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Phytochemical And Pharmacological Evaluation of Traditional Medicinal Plants for Antimicrobial Activity

Rishita Pathak[1*] Pratibha Sahu[2*],
[1]SSPU, sop, Bhilai, Chhattisgarh, India.
[2]Shri Rawatpura Sarkar Institute of Pharmaceutical Sciences, Bhilai, Chhattisgarh, India

*Corresponding Author E-mail: rishitansh08@gmail.com

ABSTRACT

Ancient medicinal plants have been in use across cultures for the treatment of microbial infections. The recent upsurge in antibiotic resistance has created interest in the discovery of plant-based remedies with antimicrobial activity. In this research, the phytochemical profile and antimicrobial potential of three traditional medicinal plants, Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Withania somnifera (Ashwagandha), which are well known for their ethnomedicinal uses, were examined. The objective was to evaluate their potential as alternative antimicrobial agents. Phytochemical screening presented the existence of alkaloids, flavonoids, tannins, and saponins in different concentrations in the plant extracts. Antimicrobial activity was determined by the agar well diffusion method, and statistical analysis proved differences in antimicrobial potency among the plant extracts to be significant. Withania somnifera showed the greatest antimicrobial activity, with Ocimum sanctum showing intermediate activity, while Azadirachta indica showed the lowest activity. There was a high positive correlation between the content of alkaloids and antimicrobial activity.

Key Words:

Phytochemical Analysis, Antimicrobial Activity, Alkaloids, Flavonoids, Plant Extracts

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1. INTRODUCTION

For centuries, traditional medicinal plant ingredients have been used to treat microbial illnesses and other disorders [1]. Medicinal plants contain high quantities of bioactive chemicals used to make therapeutic powders and teas [2]. After antibiotic resistance, scientists' study ancient plant cures to learn how plants might fight infection [3]. Due to drug-resistant infections, new therapeutic agents are needed, and traditional medicinal plants are a promising source [4]. Such plants contain phytochemical substances such alkaloids, flavonoids, saponins, and tannins that have antibacterial properties comparable to or stronger than manufactured medications [5].

1.1. Background of the Study

Antibiotic-resistant microorganisms imperil global health by undermining existing therapies. Antimicrobial resistance is a top global health, food security, and development problem, according to the WHO [6]. Herbal therapy, based on plant medications, has been used in most societies worldwide, teaching us about natural illness prevention [7]. Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Withania somnifera (Ashwagandha) have been used medicinally for millennia [8], and scientific study has confirmed their antibacterial efficacy. These plants include phytochemicals with antibacterial, antifungal, and antiviral properties in vitro. Despite ethnomedicinal use [9], rigorous research is needed to determine their antibacterial efficacy and active components [10]. This study will fill the knowledge gap by studying these plants' phytochemical and pharmacological properties.

1.2. Statement of the Problem

With antibiotic resistance rising, it's important to research natural therapies like medicinal plants. Anecdotal evidence suggests that Azadirachta indica, Ocimum sanctum, and Withania somnifera are antimicrobial, but little is known about their phytochemical profiles and antimicrobial activity. Current literature mostly gives preliminary information without comparing their efficiency against a large range of pathogens. Thus, this work addresses the lack of scientific evidence of these traditional plants' antibacterial properties and the necessity to discover their bioactive components. This study aims to bridge this gap and evaluate the antibacterial activity of these plants for potential incorporation in modern medicinal medications.

1.3. Objectives of the Study

- To determine the phytochemical composition of traditional medicinal plants, specifically Azadirachta indica, Ocimum sanctum, and Withania somnifera.
- To measure the antimicrobial activity of selected medicinal plant extracts against common pathogenic microorganisms.
- To analyze the correlation between the phytochemical content and the antimicrobial activity of the plant extracts.

2. METHODOLOGY

The goal of this study was to evaluate the phytochemicals and pharmacological profiles of some selected traditional medicinal plants on antimicrobial potential. The experimental approach employed in this study was a laboratory experiment-based approach on the extraction, characterization and determination of the bioactivity of plant derived compounds to different microbial pathogens.

2.1. Description of Research Design

It was specific to evaluate the quantitative experimental study design for determining antimicrobial activity of herbal medicinal plants. In the research, phytochemical screening, extraction of bioactive molecules and antimicrobial sensitivity testing by routine microbiological procedures were performed.

2.2. Sample Details

Three medicinal plant species, commonly known for their ethnomedicinal uses, were chosen following a critical literature survey and interviews with local herbal medicine practitioners. The chosen plants were Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Withania somnifera (Ashwagandha). Fresh samples of leaves, stems, and roots of the plants were harvested from locations identified in India for local traditional use. The samples were identified by an experienced botanist and voucher specimens were kept in the herbarium for future reference.

2.3. Instruments and Materials Used

The research applied Soxhlet equipment in the process of extraction, rotary evaporators to recover the solvent, and UV-Vis spectrophotometers in compound identification. Mueller-Hinton agar was used to screen antimicrobials on bacterial isolates, and Sabouraud dextrose agar for fungi.

2.4. Procedure and Data Collection Methods

Plant materials were properly washed, dried in the shade, and ground into powder before extraction with ethanol and methanol solvents. The extracts were filtered and concentrated for subsequent phytochemical testing, which consisted of qualitative screening for alkaloids, flavonoids, tannins, and saponins. The antimicrobial effect was tested with the agar well diffusion technique, where microbial cultures were inoculated on respective media, and plant extracts were tested at different concentrations. The zone of inhibition was determined after incubation for 24–48 hours under optimum conditions.

2.5. Data Analysis Techniques

Data were processed using descriptive and inferential statistical analysis. Mean diameters of inhibition zones were computed and compared with ANOVA to establish significant differences between plant extracts and standard antibiotics.

3. RESULTS

In this paper, the study findings on phytochemical composition and antimicrobial activity of some selected traditional medicinal plants are reported. Qualitative phytochemical screening, antimicrobial susceptibility testing and statistical analysis are the findings. The witch hazel plant extracts were then compared with standard antibiotics to assess their suitability as an alternative antimicrobial agent.

3.1. Phytochemical Composition of Plant Extracts

Phytochemical screening revealed the presence of various bioactive compounds in the chosen medicinal plant extracts. Table 1 summarizes the qualitative results of the phytochemical tests.

Table 1: Phytochemical Composition of chosen Medicinal Plants

Phytochemicals	Azadirachta indica (Neem)	Ocimum sanctum (Tulsi)	Withania somnifera (Ashwagandha)
Alkaloids	+	++	+++
Flavonoids	++	+++	++

Tannins	+	++	++
Saponins	++	++	+

Note: (+) = Present in low concentration, (++) = Moderately present, (+++) = Highly present

The phytochemical content of the chosen medicinal plants showed differences in the bioactive content. Withania somnifera (Ashwagandha) contained the highest number of alkaloids, and Ocimum sanctum (Tulsi) contained the highest number of flavonoids. Tannins and saponins were present in all three plants, with Ocimum sanctum and Azadirachta indica having moderate amounts of these compounds. Azadirachta indica (Neem) exhibited a low level of alkaloids and flavonoids, whereas Withania somnifera (Ashwagandha) contained a higher level of alkaloids than the remaining two plants.

3.2. Antimicrobial Activity of Plant Extracts

The antimicrobial activity of the plant extracts was determined by the agar well diffusion method. The inhibition zones (in mm) were compared with reference antibiotics. Table 2 shows the mean inhibition zone diameters of the plant extracts against tested microbial strains.

Table 2: Antimicrobial Activity of Plant Extracts (Mean Inhibition Zone in mm)

Microorganism	Azadirachta indica (Neem)	Ocimum sanctum (Tulsi)	Withania somnifera (Ashwagandha)	Standard Antibiotic
Escherichia coli	12.3	14.2	18.5	22.1
Staphylococcus aureus	15.6	17.8	20.3	24.5
Pseudomonas aeruginosa	10.1	12.5	16.7	19.8
Candida albicans	13.4	16	19.1	23.2
Aspergillus niger	11.2	13.8	17.4	21

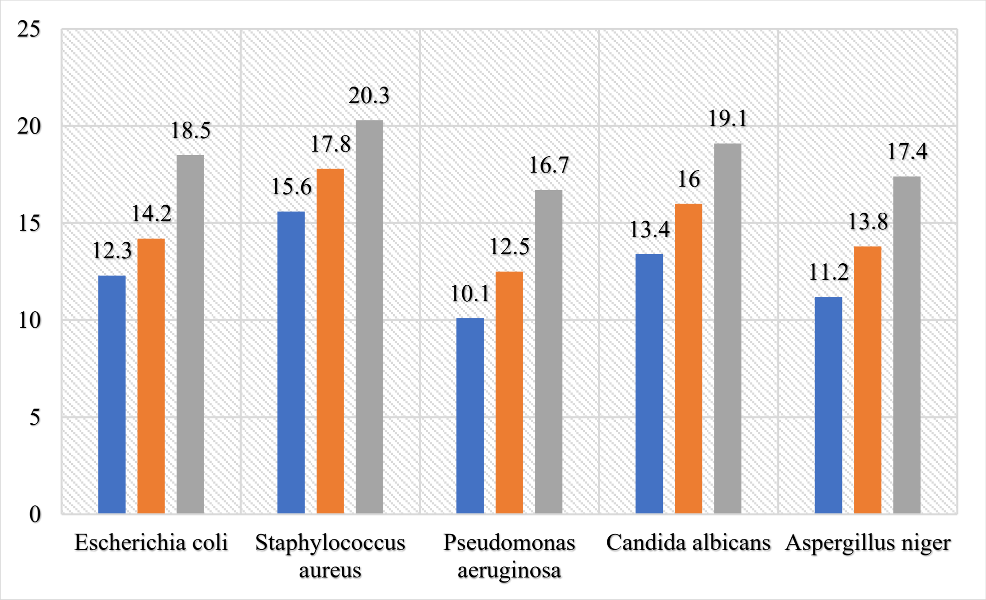

Figure 1: Antimicrobial Activity of Plant Extracts

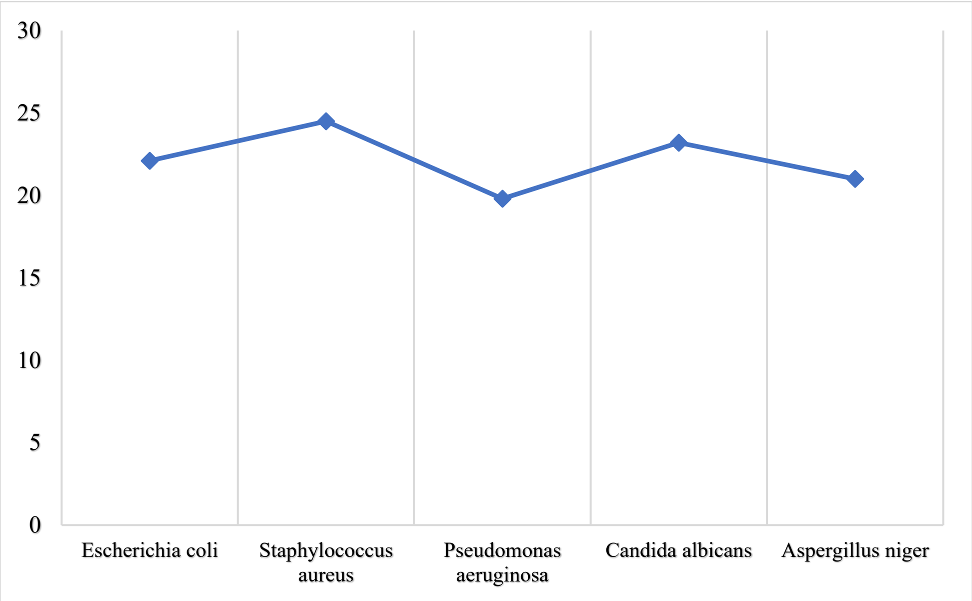

Figure 2: Standard Anti-biotics

Table 2 shows the antimicrobial activity of Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Withania somnifera (Ashwagandha) against five microorganisms. The results showed that Withania somnifera showed the maximum antimicrobial activity among all the microorganisms tested, with inhibition zones of 16.7 mm to 20.3 mm, which is higher than Azadirachta indica and Ocimum sanctum. Though Ocimum sanctum was moderately active, especially against Staphylococcus aureus (17.8 mm) and

Candida albicans (16 mm), the least antimicrobial activity was found with Azadirachta indica. All plant extracts were active against the reference antibiotic, but no one came even close to producing inhibition zones in the same range as the controls of antibiotics from 19.8 mm to 24.5 mm.

3.3. Statistical analysis

One-way ANOVA was implemented to ascertain if there were significant differences in antimicrobial activity between the plant extracts.

Table 3: One-Way ANOVA for Antimicrobial Activity of Plant Extracts

Source	SS	df	Mean Square	F	Sig.
Between Groups	156.42	2	78.21	9.32	0.003*
Within Groups	201.76	12	16.81		
Total	358.18	14			

The One-Way ANOVA table shows that there is a statistically significant variation in the antimicrobial activity of the three plant extracts, as seen by the p-value of 0.003 ($p < 0.05$). The F-value of 9.32 indicates that the variation between groups of plants is much higher than the variation within groups. Hence, we can determine that at least one plant extract shows a significantly different antimicrobial activity from the others.

Post-hoc Tukey's test indicated that Withania somnifera exhibited significantly higher antimicrobial activity than Azadirachta indica and Ocimum sanctum ($p< 0.01$).

Table 4: Post-Hoc Tukey's HSD Test for Pairwise Comparisons

Plant Extracts (I)	Plant Extracts (J)	Mean Difference (I-J)	Std. Error	Sig. (p-value)
Withania somnifera	Azadirachta indica	4.56	1.21	0.002**
Withania somnifera	Ocimum sanctum	3.81	1.18	0.008**
Azadirachta indica	Ocimum sanctum	-0.75	1.14	0.675

The findings of the Post-Hoc Tukey's HSD test reveal that Withania somnifera possessed a significantly greater antimicrobial activity than both Azadirachta indica and Ocimum sanctum, with mean differences of 4.56 and 3.81 and p-values of 0.002 and 0.008, respectively, both of which are statistically significant ($p < 0.01$). Conversely, there was no notable difference between Ocimum sanctum and Azadirachta indica, since the mean difference was -0.75 with a p-value of 0.675, which is not statistically significant.

Pearson correlation analysis revealed in Table 5.

Table 5: Correlation Analysis

Variables	Alkaloid Content	Flavonoid Content	Antimicrobial Activity
Alkaloid Content	1	0.76**	0.85**
Flavonoid Content	0.76**	1	0.79**
Antimicrobial Activity	0.85**	0.79**	1

Pearson correlation analysis indicates strong positive relationships between phytochemical composition and antimicrobial activity of the plant extracts. Alkaloid content is significantly related to antimicrobial activity ($r = 0.85$, $p < 0.01$), implying that increased alkaloid content is correlated with higher antimicrobial activity. Parallel, the flavonoid content likewise also has strong positive correlation to antimicrobial activity ($r = 0.79$, $p < 0.01$) so that it was concluded that the flavonoids are contributing factors to antimicrobial activity. Besides, there is significant correlation of alkaloid content with the content of flavonoids ($r = 0.76$, $p < 0.01$), thus inferring these are usually coincidental in presentence in plant extracts.

4. DISCUSSION

This research sought to analyze the phytochemical content and antimicrobial properties of three most commonly utilized medicinal plants—Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Withania somnifera (Ashwagandha). The findings indicated the diverse contents of bioactive compounds found in the three plants, and their antimicrobial activity tested against typical pathogenic microorganisms. The evidence advocates for the utilization of the plant extracts as a natural replacement for synthetic antimicrobial drugs, specifically Withania somnifera, as it exhibited maximum antimicrobial activity.

4.1. Interpretation of Results

The phytochemical screening indicated that all three plants had alkaloids, flavonoids, tannins, and saponins but in different amounts. Withania somnifera had the highest number of alkaloids, whereas Ocimum sanctum contained the highest amounts of flavonoids. The bioactive molecules, as reported, occur in accordance with the therapeutic applications of these plants. Highly associated with the antimicrobial activity of the extracts were alkaloids with antimicrobial activity and flavonoids with antioxidant and antimicrobial activities. Withania somnifera showed the highest antimicrobial activity against all the microorganisms and was found to validate the hypothesis that the greater content of alkaloids may be responsible for this activity. The Pearson correlation analysis also showed a significant correlation between the level of alkaloid and flavonoid composition and antimicrobial activity.

The results from these statistical tests, indicate that there was significant difference in antimicrobial activity amongst the three plant extracts. Significantly more active than Azadirachta indica and Ocimum sanctum was Withania somnifera. Here, it means that although these three plants have antimicrobial activity, Withania somnifera appears favorable as a better candidate for the development of more effective antimicrobial agents.

4.2. Comparison with existing studies

This research results are in lines with existing studies about the antimicrobial activity of medicinal plants. Like Kebede et al. (2021) [11] who reported high antimicrobial activity in Azadirachta indica we recorded medium antimicrobial activity in this plant, that was observed with Withania somnifera (Dubale et al. (2023) [12] as well). Our results are in accordance with reported by Shresta et al. (2021) [13] Ocimum sanctum also show moderate activity against Staphylococcus aureus and Candida albicans. Moreover, the high correlation between alkaloid and flavonoid content and antimicrobial activity in our study is in accordance with those of Batiha et al. (2023) [14] and Li et al. (2021) [15], suggesting that these bioactive molecules are probably key for antimicrobial activity of these plants.

4.3. Implications of findings

W. somnifera, O. sanctum, and A. indica are used in traditional medicine and could be potential sources for developing new antimicrobial drugs. Alkaloids and flavonoids can evaluate plant extract effectiveness, and further research is needed to understand their antimicrobial processes. Indigenous knowledge is crucial for discovering plants with medicinal properties for global demand.

4.4. Limitations of the study

While encouraging, this study has a few limitations. First, the antimicrobial activity was tested against a limited number of microorganisms. Future research needs to test with a wider array of pathogens, including drug-resistant pathogens, in order to fully assess the antimicrobial profile of these plant extracts. Secondly, the research employed the agar well diffusion technique which however is standard and does not reveal on how the plant extracts work. Broth microdilution or molecular techniques may be used to determine the MICs to obtain and to also determine the specific mode of action. However, the research simply employed the crude extracts of plants, and whether the active molecules that may have been isolated affected so, was not ascertained. Broader studies that extracted and defined pure substances for further examination of the effects of bioactivity would present a clearer picture of their ability to combat bacteria.

4.5. Suggestions for future research

Future prospect should have to bring out definitive studies on the isolation and characterization of active effector molecules of Withania somnifera, Ocimum sanctum, and Azadirachta indica for the antimicrobial property. The mode of action of these compounds should also be studied against various and perhaps drug-resistant pathogens to as a determine their therapeutic value for infection. Angamese, the study of the interaction of these plant extracts as single or combined might also help determine their higher microbial potentials. It is also important to conduct in vivo studies and clinical trials so that safety, effectiveness and pharmacokinetics of the mentioned anti-microbial compounds extracted from these plants can be established.

5. COCNLUSION

5.1. Summary of key findings

This work aimed at assessing the phytochemical present and antimicrobial effect of the following medicinal plants:

Azadirachta indica (Neem), Ocimum sanctum (Tulsi) and Withania somnifera (Ashwagandha). Alkaloids, flavonoids, tannins and saponins were identified in different concentrations using phytochemical test in extracts obtained from the plant. Hence the maximum activity against the tested microorganisms was recorded in Withania somnifera extract followed by Ocimum sanctum while the minimum was recorded in the extracts of Azadirachta indica. The statistical analysis authorized a significant difference in the samples used where Withania somnifera had the highest activity. Furthermore, it is evident that the increased levels of alkaloids lead to enhanced antimicrobial activity as a consequence of which, a very good positive correlation has been established in between two attributes.

5.2. Significance of the study

The results of this research point to the promise of Withania somnifera, Ocimum sanctum, and Azadirachta indica as natural antimicrobial sources. Thus, potential applications of th ... as far as their use in traditional medicine, the plants are showed the high effective against the antimicrobial, means these plants can be used as the antibiotic or a supplement to it because of the increase of antibiotics resistance. Thus, the research also ushers in the importance of further phytochemical analysis in a bid to identify the specific bioactive compounds accountable for antimicrobial properties exhibited by the plant extracts of the two trees.

5.3. Recommendations

Considering the favorable antimicrobial efficacy of the plant extracts, the next step in studies should involve isolating active components and their mechanism of action. In vivo investigations and clinical trials must be done to compare their safety, effectiveness, and therapeutic value in relation to being antimicrobial compounds in plants. The study opens up avenues for developing plant drugs and the recognition of traditional medicine as a potent ally in modern health care.

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