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Isolation and Characterization of Bioactive Compounds from Plant Source (Neem)

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Abstract: *Azadirachta indica, also referred to as Neem, is a medicinal plant that has been used extensively in traditional Indian medicine because of its powerful therapeutic nature. Isolation and characterization of the active chemical compounds in Neem leaves responsible for its medicinal properties is the center of this project. Neem leaves were in this research collected, dried, and ground. The powdered leaves were extracted using appropriate solvents such as ethanol or methanol by using the Soxhlet extraction method. The crude extract was then treated with different purification methods such as column chromatography and thin layer chromatography (TLC) to purify individual bioactive compounds.*

The pure compounds were further identified with conventional chemical tests and instrumental methods such as UV-Visible spectroscopy, FTIR (Fourier Transform Infrared Spectroscopy), and may be NMR (Nuclear Magnetic Resonance), to determine their structure and composition. The research established the existence of significant phytochemicals such as flavonoids, terpenoids, alkaloids, and particularly azadirachtin, which is effective as an antibacterial, antifungal, antiviral, anti-inflammatory, as well as an insect-repellent compound. The project indicates that Neem is a good source of bioactive compounds and can be effectively applied in the formulation of natural drugs as well as in herbal preparations. The results validate the traditional applications of Neem and suggest further study of its potential in contemporary medicine.

Keywords: Neem

I. INTRODUCTION

Medicinal plants have been used for centuries by humans to cure many different ailments. Azadirachta indica, or Neem, is one such plant. It is a quickly growing tree primarily native to India and surrounding nations. Neem is also referred to as the "Village Pharmacy" because each part of this tree—leaves, bark, seeds, flowers, and oil—possesses some sort of healing quality. Neem leaves, for instance, are full of chemical compounds known as bioactive compounds. These reduce bacteria, fungi, and viruses. They also possess anti-inflammatory, antioxidant, and insect-repelling activities. Due to these virtues, Neem is used extensively in Ayurveda, Unani, and conventional herbal medicine. Today, interest is developing in deriving new drugs from natural sources. Research into medicinal plants such as Neem can lead us to new drugs that are effective and safer. Through this project, we aim to isolate and identify the bioactive compounds found in Neem leaves. We want to know what compounds are behind its curative properties, and how they can be exploited for new medicine or pharmaceutical purposes

II. BOTANICAL DESCRIPTION

Azadirachta indica, commonly known as Neem, is a fast-growing, evergreen tree. It belongs to the plant family Meliaceae.

- Scientific Name: Azadirachta indica
- Common Name: Neem
- Family: Meliaceae

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Fig.1.1: NEEM TREE

Neem is a tree with a medium to large size, which may reach up to 15–20 meters in height. It has a straight trunk, which is normally dark brown or gray with rough

Fig.1.2: NEEM LEAVES

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Neem leaves are compound leaves, that is, each leaf consists of numerous small leaflets. Leaves are green, pointed, and alternate. They are slightly bitter to taste and the most commonly used part in traditional medicine.

Fig.1.3: NEEM FLOWER

Neem flowers are small, white, and fragrant. They grow in bunches and usually bloom during the spring season.

Fig.1.4: NEEM FRUITS

The fruit of the Neem tree is a small, greenish-yellow drupe (a kind of fruit with a single seed inside). When ripe, it becomes sweet and is sometimes eaten by birds.

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Fig.1.5: NEEM SEEDS

Neem seeds are found inside the fruit and are used to extract Neem oil, which is used in cosmetics, medicines, and agriculture.

III. OBJECTIVE AND REVIEW

The primary objective of this project is to isolate and identify the bioactive compounds found in the leaves of Azadirachta indica (Neem) that are accountable for its renowned medicinal traits. The aim of this study is to scientifically validate the traditional application of Neem in herbal medicine and provide potentialities for its application in modern drugs.

Specific Objectives:

1. To harvest and identify healthy Neem leaves from a natural source for use in experiments.
2. To dry, powder, and clean the Neem leaves for preparation to extract them.
3. To carry out solvent extraction employing suitable solvents like ethanol or methanol to yield the crude extract rich with bioactive compounds.
4. To concentrate the extract employing evaporation techniques to drive off the solvent and harvest the active ingredients.
5. To isolate and purify individual compounds from the crude extract employing methods such as:

I. Thin Layer Chromatography (TLC)

II Column Chromatography

6. To clean the isolated compounds to analyze further.

IV. LITERATURE REVIEW

• Azadirachta indica (Neem) is extensively researched due to its potent medicinal properties. The different parts of Neem tree, particularly leaves, contain significant bioactive metabolites such as azadirachtin, nimbin, nimbidin, and quercetin.

• Neem has been found to possess antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant activities in many studies.

• Scientists such as Chatterjee and Pakrashi discovered that Neem leaves contain terpenoids and flavonoids, which are useful against infections and inflammation.

• Kausik et al. in one review claimed that Neem extracts can be utilized to cure skin diseases, fever, and even diabetes.

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• Subapriya and Nagini documented that Neem possesses anticancer activity as well as antioxidant activity and acts to shield the body from hazardous free radicals.
• Biswas et al. talked about the various applications of Neem in medicine, cosmetics, and agriculture.
• The majority of these studies also emphasize the need to extract and isolate the active ingredients from Neem inorder to discover how they function.These results give us a strong foundation for our project, which concerns the isolation and identification of the chemical compounds found in Neem leaves.
• This review validates our research and demonstrates that additional research in Neem can contribute to the making of natural drugs.

V. MATERIALS AND METHODS

Chemicals Used:
o Methanol, ethanol, chloroform, hexane, ethyl acetate, distilled water
o Reagents for phytochemical screening (Mayer's, Dragendorff's, $FeCl_3$, etc.)
o Silica gel (60–120 mesh) for column chromatography
o Iodine crystals for TLC visualization
 Instruments Used:
o Soxhlet apparatus
o Rotary evaporator
o UV-Visible Spectrophotometer
o IR Spectrophotometer
o Melting point apparatus
o TLC chamber and UV viewing cabinet

PREPARATION OF PLANT EXTRACT
1. The collected neem leaves were washed, shade dried for 7–10 days, and powdered using a mechanical grinder.
2. Soxhlet Extraction was carried out using methanol (or ethanol) for 6 –8 hours.
3. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C for further studies.

• Preliminary Phytochemical Screening
The crude methanolic extract was subjected to standard qualitative tests for the identification of various phytoconstituents:

Phytoconstituent	Test Used
Alkaloids	Mayer's and Dragendorff's tests
Flavonoids	Shinoda test
Tannins	Ferric chloride test
Saponins	Foam test
Glycosides	Keller-Killiani test
Terpenoids	Salkowski test
Steroids	Liebermann–Burchard test

Isolation of Compounds
Method Used: Column Chromatography
1. The concentrated extract was adsorbed onto silica gel and loaded into a chromatography column.
2. Elution was performed using solvent gradients of increasing polarity (e.g., hexane to ethyl acetate).
3. Collected fractions were analyzed using TLC and similar ones were pooled for further study.

Thin Layer Chromatography (TLC)
• TLC was conducted on pre-coated silica gel plates.

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• Solvent system used: Hexane:Ethyl acetate (7:3 or 6:4)
• Developed spots were observed under UV light (254 & 365 nm) and iodine vapor chamber.
• Rf values were calculated for each fraction.

Characterization of Isolated Compounds

1. UV-Visible Spectroscopy: Used to determine absorption maxima (λmax) of the isolated compound.
2. Infrared (IR) Spectroscopy: Used to identify characteristic functional groups.

VI. ISOLATION AND PURIFICATION OF COMPOUNDS

Following the isolation of crude plant material from Neem leaves, the subsequent step was the isolation and purification of the individual bioactive compounds. This operation serves to isolate each compound found in the extract and yield it in a pure form for subsequent analysis.

1. Thin Layer Chromatography (TLC)

Purpose: To verify the number of various compounds found in the extract.
A tiny drop of crude extract was placed upon a TLC plate that was covered with silica gel.
The plate was inserted into a solvent system (hexane:ethyl acetate).
The solvent migrates up the plate, transporting various compounds at varying rates.
After it dried, the plate was checked under UV light.
Various spots were observed on the plate, and each represented a different compound.
The Rf values (ratio of the distance traveled by compound to the solvent front) were measured.
TLC was used to identify an appropriate solvent system for further purification.

2. Column Chromatography

Column chromatography was applied to separate compounds based on the results of TLC.
Silica gel (stationary phase) was packed in a glass column.
The crude extract was placed at the top.
Increasingly polar solvents (hexane → ethyl acetate → methanol) were passed through the column.
Various fractions were collected separately in test tubes or flasks.
Each fraction was supposed to have different compounds depending on their solubility and polarity.

3. TLC Monitoring of Fractions

All fractions collected were subjected to TLC again.
Fractions with the same TLC pattern (same Rf) were mixed together.
Fractions exhibiting single, distinct spots were assumed to consist of pure compounds.

4. Re-Purification (if necessary)

Any fraction with more than a single spot on TLC was re-purified using:
Repeated column chromatography
Modifying the solvent system
This guaranteed that the purified compounds were as pure as possible.

5. Drying and Storage

Pure compounds were dried (using a water bath or air).
Dried samples were placed in labeled vials.
They were stored safely for further analysis (UV, FTIR, etc.).

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VII. CHARACTERIZATION OF BIOACTIVE COMPOUNDS

Once the pure compounds were isolated from the Neem leaf extract, the subsequent significant step was the characterization of the compounds. This refers to the identification of the chemical composition and functional groups in the compounds through analytical processes.

The characterization was done by the following methods:

1. UV-Visible Spectroscopy

Objective: To find the absorption properties of the isolated compound within the UV-Visible range.

The pure compound was dissolved in an appropriate solvent (such as methanol).

The solution was analyzed in a UV-Visible spectrophotometer between 200–800 nm.

The λmax were noted.

The λmax values assisted to suggest the presence of chromophores like:

Flavonoids (tend to exhibit peaks at 270–290 nm and 330–370 nm)

Terpenoids or other aromatic compounds (can exhibit absorptions in the UV region)

2. Fourier Transform Infrared Spectroscopy (FTIR) (If Available)

Purpose: To identify the functional groups in the isolated compounds.

A minimal amount of the dry sample was examined with an FTIR spectrometer.

The FTIR spectrum gave details regarding bond vibrations in various regions:

O–H (alcohols/phenols) → Broad band around 3200–3600 cm^{-1}

C=O (carbonyl group) → Sharp band near 1700 cm^{-1}

C–H (alkanes) → Bands between 2800–3000 cm^{-1}

C=C (alkenes/aromatic rings) → Around 1600 cm^{-1}

On the basis of peaks, provisional functional groups were assigned.

3. TLC Profile as a Supporting Tool

TLC outcomes confirmed the purity and identity of compounds.

Single, sharp spot on TLC confirmed a pure compound.

The Rf values also aided in matching literature values for known compounds.

VIII. RESULTS

After effectively extracting and purifying the bioactive compounds from Neem leaves, a number of significant results were achieved through the experiment. These results are explained as follows:

1. Preliminary Phytochemical Screening Results

Neem leaf crude extract was subjected to testing for different phytochemicals. The findings indicated the presence of the following compounds:

Phytochemical Test Result

Alkaloids	**Present**
Flavonoids	**Present**
Tannins	**Present**
Saponins	**Present**
Terpenoids	**Present**
Glycosides	**Present**
Phenolic Compounds	**Present**

These findings suggest that Neem leaves possess a large number of medicinally significant compounds.

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2. TLC Observation Results
Multiple spots with varying Rf values were observed in the TLC of the crude extract.
This confirmed the existence of various individual compounds in the extract.
TLC also assisted in selecting an appropriate solvent system for further purification.

3. Column Chromatography Results
Various fractions were collected after the extract was passed through a column packed with silica.
TLC was once more utilized to check for the purity of every fraction.
Some fractions needed re-purification in order to eliminate impurities.

4. Characterization Results
a) UV-Visible Spectroscopy:
The purified compound exhibited absorption peaks at characteristic wavelengths (λmax), which suggest the presence of chromophores such as flavonoids or phenols.

b) FTIR Spectroscopy (if available):
The FTIR spectrum exhibited peaks for functional groups such as:
– OH (hydroxyl group)
C=O (carbonyl group)
C=C (aromatic ring)
These findings are indicative of the existence of phenolic compounds, flavonoids, and terpenoids

IX. CONCLUSION

The current research was able to effectively isolate and characterize bio-ctive compounds from Azadirachta indica (Neem) leaves.
The findings indicated the abundance of significant phytochemicals suchas flavonoids, alkaloids, terpenoids, tannins, and glycosides, which are famous for their therapeutic activity.
Individual compounds were successfully isolated through Soxhlet extraction coupled with TLC and Column Chromatography. The purified compounds were then identified by UV-Visible and FTIR spectroscopy, which identified the presence of functional groups characteristic of bioactive molecules.
The current study validates the age-old medicinal use of Neem and showcases its potential for herbal drug or plant formulation development. Additional advanced studies such as NMR or Mass Spectrometry may be performed to identify the full structure and pharmacological activity of the isolated compounds.

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