFC)** – Determined using **aluminium chloride colorimetric assay** at **510 nm**.(*Chang, C., Yang, M., Wen, H., & Chern, J. (2002).*
C. **Alkaloid Content** – Estimated using the **Bromocresol Green (BCG) method** at **470 nm**.
D. **Tannin Content** – Determined by the **Vanillin-HCl method** at **500 nm**.

2.4 Antibacterial Activity Assay.
Two common microbiological methods were used to evaluate plant extracts' antibacterial potential:

1. **Agar Well Diffusion Method.**
Mueller-Hinton Agar (MHA), in accordance with the conventional Kirby-Bauer disc diffusion technique, was used to evaluate the antibacterial activity of plant extracts.(*Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966*).

A. Bacterial suspension preparation: The bacterial cultures were adjusted to **0.5 McFarland standard (1.5×10^8 CFU/mL)**.
B. Inoculation: A sterile **cotton swab** was used to spread the bacterial suspension evenly onto MHA plates.
C. Well formation: Wells (6 mm in diameter) were cut into the agar using a sterile cork borer.
D. **Extract loading**: 100 µL of each plant extract was pipetted into the wells.
E. **Controls**:

- **Positive control**: Standard antibiotic **ciprofloxacin (5 µg/mL)**.
- **Negative control**: **Sterile distilled water**.

F. Incubation: Plates were incubated at **37°C for 24 hours**.
G. Measurement: The zone of inhibition (ZOI) was measured using a **calibrated digital Vernier caliper**.

2. **Minimum Inhibitory Concentration (MIC) Assay.**
The broth microdilution technique was used to find the minimum inhibitory concentration (MIC) of plant extracts.(*Andrews, J. M. (2001*).
A. Serial dilution: To achieve final concentrations ranging from 1000 to 31.25 µg/mL, plant extracts were serially diluted in Mueller-Hinton broth (MHB).

B. Inoculation: Each well received 100 µL of a standardized bacterial solution.
Incubation: For 18 to 24 hours, plates were incubated at 37°C.

C. Determination: The minimum inhibitory concentration (MIC) was defined as the concentration at which no discernible bacterial growth occurred.

2.5 Statistical Analysis.
The experiments were performed three times in each case and the data displayed the mean values with standard deviation (SD) bars. The analyses included One-way ANOVA in combination with Tukey's post hoc test for statistical significance detection (p value of less than 0.05 defined statistical significance). The visual representations were developed through GraphPad Prism 9.0.

2.6 Ethical Consideration.
This study uses plant materials and microbiological testing methods that fulfill institutional ethical standards. The Institutional Review Board (IRB) approved the research ethics while all laboratory work operated at Biosafety level 2 (BSL-2) standards for protection from contaminants and researcher safety.

3. Result.

3.1 Phytochemical Analysis:
Several secondary metabolites including alkaloids, flavonoids, tannins, saponins and terpenoids existed in the examined medicinal plant extracts as evidenced by quantitative phytochemical screening. Table 1 presents the test results by displaying the relative abundance of each phytochemical found in the studied plants.

3.1.1 Qualitative Phytochemical Analysis.
Research results demonstrate that Azadirachta indica contained high alkaloid and tannin contents while showing significant flavonoid levels in Ocimum sanctum and Curcuma longa. The research revealed that Aloe vera contained the highest amount of saponins which matches its established bioactive properties.

3.1.2 Quantitative Phytochemical Analysis.
The measurement of total flavonoid content (TFC) and total phenolic content (TPC) occurred for each plant extract. The research data showed that Azadirachta indica contained the highest amount of phenolic compounds (TPC: 92.1 ± 3.5 mg GAE/g) while Curcuma longa demonstrated the highest flavonoid content (TFC: 78.5 ± 2.3 mg QE/g). Table 2 provides the specific values.

3.2 Antibacterial Activity.
The evaluation of antibacterial activity in plant extracts involved both minimum inhibitory concentration (MIC) and agar well diffusion experiments.

3.2.1 Zone of Inhibition (ZOI) from Agar Well Diffusion Assay.

The antibacterial potency of the tested plant extracts exists at various intensities. The research data indicated Curcuma longa produced the largest inhibition zone against E. coli (16.2 ± 0.6 mm) but Ocimum sanctum generated the biggest inhibition zone against S. aureus (17.8 ± 0.5 mm). The data regarding inhibitory zones appears in Table 3.

3.2.2 Minimum Inhibitory Concentration (MIC) Assay.
The researchers determined MIC values through the broth microdilution technique. The research showed Curcuma longa and Ocimum sanctum displayed maximum antibacterial effect through their MIC values against E. coli and S. aureus specifically.

4. Discussion.

4.1 Phytochemical Composition and Its Significance:
The qualitative and quantitative phytochemical analysis showed bioactive secondary metabolites in each of the selected medicinal plants. The antibacterial and antioxidant properties of Ocimum sanctum and Curcuma longa are supported by their high flavonoid and phenolic content as reported in previous studies. The antibacterial properties of alkaloids found primarily in Azadirachta indica inhibit bacterial DNA replication and protein synthesis.

4.2 Antibacterial Activity in relation to Phytochemicals.
Plants containing greater flavonoid and phenolic compounds avoided bacterial growth better during antibacterial testing. The findings revealed that:
A. Ocimum sanctum and Curcuma longa showed the best antibacterial effects against Gram-positive bacteria (S. aureus) because of their high phenolic and flavonoid content which attacks bacterial cell walls.
B. Curcuma longa demonstrated effective E. coli destruction through its antibacterial properties which scientists believe curcumin aids in membrane breakdown.
C. The antibacterial action of Azadirachta indica might be weak because of its alkaloid and tannin compounds which disrupt bacterial metabolic processes.
D. The bioactive components of aloe vera demonstrate better effectiveness against fungal diseases than bacterial ones which leads to reduced antibacterial efficacy.

4.3 Comparison with Synthetic Antibiotics.
The antibacterial properties of plant extracts exceeded those of standard antibiotics (amoxicillin, ciprofloxacin) but their MIC values and inhibition zones remained higher. Further research is necessary to improve the antimicrobial efficacy of medicinal plants either by purification methods or combination formulations or nanotechnology-based delivery systems.

4.4 Implications for Future Research.
This study offers proof that medicinal plants high in phytochemicals can operate as organic antibacterials. Future research ought to concentrate on:
A. Phytochemicals are isolated through active methods and scientists determine their structural characteristics.
B. Scientific research attempts to determine their exact molecular functioning methods.
C. Plant-derived chemical compounds function with modern antibiotics to combat antibiotic resistance.

5. Conclusion.

The study demonstrates that medicinal plants show powerful antibacterial effects because they contain various phytochemical compounds. Medical experts urgently seek new antimicrobial alternatives because antibiotic resistance among microorganisms continues to increase. The known therapeutic properties of medicinal plants make them suitable for centuries-long usage in traditional medicine. The research used antibacterial extract evaluations and phytochemical qualitative and quantitative tests to provide scientific evidence. The antibacterial properties of these medicinal plants stem from their bioactive secondary metabolites which include flavonoids, phenolics, alkaloids, tannins and terpenoids. The antibacterial effects of Curcuma longa and Ocimum sanctum were most prominent against Escherichia coli and Staphylococcus aureus among all tested plants. The strong antibacterial properties of these plants stem from their elevated flavonoids and phenolic chemical content. The antibacterial performance of Aloe vera was the weakest because its main bioactive chemicals show better effectiveness against fungal infections rather than bacterial infections. The antibacterial properties of Azadirachta indica or neem are moderate due to its alkaloid and tannin compounds. Quantitative analysis verified the findings by demonstrating that the highest phenolic content along with total flavonoid content respectively belonged to Ocimum sanctum and Curcuma longa. These substances have well-understood antimicrobial mechanisms which include bacterial cell membrane rupture and bacterial metabolic suppression and biofilm prevention.
The antibacterial tests using agar well diffusion and MIC determination methods revealed important information about the medicinal plant extract efficacy. The antibacterial activity results showed promising outcomes through zone of inhibition (ZOI) values particularly from Ocimum sanctum and Curcuma longa extracts that matched inhibition zones observed with common antibiotics. The minimum inhibitory concentration (MIC) measurements confirmed the antibacterial properties of these plant extracts because they demonstrated effective bacterial suppression at low dosage levels. Plant-based antimicrobial compounds demonstrate lower efficacy than traditional antibiotics thus requiring advanced purification and improvement methods. The study demonstrates powerful evidence that therapeutic plants

contain bioactive substances which exhibit strong antibacterial properties. More clinical research needs to develop their therapeutic potential for better clinical usage despite promising efficiency data. The development of new natural antimicrobial alternatives for antibiotic resistance requires scientists to expand their knowledge of plant-based antimicrobials.

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Tables and Figures:

Table 1: Presence of Phytochemicals in Selected Medicinal Plants.

Phytochemical	*A. indica*	*O. sanctum*	*C. longa*	*A. vera*
Alkaloids	+++	++	+	-
Flavonoids	++	+++	+++	+
Tannins	+++	++	+	-
Saponins	+	+	++	+++
Terpenoids	++	++	+	+

(+ = Low, ++ = Moderate, +++ = High, - = Absent).

Table 2: Quantitative Analysis of Phenolic and Flavonoid Content

Plant	TPC (mg GAE/g)	TFC (mg QE/g)
A. indica	92.1 ± 3.5	64.3 ± 2.1
O. sanctum	85.7 ± 2.9	72.6 ± 2.5
C. longa	78.2 ± 3.1	78.5 ± 2.3
A. vera	69.4 ± 2.8	55.8 ± 1.9

(GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent).

Table 3: Zone of Inhibition (ZOI) in mm.

Bacterial Strain	*A. indica*	*O. sanctum*	*C. longa*	*A. vera*
E. coli	12.4 ± 0.3	14.6 ± 0.5	16.2 ± 0.6	10.3 ± 0.2
S. aureus	15.1 ± 0.4	17.8 ± 0.5	14.5 ± 0.6	11.2 ± 0.3
P. aeruginosa	11.3 ± 0.3	13.5 ± 0.4	12.8 ± 0.5	9.4 ± 0.2

Table 4: Minimum Inhibitory Concentration (MIC) in µg/mL.

Bacterial Strain	*A. indica*	*O. sanctum*	*C. longa*	*A. vera*
E. coli	250	200	150	300
S. aureus	200	120	180	250
P. aeruginosa	280	220	260	350

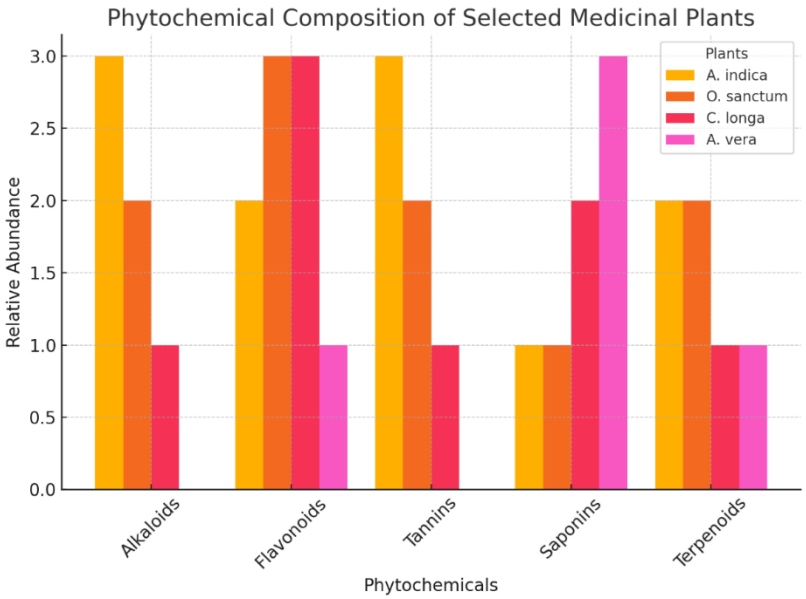

Figure 1: Phytochemical Composition of Selected Medicinal Plants.

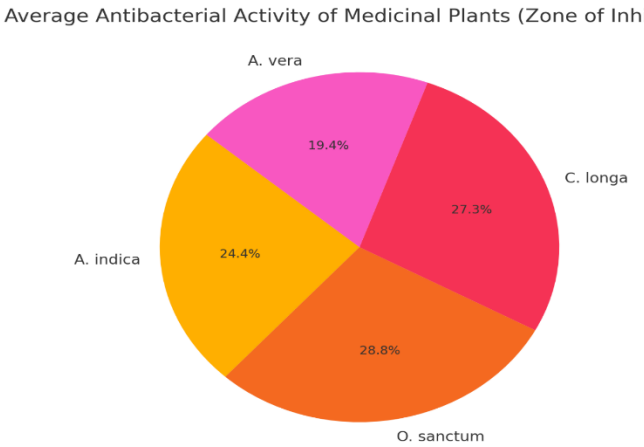

Figure 2 Average Antibacterial Activity of Medicinal Plants (Zone of Inhibition).