

Exploring the Properties and Extraction of Neem Leaves

Shrinkhala Rai*

Abstract

Neem (Azadirachta indica) is a versatile medicinal plant, for its diverse therapeutic properties. This abstract provides a comprehensive overview of recent research focusing on phytochemical analysis, biochemical characterization, and antimicrobial susceptibility testing of Neem extracts. Utilizing the different from of chemical used in it, phytochemical analysis identified a plethora of secondary metabolites within Neem, including alkaloids, flavonoids, terpenoids, steroids, saponin, tannins, quinone, and phenolic compounds, indicative of its complex chemical composition. These bioactive constituents contribute significantly to Neem's pharmacological effects, highlighting its potential as a valuable natural remedy in healthcare. Biochemical analysis further elucidated Neem's enzymatic activities, particularly its antioxidant potential. Various enzymes involved in antioxidant pathways were identified, emphasizing Neem's ability to combat oxidative stress-related disorders and promote overall health and well-being through its antioxidant properties. Antimicrobial susceptibility testing demonstrated the potent efficacy of Neem extracts against a broad spectrum of pathogens, including bacteria, fungi, and parasites. Neem's antimicrobial activity can be attributed to its diverse bioactive constituents, which exhibit inhibitory effects on microbial growth and proliferation. These findings underscore Neem's potential as a natural alternative for antimicrobial therapy, offering promising avenues for the treatment and prevention of infectious diseases. In conclusion, this abstract underscore the importance of Neem in modern pharmacology, highlighting its phytochemical diversity, biochemical attributes, and potent antimicrobial properties. The results emphasize the need for further research and exploration of Neem's therapeutic potential in healthcare and pharmaceutical industries, paving the way for the development of novel Neem-based therapeutics.

Keywords: Neem, Azadirachta indica, phytochemical analysis, biochemical characterization, antimicrobial susceptibility testing, medicinal plant, natural remedies, therapeutic potential, pharmacology.

INTRODUCTION

Azadirachta indica, the scientific name for neem, is a multipurpose tree that is indigenous to the Indian subcontinent. Because of its amazing qualities, it has been respected for generations. This tree is evergreen and can be found all over the world (Figures 1, 2).

According to the World Health Organization, "good-health" is a condition in which one's physical and mental well-being are unaffected by illness or disease [1]. This state was referred to in ancient Sanskrit as "Nimba" [2], which eventually became Neem. These days, the term "neem" refers to the Azadirachta indica (neem) tree, which was formerly believed to bestow "good health" upon those who consumed it [1, 3].

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Plant-based natural substances can be used to cure and prevent diseases by changing genetic pathways, boosting antioxidant activity, and halting the growth of bacteria. Several plants continue to be

the focus of extensive research in the field of disease control due to their low side effects and economical nature. It is commonly known that allopathic drugs are expensive and detrimental to normal tissues as well as other biological processes. It is commonly known that a large number of pharmacologically active drugs originate from natural sources, such as medicinal plants.

Azadirachta indica for almost 2000 years, the Indian subcontinent has been aware of a just, popularly known as neem, as one of the most adaptable medicinal herbs, with a diverse range of biological activity. The following is its taxonomic position:

Order–Rutales

Suborder-Rutales

Family-Meliaceae (Mahogany family)

Subfamily–Melioidae

Tribe–Meliceae

Genus-Azadirachta

Species-Indica

Azadirachta indica is the latinized name for neem, which literally translates to "the free tree of India" (Azadi = free, diracht = tree) because to its inherent immunity to pests and diseases. The neem tree is revered as a "divine tree," "heal all," "village dispensary," and "nature's drugstore" in addition to being known as a "sarvaroga nivarini," or the cure-all for all ailments.

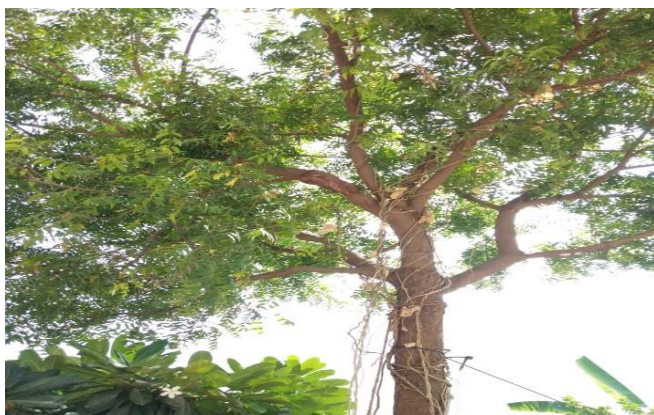


Figure 1. Neem Tree.



Figure 2. Leaves of Neem.

Biologically active compounds called limonoids, which include azadirachta indica and melia azadirachta, are found in the meliaceae family. Nimbin and Nimbidin are further bioactive compounds possessing anti-inflammatory, anti-bacterial, and anti-fungal characteristics.

The phytochemical analysis of the neem plant's crude leaf extract was conducted using the techniques and protocols [4, 5]. The current study set out to investigate the antibacterial activity of neem (*Azadirachta indica*) crude leaf extract and assess its potential as a source of bioactive chemicals for chemotherapeutic research.

Through extensive research, several of the phytochemicals found in neem plants have been extracted, measured, and identified. Most traditional medical literature advocate extract from *Azadirachta indica*, also known as dogonyaro in some parts of Nigeria. The leaves have been used as a medication for fever, eczema, and diabetes. Determining the phytochemical components of the neem plant and connecting them to some of its traditional uses was the aim of this study.

The majority of urban Bangladeshis, Indians, and Nepalese clean their teeth with neem twigs. The leaves' juice is used as a tonic to boost appetite and get rid of intestinal worms.

These extracts have the potential to cause a variety of cellular and molecular effects, such as detoxification, free radical scavenging, DNA repair, cell cycle alteration, immune surveillance, mitigation of programmed cell death and autophagy, anti-inflammatory, anti-angiogenic, and anti-metastatic activities, as well as the capacity to modulate different signaling pathways [1, 6].

MATERIALS AND METHOD

Collection of Samples

Fresh leaves of Neem Plant were collected on 4 October 2023, from India in Lucknow state from local area of spring garden. It was identified by prof. Kanchan Batt, a prof. of Goel institute of technology and management Lucknow.

Sample Preparation

After three weeks of air drying at room temperature, it was transferred to a motor pistol and ground into a crude extract. In order to prevent infection, it was thoroughly cleaned and dried. The leaf was ground to the necessary fineness.

Extraction

5g of the sample were weighed and put into a reagent bottle for the aqueous extraction process, which involved zeroing the weighing balance and setting the filter papers on top of it. To help with extraction and to soak, 50 milliliters of distilled water were added. Following a few minutes, the filtrate was properly filtered through filter paper and subjected to a series of tests using various reagents to ascertain the quantity and presence of tannins, alkaloids, saponins, terpenoids, quinone, flavonoids, steroids, and phenols.

The filter paper was placed on the weighing balance after the balance had been zeroed for the purpose of extracting ethanol. After weighing the sample (0.5 g) into a 250 ml beaker, 50 ml of 90% ethanol was added, and the mixture was covered. For optimal extraction, ethanol was extracted using a closed system.

Phytochemical Screening

Test for Alkaloids

The technique was used to check for alkaloids in neem plants. After adding 2 milliliters of the sample to 200 microliters of Wagner's reagent (a potassium iodide solution), the presence of an alkaloid was established by the production of red precipitate. The so-called Wagner's test verifies the presence of alkaloids by forming a red color.

Test of Tannins

The method was adopted in testing for tannins in neem plant. A quantity of 2 ml of plant extract added few drops of dilute ferric chloride solution, formation of black coloured precipitate confirmed indicating the presences of Tannins.

Test of Flavonoid

The method was adopted in testing for flavonoid in neem plant. A quantity of 2 ml of the plant extract, 200 μ l NaOH was added, formation of yellow coloured precipitate confirmed indicating the presences of Flavonoid.

Test of Quinone

The method was adopted in testing for quinone in neem plant. A quantity of 1 ml of the plant extract, 1 ml conc. H_2SO_4 was added, formation of red coloured precipitate confirmed indicating the presences of Quinone.

Test of Phenol

The procedure was used to check for phenol in neem plants. One milliliter of the plant extract and two hundred microliters of $FeCl_3$ were combined, and the production of a crimson precipitate was verified, signifying the existence of Quinone.

Test of Coumarin

The method was adopted in testing for coumarin in neem plant. A quantity of 1 ml of the plant extract, 1 ml NaOH was added, formation of yellowed coloured precipitate confirmed indicating the presences of Coumarin.

Test of Steroid

The method was adopted in testing for steroid in neem plant. A quantity of 1 ml of the plant extract, 1 ml $CHCl_3$, 200 microlitre H_2SO_4 was added, formation of brown coloured ring precipitate confirmed indicating the presences of Steroid.

Test of Saponins–foam Test

The technique was used to check the neem plant for saponins. After adding 1 ml of the plant extract and 2 ml of water to a test tube and giving the combination a good shake, the presence of saponins was confirmed by the production of foam.

Biochemical Screening***Test of Tannins***

The technique was used to check the neem plant for tannins. After adding 1 milliliter of the plant extract and 1 milliliter of ferric chloride, the creation of a brownish-black precipitate color was verified, confirming the presence of tannins.

Test of Flavonoids

The procedure was used to check for flavonoids in neem plants. One milliliter of the plant extract, a few drops of diluted sodium hydroxide, and the appearance of a yellow color when adding a few drops of diluted acid were proven to produce a colorless precipitate color, signifying the presence of flavonoids.

Test of Saponins

The technique was used to check the neem plant for saponins. After adding 1 milliliter of the plant extract and 20 milliliters of water to a test tube and shaking the mixture vigorously, a colorless foam was confirmed to form, signifying the presence of saponins.

Test of Alkaloid

The technique was used to check for alkaloids in neem plants. After adding 1 milliliter of the plant extract and Mayer's reagent, the presence of alkaloids was confirmed by the production of a yellow cream precipitate color.

Test of Terpenoid

The technique was used to test the neem plant for terpenoid. After adding 1 milliliter of the plant extract and 1 milliliter of H₂SO₄, the creation of a reddish brown precipitate color was verified, signifying the existence of terpenoid.

Test of Glycosides

The procedure was used to test the neem plant for glycosides. Once one milliliter of the plant extract, glacial acetic acid, and FeCl₃ were added, the presence of glycosides was confirmed by the production of a violet precipitate color.

Test of Phenols

The technique was used to check for phenols in neem plants. After adding 1 milliliter of the plant extract and 1% FeCl₃, the production of a violet/red brown precipitate color was verified, confirming the presence of phenols.

Antimicrobial Susceptibility Test

The disc diffusion method was used to test the antibiotic susceptibility profiles of bacteria against 14 commercially available antibiotics, including vancomycin, ciprofloxacin, ampicillin, gentamycin, azithromycin, sulphatrimethoprim, cephalixin, doxycycline, streptomycin, oxyacillin, erythromycin, telcycline, chloramphenicol, and nalidixic acid (Hi Media, India). Results of antimicrobial testing were classified as susceptible, intermediate, and resistant based on CLSI (2010)'s zone diameter interpretative standards [7].

RESULT AND DISCUSSION

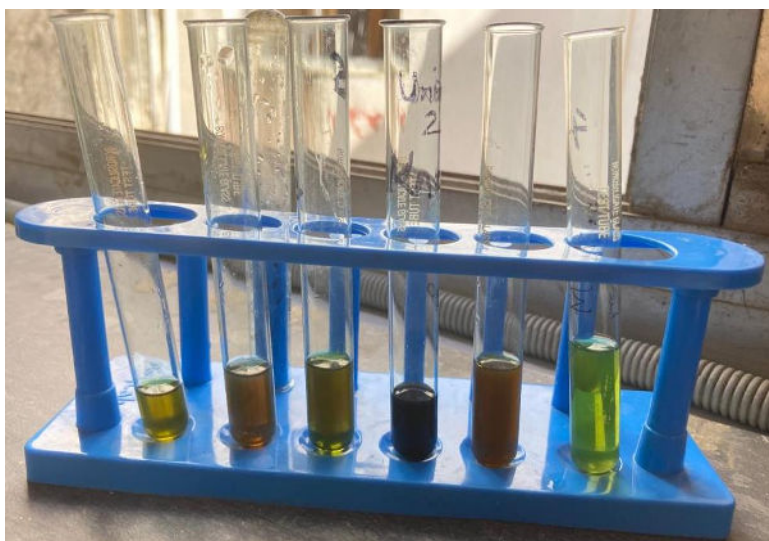
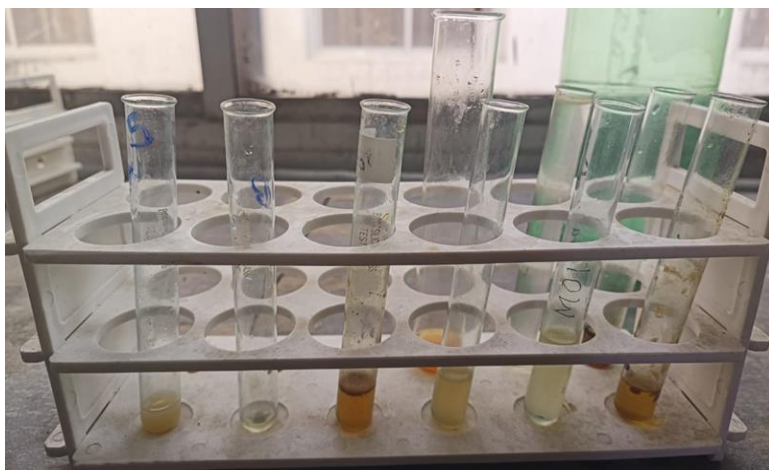
The findings of the neem plant's qualitative investigation (*Azadirachta indica*). Recent years have seen a significant increase in the amount of research on phytochemistry and biochemistry of plants, with a focus on their leaves, bark, roots, seeds, and stems [8]. The pharmacological and pharmaceutical industries make substantial use of these phytochemicals and biochemicals, which have been shown to have numerous biological and therapeutic effects. Because *Azadirachta indica* may address a wide range of human difficulties, it has gained importance as a herb. (Tables 1, 2) demonstrate that the most abundant chemical compounds in the aqueous extract of *A. indica* are saponins, steroids, and terpenoid compounds; in contrast, tannins and glycosides, alkaloids, flavonoids, phenol, and other compounds are perceived, and color confirms the presence of these compounds in neem (Figure 3).

Table 1. Phtochemical tests.

Bioactive compound	Result
Tannins	Black/present
Flavanoid	Yellow/present
Quinone	Red/present
Phenol	Blue green/present
Coumarin	Yellow/present
Steroid	Brown ring/present
Terpenoid	Red brown/present
Saponin	Colourless/present
Alkaloid	Red/not present

Table 2. Biochemical tests.

Bioactive compound	Result
Tannins	Brownish black/present
Flavonoids	Colourless/present
Saponins	Foam layer/colourless/present
Alkaloids	Yellow cream/present
Terpernoid	Reddish brown/present
Glycosides	Violet colour/not present
Phenols	Violet/not present

**Figure 3.** Results of Phytochemical tests.**Figure 4.** Result of biochemical tests.

Phytochemical Test

Bioactive compounds in neem leaves play various roles in our body. Anti-inflammatory compounds in neem leaves may help reduce inflammation and acne, while antifungal compounds may help treat fungal infections. Antioxidants in neem leaves may help protect against cell damage and skin aging. Neem leaf also has anti-lipidemic properties, which may help reduce high cholesterol levels and improve cardiovascular health. Additionally, neem leaf is anti-cancer properties due to its flavonoid content. Overall, bioactive compounds in neem leaves can have various health benefits, both topical and internal (Figure 4).

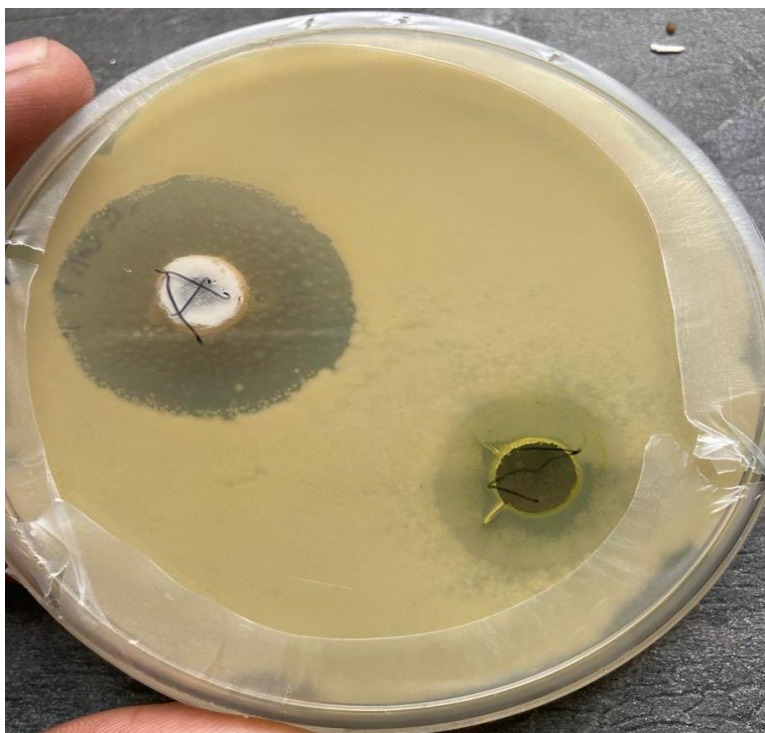


Figure 5. Result of antimicrobial susceptibility test.

Biochemical Testing

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test on neem leaves is a way of determining the activity of neem extracts against different microorganisms. The test involves placing microorganisms on a medium containing a known concentration of neem extract, then reading the results and assessing the effectiveness of the neem extract against the microorganisms. This test can be used to determine which pathogens neem leaf extract is most effective against, and at what concentrations. Additionally, it can also help in understanding the antifungal properties of neem (Figure 5).

To find out how well an antimicrobial agent works against a certain bacterium or fungus, antimicrobial susceptibility testing is essential. Using a laboratory technique called biochemical screening, bacteria are identified based on their capacity to use particular nutrients. This information is then utilized to diagnose diseases and choose the best course of therapy. The technique of discovering bioactive molecules in plants—which have been proven to have a variety of therapeutic qualities, including antibacterial activity—is known as phytochemical screening.

CONCLUSION

Antimicrobial susceptibility testing, biochemical screening, and phytochemical screening are all methods used to assess the chemical makeup and potential antimicrobial activity of various compounds [9]. Antimicrobial susceptibility testing determines the ability of a substance to inhibit the growth of bacteria, while biochemical screening and phytochemical screening focus on identifying specific chemical groups within a substance that may be responsible for its antimicrobial effects. The main conclusions of these tests are the identification of the specific compounds present, their potential to inhibit microbial growth, and their potential for use as antimicrobial agents [10, 11].

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