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Investigation of the Correlation Between FTIR Spectral Characteristics and Antimicrobial Activity (MIC) of Neem (*Azadirachta indica*) Extracts Obtained by Different Extraction Methods

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Abstract

Background: Neem (*Azadirachta indica*) contains diverse bioactive compounds with established antimicrobial properties, and the efficiency of extracting these compounds depends strongly on the solvent polarity.

Objectives: This study aimed to investigate the correlation between Fourier-transform infrared (FTIR) spectral characteristics and antimicrobial activity, specifically the minimum inhibitory concentration (MIC), of neem extracts obtained using different extraction solvents.

Methods: Neem samples were collected from the Sistan and Baluchestan region, shade-dried, and extracted using different solvents, including ethanol and acetone. Antimicrobial activity against *Staphylococcus aureus* strains was assessed, and the MIC and minimum bactericidal concentration (MBC) values were determined using the microdilution method. Chemical functional groups were analyzed by FTIR spectroscopy.

Results: FTIR analysis identified key functional groups, including hydroxyl (-OH) and carbonyl (C=O) groups. A strong correlation was observed between the intensities of these peaks and antimicrobial potency. The acetone extract exhibited the highest antibacterial activity and showed greater absorption intensity at specific FTIR wavenumbers.

Conclusions: Neem antimicrobial activity may be associated with bioactive compounds such as tannins, flavonoids, and terpenoids. These findings indicate that the extraction solvent influences the yield of these compounds and, consequently, antimicrobial efficacy.

Keywords: Neem, Azadirachta Indica, Fourier Transform Infrared Spectroscopy, Minimum Inhibitory Concentration, Antimicrobial Activity, Solvent Extraction

1. Background

Neem (*Azadirachta indica* A. Juss.), a tropical evergreen tree in the Meliaceae family, is widely recognized in traditional medicine for its pharmacological properties (1, 2). Native to the Indian subcontinent and naturally distributed in the warm regions of southern and southeastern Iran, neem contains a diverse range of secondary metabolites, including glycosides, coumarins, tannins, azadirachtin, nimbin, and various polyphenolic compounds (3, 4).

Various parts of the neem tree, including the leaves, bark, and fruit, are used to treat conditions such as diabetes, hypertension, and certain types of cancer (5). Recently, interest has grown in using plant-derived essential oils and extracts as alternatives to synthetic antimicrobial agents because of their antifungal and antibacterial properties (6). Tannins, which are high-molecular-weight phenolic compounds, contribute

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importantly to this activity by binding to proteins and microbial cell-wall components, thereby inhibiting microbial growth (7).

The efficiency of extracting these bioactive compounds depends strongly on the polarity of the solvent used (8). Therefore, identifying an optimal solvent is important for maximizing antimicrobial activity.

2. Objectives

This study aimed to evaluate the correlation between the FTIR spectral characteristics and the MIC of neem extracts to establish a rapid, nondestructive method for predicting antimicrobial potency.

3. Methods

3.1. Plant Preparation and Extraction

Neem leaves were collected from the Sistan and Baluchestan region, washed with distilled water, and shade-dried. The samples were then dried in an oven at 50°C for 24 hours, powdered, and stored at -80°C. The powder was mixed with solvents (eg, ethanol and acetone) at a 1:10 ratio (10 g of sample per 100 mL of solvent) and processed in an ultrasonic bath (Kodo2040, Japan) for 20 minutes. The suspension was homogenized on a shaker (SK-0180-Pro, USA) at 200 rpm for 24 hours at room temperature. After centrifugation at 3500 g for 35 minutes (5810, Germany), the supernatant was filtered through a 0.45-µm Whatman filter and concentrated using a rotary evaporator at 70°C and 120 rpm.

3.2. Antimicrobial Activity Assay

Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) were prepared according to standard protocols and sterilized by autoclaving at 121°C for 35 minutes. Ten strains of *Staphylococcus aureus* were isolated from native sheep in the Sistan region and cultured on blood agar. The MIC and MBC were determined using the microdilution method.

3.3. FTIR Spectroscopic Analysis

The chemical functional groups of the extracts were analyzed using an FTIR spectrometer. Spectra were recorded over the range of 4000 to 1000 cm^{-1}, and shifts in peak positions and intensities were compared among extracts to assess correlations with antimicrobial activity.

4. Results

4.1. Antimicrobial Activity

The extracts exhibited inhibitory activity against *S. aureus*. The lowest MIC was 5.25 mg/mL for two strains, whereas the highest MIC was 100 mg/mL for one strain (Table 1).

Table 1. MIC and MBC of Neem Extracts Against *S. Aureus* Strains

Strain Code	MIC (mg/mL)	MBC (mg/mL)
1	50	100
2	100	200
3	25	50
4	50	100
5	5.25	10.5
6	50	100
7	25	50
8	50	100
9	25	50
10	5.25	10.5

4.2. FTIR Analysis

The FTIR spectra of the extracts showed prominent peaks in the 3000 - 3500 cm^{-1} region, corresponding to hydroxyl (-OH) groups. A decrease in wavenumber and changes in peak intensity were observed across different solvents, suggesting differences in the concentration of phenolic compounds. Peaks in the 1600 - 1700 cm^{-1} region were attributed to carbonyl (C=O) groups. The intensity of these peaks correlated positively with antimicrobial activity, with the acetone extract showing the strongest absorption.

5. Discussion

The results of this study confirm the antimicrobial activity of neem extracts against *S. aureus*. This activity is primarily attributed to tannins and phenolic compounds, which disrupt the microbial cell membrane. Specifically, compounds such as nimbidin and nimbin increase membrane permeability, leading to leakage of cytoplasmic contents and cell death (9, 10).

The correlation between FTIR peaks, specifically the -OH and C=O groups, and MIC values suggests that these functional groups may be important in the antimicrobial mechanism. This finding is consistent with previous research showing that hydroxyl groups are associated with stronger inhibition of bacterial growth (11). Furthermore, our results indicate that acetone is a more efficient solvent than the other tested solvents for extracting these bioactive phenolic compounds, resulting in lower MIC values.

Inhibition of vital enzymes, such as ATPase and DNA gyrase, by azadirachtin and quercetin may further explain the bactericidal effect observed in this study (12). Overall,

the combined use of FTIR and antimicrobial assays provides a useful approach for the rapid screening of medicinal plant extracts.

In a study investigating the antimicrobial activity of several medicinal plants against standard bacteria, F. subpinnata inhibited *S. dysenteriae*, with an inhibition-zone diameter of 6 mm (13).

Saeidi et al. reported that *P. harmala* and *M. comminus* L. extracts showed strong antimicrobial activity against *S. aureus*, *Escherichia coli*, and *Enterobacter cloacae* (14).

A study investigating the antimicrobial activity of plants collected from the Sistan region reported that the lowest inhibitory concentration of pecan extract against *Vibrio cholerae* was 3.1 ppm and the highest inhibitory concentration was 6.25 ppm (15).

In a study investigating the effects of *Mentha piperita* and *Zataria multiflora* essential oils, the minimum and maximum inhibitory concentrations required to inhibit *Pseudomonas aeruginosa* were 1.25 and 5 mg/mL, respectively (16).

In a study investigating the antimicrobial properties of several extracts against standard bacterial strains, *P. nigrum* and C. cyminum L. showed inhibitory activity against the Gram-positive bacterium *Bacillus cereus* and the Gram-negative bacteria *Proteus mirabilis*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae* (17).

5.1. Conclusions

This study demonstrates that neem extracts exhibit antimicrobial activity against *S. aureus*. The correlation between FTIR spectral intensity and MIC values highlights the potential importance of phenolic and carbonyl groups in the plant's bioactivity. These findings support further investigation of neem extracts for the development of antimicrobial agents against *S. aureus* infections.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

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