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Comparative Evaluation of Neem Leaf Extract and Powder as Natural Antibacterial Agents Against Staphylococcus Aureus

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Azadirachta indica possess antiviral, antimicrobial, antioxidant, and antimalarial qualities due to a rich source of bioactive compounds. The study was conducted to find out the antibacterial activity of methanol extracts and powdered leaves of A. indica against Staphylococcus aureus. 25 g of finely ground powdered dried leaves was dissolved in 100 ml of methanol for an hour. To prepare the methanolic extract, 25 g of powdered leaf was mixed with methanol and allowed to stand for a week. Different concentrations of leaf extract and powder, ranging from 0.25, 0.3, 0.35, 0.4, 0.5, 1, 1.5, and 2.0 mg, were prepared after filtration and tested against cultured strains of bacteria, using both agars well diffusion and disc diffusion methods. The antibacterial evaluation was done by measuring the diameter of inhibition zones. According to statistical data, at a concentration of 0.25 mg/mL, the methanol neem powder showed the highest zone of inhibition (3.6 mm), while the lowest inhibition zone (0.7 mm) was recorded at 2 mg/mL. In comparison to the methanol extract, the powder shows greater effectiveness against S. aureus. At 2 mg/ml, methanol extract showed minimum inhibitory concentration (MIC) which is found to be 6.2 µg/ml, whereas the highest inhibitory value at 0.25 mg/mL was 0.6 µg/ml. Along with flavonoids and tannins, which enhance effectiveness by damaging bacterial cell walls, inhibiting enzymes, and generating oxidative stress, the strong antibacterial activity of neem is mainly attributed to its major bioactive compounds i.e Limonoids (azadirachtin, nimbin, nimbolide). For developing natural antibacterial drugs, Neem can be considered a very promising source due to its remarkable antibacterial potential.

Keywords: Neem Plant, Antibacterial Activity, Leaves Powdered, Leaves Extract, Infectious Disease

Introduction:

Medicinal plants have long served as essential sources of therapeutic compounds and have played a pivotal role in healthcare systems. Historically, well before the advent of modern pharmaceuticals, humans relied on plants for the treatment of various ailments, guided by instinct, sensory cues, and accumulated experiential knowledge [1].

Azadirachta indica, commonly known as Neem, belonging to the family Meliaceae has been used in old medicines to relieve pain and against infections, skin problems, and dental diseases. Neem also has anti-inflammatory properties, which help reduce acne and nourish the skin. And promote growth in animals. It is also particularly effective against various bacterial infections. Neem contains several bioactive compounds that possess multiple properties like antiviral, antitumor, anti-inflammatory, antimicrobial, antioxidant, and antimalarial.

Research has demonstrated that neem extracts can inhibit the growth of up to 21 foodborne pathogens, indicating their strong potential for use in controlling foodborne illnesses and preventing spoilage. Studies have also demonstrated the antibacterial potential of neem bark, leaves, seeds, and fruits [2].

Staphylococcus aureus, a Gram-positive bacterium belonging to the phylum Bacilli, is commonly found on the human skin and in the upper respiratory tract. Although it is part of the normal microbiota, it can become pathogenic and cause a wide range of infections, from minor skin conditions to severe systemic diseases. It is a major cause of serious infections leading to significant illness and death [3].

Objective:

The present study was carried out to find the comparative antibacterial potential of Neem extract and powder against Staphylococcus aureus, to evaluate bacterial growth inhibition potential.

Novelty Statement:

This study is novel in providing a direct comparison between neem leaf extract and leaf powder against Staphylococcus aureus under identical experimental conditions. By evaluating both processed and minimally processed neem forms, the work highlights their relative antibacterial potential and practical applicability as cost-effective, plant-based alternatives to synthetic antibiotics.

Research Design and Methodology:

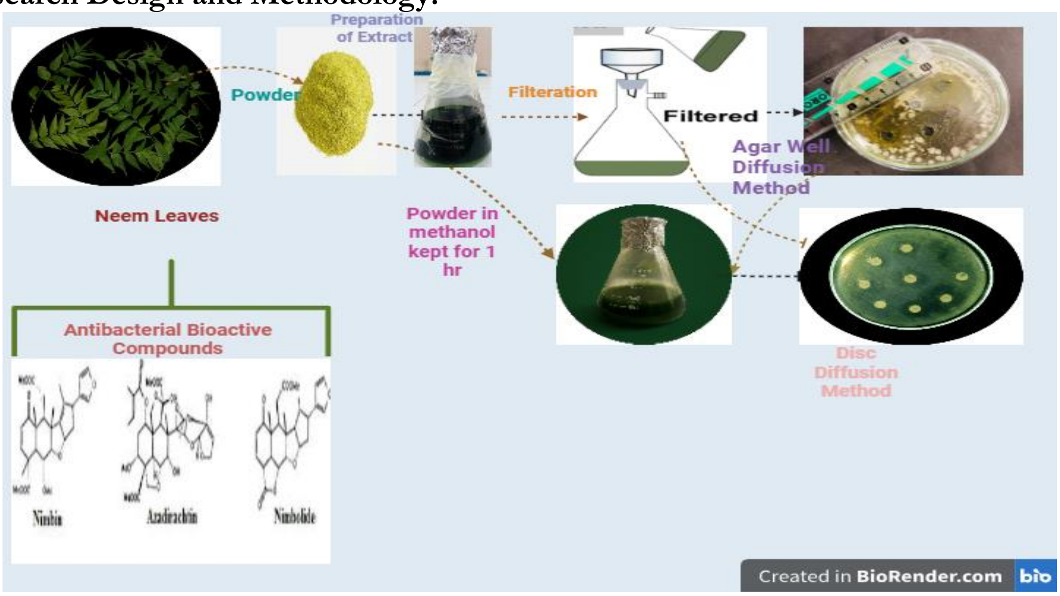

Figure 1. Flowchart illustrating the antibacterial activity of leaf powder and leaf extract against S. aureus.

Experiment Set up:

To evaluate the antibacterial activity of methanolic neem extract and neem powder against Staphylococcus aureus, an experiment was carried out at the School of Biology, Minhaj University, Lahore. All collected samples were transported to the research laboratory for detailed analysis.

Collection and Identification of Leaves of Selected Plant:

Fresh leaves of Azadirachta indica were collected from Tehsil Daska. The plant material was authenticated by a taxonomist at Minhaj University, Lahore, to ensure correct identification.

Drying and Grinding of Leaves of Selected Plant:

The collected neem leaves were washed thoroughly. After cleaning, leaves were air-dried at room temperature for about fifteen days until completely moisture-free. Once dried, the leaves were finely ground using a blender and kept in an airtight container for storage for further use.

Plant Extract Preparation:

For the preparation of the methanolic extract, 25 grams of neem leaf powder were soaked in 100 mL of methanol for one week. The mixture was then filtered through filter paper, and this filtrate was collected for antibacterial testing.

Preparation of Plant Leaves Powder:

For the preparation of the neem powder solution, 25 grams of leaf powder were dissolved in 100 mL of methanol and allowed to stand for one hour. The resulting solution was then used for the antibacterial analysis.

Determination of Antibacterial Activity:

Collection of Bacterial Isolate:

The bacterial strain Staphylococcus aureus was obtained from the First Fungal Bank of Lahore and maintained under suitable conditions in the laboratory of Minhaj University Lahore. The bacterium was selected for testing due to its clinical significance and its involvement in diseases such as diarrhea and urinary tract infections.

Nutrient Agar Media Preparation:

Nutrient agar medium was prepared by dissolving 5 g of peptone, 3 g of yeast extract, and 5 g of sodium chloride in 500 mL of distilled water. Subsequently, 15 g of agar was added, and the medium was sterilized by autoclaving. After pouring into 20 Petri dishes, the sterilized medium was left to solidify. Observations were made after 24 hours of incubation [4].

Agar Well Diffusion Method:

The agar well diffusion method was used to evaluate the antibacterial potential. By using the streaking method, nutrient agar plates were inoculated with the test bacterium. Having a diameter of 8mm, wells were punched into agar and filled separately with Neem methanol extract and powder solution. Control wells were also prepared, one containing methanol as the negative control and another with the antibiotic chloramphenicol as the positive control. Each well was loaded with 50–100 µL of positive and negative controls. The plates were incubated at a temperature of 37 °C for 24 hours, and inhibition zones were measured in millimeters [5].

Disc Diffusion Method:

Agar plates were inoculated with the bacterial culture. Sterile filter paper discs impregnated with the plant extract or antibiotic were placed on the agar surface. After incubation, the antibacterial activity was determined by measuring the diameter of the inhibition zones around each disc [6].

Minimum Inhibitory Concentration (MIC):

From the test tubes containing 10 mL of nutrient broth, the MIC was determined. The plant extract was serially diluted to achieve concentrations of 0.25, 0.3, 0.35, 0.4, 0.5, 1.0, 1.5, and 2.0 mg/mL. Each tube received 10 µL of bacterial suspension standardized to the McFarland 0.5 turbidity standard. Control tubes included, one containing only broth (negative control) and another containing broth with bacteria but no extract (positive control). The incubation was done for 24 hours at 37°C. The minimum inhibitory concentration was referred to as the lowest concentration that inhibits bacterial growth.

Results:

Agar Well Diffusion Assay of Methanolic Neem Leaf Extract and Powder against S. aureus:

The experimental results revealed that the antibacterial effects of the plant's leaf powder and methanolic extract varied against the tested bacterial strain. The inhibitory zones, measured in millimeters, indicated that the methanolic leaf extract showed the highest inhibition (3.6 mm) at a concentration of 0.25 mg/mL. A moderate inhibition zone (2.0 mm) was observed at 0.4 mg/mL, while the lowest inhibition (0.7 mm) occurred at 2 mg/mL, as illustrated in Figure 2.

Neem leaves powder extract prepared for an hour exhibited the highest antibacterial activity against S. aureus, showing a maximum inhibition zone of 2.8 mm at a concentration of 0.25 mg/mL. At 0.4 mg/mL, the inhibition zone decreased to 1.7 mm, while the lowest activity was recorded at 2 mg/mL with a zone of 0.8 mm, as shown in Figure 3.

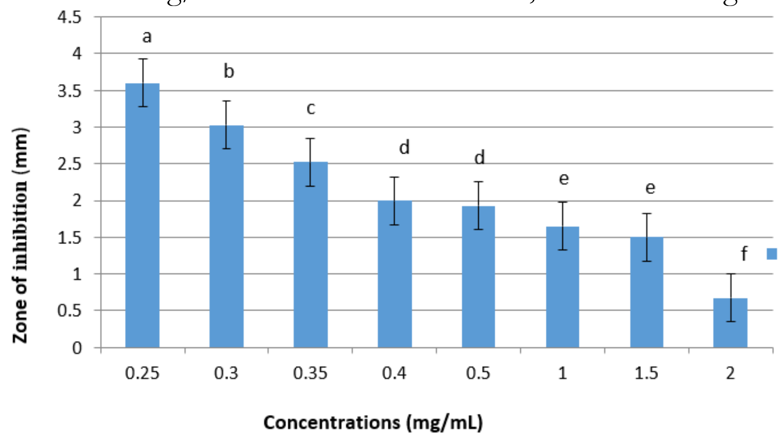

Figure 2. Assessment of the antibacterial activity of methanolic leaf extracts at varying concentrations (0.25–2 mg/mL) against Staphylococcus aureus. Means were shown by all bars, while standard errors were shown by vertical lines on bars. Variance was shown by letters a to f.

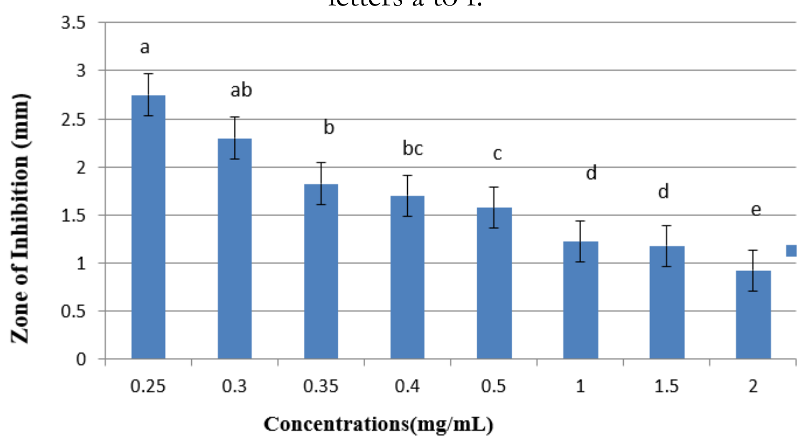

Figure 3. Assessment of the antibacterial activity of methanolic leaf powder extracts at varying concentrations (0.25–2 mg/mL) against Staphylococcus aureus. Means were shown by bars, while standard errors were shown by vertical lines on bars. Variance was shown by letters a to e.

Disc Diffusion Assay of Methanolic Neem Leaf Extract and Powder Against S. Aureus:

The leaf extracts of the selected plant exhibited the highest inhibition zone of 2.5 mm at a concentration of 0.25 mg/mL. A moderate inhibition zone of 2.0 mm was observed at 0.4 mg/mL, while the lowest inhibition zone (1.7 mm) was recorded at 2 mg/mL, as shown in Figure 4.

The potential of A. indica leaf powder was tested against S. aureus. The results showed that the extract produced the largest zone of inhibition, measuring 2.6 mm, at a concentration of 0.25 mg/mL. The zone slightly decreased to 2.4 mm at 0.4 mg/mL, while the lowest inhibition zone of 1.4 mm was observed at 2 mg/mL, as illustrated in Figure 5.

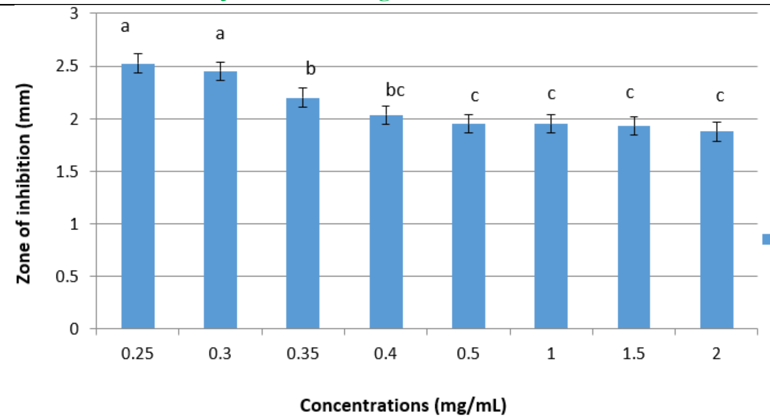

Figure 4. Assessment of the antibacterial activity of methanolic leaf extracts at varying concentrations (0.25–2 mg/mL) against Staphylococcus aureus. Means were shown by bars, while standard errors were shown by vertical lines on bars. Variance was shown by letters a to c.

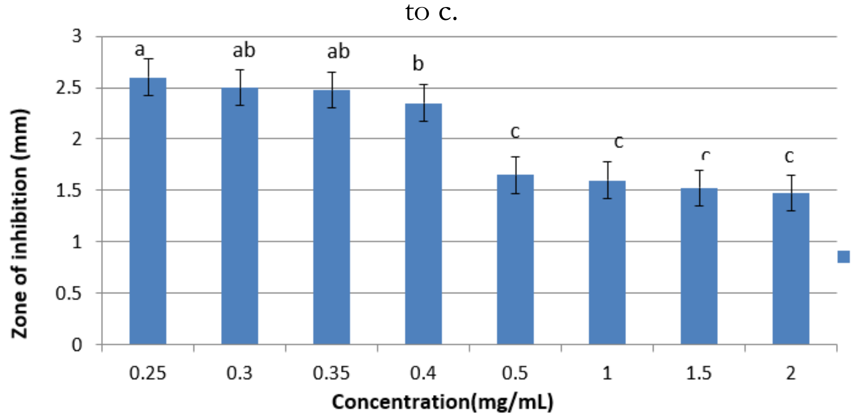

Figure 5. Assessment of the antibacterial activity of methanolic leaf extracts at varying concentrations (0.25–2 mg/mL) against Staphylococcus aureus. Means were shown by bars, while standard errors were shown by vertical lines on bars, and variance was shown by letters a to c.

Minimum Inhibitory Concentration (MIC):

The lowest inhibitory value is shown by the methanolic extract against S. aureus which was 4.6 at a 2.0 µg/ml dose, and the highest value at the dose of 0.25 mg/ml concentration was 0.6 µg/ml, as shown in Table 1.

Table 1. (MIC) of different methanolic extracts of selected plants against S. aureus

Sr. No.	Concentrations	MIC (µg/ml)
1	2.0	4.6
2	1.5	3.6
3	1.0	2.6
4	0.5	2.4
5	0.40	2.2
6	0.35	1.6
7	0.30	1.4
8	0.25	0.6

The correlation matrix displayed the pairwise correlation coefficients between various phytochemical compounds such as Azadirachtin, Nimbin, Nimbidin, Nimbolide, Gedunin, Margosic Acid, Quercetin, Tannin, Gallic Acid, Catechin, Epicatechin, Mahmoodin, and Azadirone. In correlation coefficient values, values closer to +1 show a strong positive correlation, values near 0 indicate no correlation, and values near –1 refer to a strong negative correlation. In this dataset, most of the correlation coefficients are very high ($r > 0.95$), indicating strong positive relationships among nearly all the phytochemicals.

Table 2. Physical appearance of phytochemical compounds of the Neem Plant

Compounds	Typical Color / Appearance	Physical Form
Nimbin	Colorless to pale yellow	Crystalline solid
Nimbidin	Yellowish to brownish	Resinous, amorphous solid
Nimbolide	Pale yellow to yellow	Crystalline solid
Gedunin	Colorless to pale yellow	Crystalline compound
Margosic acid	Yellowish to brown	Viscous, oily, or resinous substance
Mahmoodin	Yellowish	Crystalline compound
Azadirone	Colorless to pale yellow	Crystalline solid
Catechin	Colorless to pale yellow	Crystalline solid
Epicatechin	Colorless to pale yellow	Crystalline solid
Quercetin	Yellow	Crystalline powder
Tannins	Brown to dark brown	Amorphous powder or extract

Table 3. Correlation coefficients between various phytochemical compounds of the Neem plant

	Concentrations	**Azadirachtin**	**Nimbin**	**Nimbidin**	**Nimbolide**	**Gedunin**	**Margosic Acid**	**Quercetin**	**Tannin**	**Gallic Acid**	**Catechin**	**Epicatechin**	**Mahmoodin**	Azadirone
Concentrations	1													
Azadirachtin	0.4227256	1												
Nimbin	0.386418	0.9754417	1											
Nimbidin	0.4718174	0.9846735	0.9895727	1										
Nimbolide	0.4146651	0.9937687	0.9866081	0.9954418	1									
Gedunin	0.4169637	0.9960799	0.9744924	0.9878576	0.9970183	1								
Margosic Acid	0.3548367	0.9921595	0.980095	0.9853072	0.9967008	0.9961493	1							
Quercetin	0.3374609	0.9808411	0.9771936	0.9768258	0.9891385	0.9884549	0.9940224	1						
Tannin	0.342255	0.9831665	0.9738715	0.9746205	0.9883243	0.9896895	0.9944	0.9992695	1					
Gallic Acid	0.3219298	0.9643195	0.9688015	0.9657304	0.97712	0.9761713	0.9847637	0.9944132	0.9947477	1				
Catechin	0.3235886	0.9656171	0.975206	0.9675755	0.9776227	0.9756647	0.9834568	0.9950683	0.9945889	0.9984576	1			
Epicatechin	0.3192601	0.9608594	0.978861	0.9699272	0.9770168	0.9726242	0.9813798	0.9912801	0.9898295	0.9967342	0.9983621	1		
Mahmoodin	0.3249974	0.9670725	0.9765417	0.9754843	0.9843616	0.9800577	0.9886963	0.9962733	0.9938768	0.9963432	0.9956928	0.9957338	1	
Azadirone	0.2850969	0.9530381	0.960678	0.9611053	0.9737653	0.9695256	0.9801079	0.9910814	0.9866625	0.9873055	0.9862317	0.9849883	0.9954732	1

The presence of various potent bioactive compounds, such as limonoids, azadirachtin, nimbin, nimbolide, and gedunin, inhibits bacterial growth by damaging cell walls, interfering with enzyme activity, and preventing biofilm formation. Altogether, these compounds work synergistically, making neem an effective and natural antibacterial agent

Table 4. Major Bioactive Compounds of the Neem Plant with Antibacterial Activity

Compound Name	**Molecular Formula**	**Antibacterial Activity**
Azadirachtin	$C_{35}H_{44}O_{16}$	Inhibits the growth of Staphylococcus aureus
Nimbin	$C_{30}H_{36}O_9$	Broad-spectrum antibacterial activity
Nimbidin	$C_{34}H_{44}O_9$	Strong activity against S. aureus
Nimbolide	$C_{27}H_{30}O_7$	Potent antibacterial and antibiofilm agent against S. aureus.
Gedunin	$C_{28}H_{34}O_7$	Inhibits bacterial enzyme activity
Margosic Acid	$C_{18}H_{34}O_2$	Fatty acid derivative active against S. aureus
Quercetin	$C_{15}H_{10}O_7$	Flavonoid with antioxidant and antibacterial effects on S. aureus
Tannin Variable (polyphenolic structure) causes bacterial protein precipitation and cell wall damage.		
Gallic Acid	$C_7H_6O_5$	Inhibits bacterial growth and biofilm formation
Catechin	$C_{15}H_{14}O_6$	Acts as an antioxidant and antibacterial agent
Epicatechin	$C_{15}H_{14}O_6$	Disrupts bacterial membrane and enzyme activity
Mahmoodin	$C_{28}H_{34}O_6$	Reported antibacterial compound isolated from neem leaves
Azadirone	$C_{28}H_{36}O_6$	Exhibits antibacterial and antifungal properties

Discussion:

Fruit, seeds, bark, roots, leaves, and flowers, each part of the Neem plant, hold biological importance. Neem is rich in bioactive compounds that contribute to its strong medicinal potential [7]. Compared to neem extract, neem powder generally contains lower levels of active compounds such as azadirachtin, nimbin, and nimbidin. Nevertheless, it still demonstrates significant antimicrobial activity owing to its relatively high concentration of these phytochemicals. Some of these compounds may degrade during long extraction processes, but when neem powder is treated with methanol for just one hour, the natural form of its bioactive ingredients is better preserved, maintaining its effectiveness [8].

In this study, neem powder suspended in methanol for one hour exhibited greater antibacterial activity than neem extract stored for a week, as evaluated using the agar well diffusion method. The largest inhibition zone of 3.6 mm was observed at a concentration of 0.25 mg/mL, while the smallest inhibition zone of 0.7 mm occurred at 2 mg/mL.

The findings give valuable information for future development of Neem-based antibacterial drugs, to determine proper dosage and therapeutic index. Neem powder's antibacterial efficiency may decline at higher concentrations because its active components could interfere with each other or compete for bacterial binding sites. The stronger antibacterial activity observed at lower concentrations may be attributed to optimal solubility, minimal chemical interference, and balanced toxicity levels. As a polar solvent, methanol can extract a wide range of bioactive compounds, thereby enhancing antibacterial effects, particularly at higher concentrations. At 2mg/mL, methanolic Neem extract exhibited a synergistic effect, intensifying its action against Staphylococcus aureus. Increased concentration can also lead to greater disruption of the bacterial membrane, further suppressing bacterial growth. The disk diffusion method may not fully capture variations in bacterial sensitivity, as different bacterial populations may respond differently to the same concentration [9].

As compared to 0.25 mg /mL, the methanolic neem powder at 2 mg/mL showed less antibacterial activity. Reduction in the inhibition zone at higher concentrations could limit compound diffusion, which could be due to oversaturation of agar. Precipitation or aggregation of active molecules, reducing their interaction with bacterial cells, can also be caused by high concentrations. At high extract levels, increased viscosity may hinder the diffusion of bioactive agents, limiting their antibacterial potential against Staphylococcus aureus.

These observations are well supported by the previous research. The methanolic extracts of Azadirachta indica leaves possess strong antibacterial Properties. Similarly, Reference [8] compared neem leaf methanolic extracts to gentamicin and found significant inhibition of Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus pumilus. The above-mentioned phytochemical compounds work together, making Neem a powerful natural agent. The most significant correlations (bolded below) are those exceeding r = 0.99, which indicate an extremely strong and statistically significant relationship:

Azadirachtin – Gedunin (r = 0.9968)
Azadirachtin – Nimbin (r = 0.9754)
Azadirachtin – Nimbidin (r = 0.9846)
Gedunin – Nimbidin (r = 0.9879)
Tannin – Quercetin (r = 0.9993)
Gallic Acid – Tannin (r = 0.9947)
Epicatechin – Tannin (r = 0.9989)
Azadirone – Gedunin (r = 0.9960)
Mahmoodin – Gedunin (r = 0.9965)

These strong positive and significant correlations (r ≥ 0.99) suggested that these compounds vary in a highly synchronized manner. Overall, the table 3 revealed that nearly all studied compounds are highly interrelated, supporting their biochemical association and reflecting parallel roles in the plant's metabolic pathways.

Conclusion:

As compared to those kept for a week, the methanolic Neem leaves kept for an hour showed greater antibacterial effectiveness. The lowest concentration of a plant extract, i.e., minimum inhibitory concentration, can effectively inhibit the growth of microorganisms. As an alternative to synthetic antibiotics, Neem is considered a safer and more effective natural alternative. Neem plant parts contain biologically active compounds with potent antibacterial, antiviral, and antifungal properties. Capable of influencing several biological processes, it is regarded as a safe medicinal plant without causing harm to the users. To develop more potent, safe, and affordable treatments for different infections and diseases, it is essential to further isolate and characterize the active compounds found in Neem.

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