

TAXONOMICAL, MORPHOLOGICAL, PHARMACOLOGICAL, AND CHROMATOGRAPHIC ASPECTS OF AZADIRACHTA INDICA (NEEM) LEAVES EXTRACT

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Article Received on
25 Nov. 2023,

Revised on 16 Dec. 2023,
Accepted on 06 Jan. 2024

DOI: 10.20959/wjpr20232-30973



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ABSTRACT

To research neem and assess the impact of neem leaf extract in the forms of aqueous, ethanolic, and methanol. The side effects of drugs derived from plants are extremely minimal. Several neem parts have yielded the isolation of over 140 compounds. There have been descriptions of the entire neem tree, including the bark, leaves, flowers, seeds, and fruits; in particular, inflammation, infection, fever, skin conditions, and dental issues. The therapeutic benefits of neem leaf in particular have been documented. Based on currently available published data, this review represents the pharmacology, pharmacognosy, and various molecular aspects of *Azadirachta indica* that may be useful shortly. This review provides a framework or opens doors for several studies that will become widely known shortly.

KEYWORDS: Pharmacology, Pharmacognosy, *Azadirachta indica*, Pharmacological, Chromatographic.

INTRODUCTION

Azadirachta indica A. Juss, commonly referred to as “Neem”, is revered as a holy tree and has been used in traditional medicine.^[1,2]

Ayurveda and other medicinal systems like Unani, Chinese, and Europe have announced the neem tree as the “Panacea of all disease”.

For more than 2000 years, *Azadirachta indica* A. Juss has one the plant used in India and other neighboring countries. Neem has a wide range of biological activity and is classified as the most versatile plant.^[3]

The mahogany tree i.e. *Azadirachta indica*, Indian lilac; belongs to the Meliaceae family. Neem belongs to the genus *Azadirachta* and neem is indeed native to the subcontinent of India.^[4]

De Jussieu introduced the neem tree as *A. indica* in 1830. The taxonomic classification of neem is as follows: Suborder: Rutinae; Order: Rurales; Genus: *Azadirachta*; Family: Meliaceae; Subfamily: Melioideae, India is the species. Three species of *Azadirachta* A. Juss belong to the genus well- -characterized, by an Indo-Malayan origin.^[13]

The original home of *Azadirachta indica* in South India and Myanmar. Finding out that it's grown on Iran's southern coast is fascinating. Neem is utilized as a natural pesticide, insecticide, and agent of agro chemistry.^[20]

It is found in at least 78 countries worldwide, including Asia, Africa, Australia, America & Europe and it is native tree to India, Indonesia, Malaysia, Myanmar, Pakistan, Nepal, Thailand, etc.^[1] Neem trees have been found in many exotic countries, including Algeria, Angola, Barbuda, and Sudan.^[5]

Every part of the neem tree, including the seeds, flowers, twigs, leaves, roots, and barks, has its medicinal use for humans.

The neem tree has the potential to reach a height of about 20 meters and it remains green & vibrant throughout the year. The neem tree leaves are arranged alternately on the branches, and each leaflet can have around 8-19 leaves and they grow in the months of March to April. The leaves do have a distinct bitter flavour.^[6]

Like the leaves of most tropical trees, neem leaves contain bioactive chemicals that could influence how well nutrients are utilized.^[18,19]

In the African subcontinent, *Azadirachta indica* is a highly beneficial traditional medicinal herb. Every component of the tree possesses some therapeutic qualities that can be applied to cure a variety of illnesses.^[37,38]

Because of their hepatoprotective, immunomodulatory, antihyperglycemic, anti-ulcer, anti-inflammatory, anti-bacterial, anti-viral, anti-oxidant, anti-fungal, carcinogenic qualities and anti-mutagenic properties, neem tree leaves have long been used in medicinal formulations.^[7,8,9,10]

It also possesses antimalarial and pregnancy-interrupting qualities.^[21]

The leaves are used to make infusions and teas that are used to treat intestinal issues, headaches, malaria attacks, heartburn, and intestinal complaints. They are also used as an insect repellent. It was also used as a laxative, and diuretic, for diabetes, as well as numerous skin diseases.^[40]

In India, people frequently use neem tree twigs to brush their teeth. In addition, neem tree branches are among the most effective dental treatments in conventional medicine, even though users find them to be a little uncomfortable.^[39]

The neem tree's seed has the most oil content of any of its parts. About 45% of it is made up of oil, which also includes stearic, palmitic, linoleic, and arachidic acids.^[11]

The neem tree is resistant to drought and grows well in regions with subtropical temperatures, with an annual rainfall of 400 in the arid Southern districts and coastal regions.^[12]

Proteins, carbohydrates, sulfurous compounds, polyphenolics, dihydrochalcone, coumarin, tannins, zadirachtin, nimbin, nimbidine, diterpenoids, and triterpenoids are among the majority of chemical compounds that are found in a selected plant.^[14,15] Azadirachtin, nimbin, and nimbidine are the most common active ingredients found frequently in neem among these.^[14]

Neem leaves are used to treat chicken pox, and the oil extracted from them is used in cosmetics, medicines, and pest control, among other applications. Neem is used as a pesticide and has anticoccidial effects on broiler chickens.^[16,17]

It has been noted that ethanolic neem leaf extract (NLE) increases the expression of proapoptotic genes, such as caspase-3 and caspase-8, and inhibits the expression of Bcl-2 and mutant p53 in cancer cells induced by 7,12-dimethylbenz(a)anthracene (DMBA).^[22,23]



PLANT BOTANY

Common Names- Indian lilac, nimtree, margosa, neem.

Vernacular Names

- Danujhada, Limbado, Limbra, Limdo in Gujarati,
- Nim and Nimgachh in Bengali,
- Nim and Nimb in Hindi,
- Picumarda, Arista, Nimba, Nimbah in Sanskrit,
- Margosa tree, Neem tree, Indian lily in English,
- Kaybevu, Bivu, Bemu, Bevinamara in Kannada,
- Bakam, Drekh, and Nim in Punjabi.

TAXONOMY OF AZADIRACHTA INDICA^[24]

- Plantae is the **division** of the vascular plant **phylum** Magnoliophyta.
- **Class:** Ratales; **Order:** Dipsacales
- **Subclass:** Rutinae
- The Meliaceae **family**
- **Subfamily:** Melioideae
- Azadirachta is **genus**

- **Species:** Indica.

MORPHOLOGY OF NEEM LEAVES

- When the leaves are fully grown, they are 20-40 cm long, have 10-20 leaflets, are alternate, petiolated, clustered at the tips of the branches, unevenly pinnate, glabrous, and dark glossy green.^[36]
- The leaves are sickle-shaped, slightly denticulate, and measure 5-10 cm in length and 1.2-4 cm in width.^[36]
- Base - Inequitable
- Colour – A deep, glossy green
- Odour – Generally
- Taste- Bitter.

EXTRACTION OF NEEM LEAVES

Definition of Extraction- To extract medicinal plants, the active ingredients such as steroids, glycosides, alkaloids, flavonoids, terpenes, and saponins – must be separated from the inert or inactive portions of the plant, applying a suitable solvent and standard extraction methods.^[25,26,27,28]

Menstruum used in the extraction of Neem leaves

- **Water** – Water is the most polar solvent that is used to extract many polar compounds.^[34,35]

Advantages: Benefits include its broad range of solubility, low cost, nontoxicity, inflammability, and high polarity.^[34,35]

Disadvantage: It encourages the growth of mold and bacteria, can result in hydrolysis, and requires excessive heat to concentrate the extract.^[34,35]

- **Alcohol-** It is polar by nature and can extract polar secondary metabolites. It is mixed with water as well.^[34,35]

Advantages: At concentrations above 20%, it acts as a self-preservative. Low concentrations are nontoxic, and only a little heat is needed to concentrate the extract.^[34,35]

Disadvantages: It is flammable and volatile; and insoluble in wax, gums, or fats.^[34,35]

MACERATION METHOD OF EXTRACTION

- In this extraction process, coarsely ground medicinal material- leaf, stem, or root bark- is used.^[26,28,31,32,33]
- A container is filled with leaves.^[26,28,31,32,33]
- The menstruum is poured over the drug material until it is fully covered.^[26,28,31,32,33]
- Following that, the container is sealed and kept in place for three days or longer.^[26,28,31,32,33]
- To guarantee full extraction, the contents are periodically swirled and, if placed inside a bottle, shaken.^[26,28,31,32,33]
- Filtration is used to separate the micelle from the marc after extraction.^[26,28,31,32,33]
- The micelle is then removed from the menstruum by evaporating it over a water bath or in an oven.^[26,28,31,32,33]

This is a very practical method that works well with thermolabeled plant material.



HOME REMEDIES OF NEEM LEAVES

A home remedy is defined as an essential pre-planned medication or tonic taken regularly with questionable viability and no expert supervision or solution.

- **Neem oil:** Neem leaves have a very high calcium and other mineral content. Neem oil is advantageous for arthritis sufferers because it lessens inflammation and eases pain.^[41]
- **Natural insecticide:** Neem leaves have a strong insect and mosquito-repellent effect. Neem leaves can be burned to keep mosquitoes out of your house and surrounding areas. The majority of people use this trick to remove various insects from the cattle.^[42]

- **Dental health:** Chewing neem leaves helps keep your teeth in good condition. It reduces tooth decay, eliminates harmful bacteria in your mouth, helps you breathe more easily, keeps infections away from your gums, and preserves your saliva's pH level.^[42]
- **Boost immunity:** Neem tea helps the body fight off illnesses like fever and colds. When it comes to treating the body's malaria-affected cells, neem tea can be just as beneficial as quinine.^[43]
- **Hair mask:** Neem leaves your hair feeling incredibly healthy and strong. Additionally, it can be used to treat fungal infections on your scalp and dandruff.^[44]

PHARMACOLOGICAL ACTIVITY OF NEEM LEAVES

Antioxidant Activity: By regulating a variety of biological processes, the leaves, bark, roots, seeds, and flowers of neem plants can help manage disease. According to the findings, extracts from leaves, flowers, and stem bark have strong antioxidant properties.^[46]

Free radicals are stabilized and rendered inactive by antioxidants, frequently before they assault biological cell targets.^[47]

It has been noted that of the neem leaf extracts in chloroform and methanol, the latter has substantially more antioxidant qualities.^[47]

Aqueous neem leaf extract was found to reduce the frequency of rat bone marrow micronuclei and chromosomal aberrations induced by MNNG.^[48] Neem leaves ethanolic extract pretreatment drastically reduced the 7,12-dimethyl-benz(a)-anthracene (DMBA) genotoxic effects and the carcinogenic MNNG in mice and correspondingly, in hamsters.^[49]

Antihypertensive and Antihyperglycaemic Activity: It has been observed that an alcoholic neem leaf extract significantly and dose-dependently lowers blood pressure.^[50]

It has been discovered that aqueous neem leaf extract lowers blood sugar and guards against hyperglycemia brought on by glucose and adrenaline.^[51]

Neem leaves may help with irregular heartbeats, lessen clotting and circulatory strain, and possibly even lower bad cholesterol levels.^[53,54]

Wound-healing effect: The process of restoring damaged tissue, organs, and biological systems to normal function is known as the wound-healing effect. A series of events will begin after an injury to help the skin heal and return to its normal structure.^[55,56,57,58]

Azadirachta indica leaf extracts stimulate wound healing activity by increasing neovascularization and inflammatory response.^[52]

The active ingredients in neem, including nimbidin, nimbin, and nimbidol, help to speed up the healing process.

Hepatoprotective Activity: The elevated levels of AST, ALT, and gamma-GT were significantly reduced by leaf extract. Additionally, it emerged that paracetamol-induced liver necrosis was lessened, as indicated by macroscopic and histological observations.^[59]

Antitubercular medications' hepatotoxic effects are prevented and even reversed by leaf extract.^[60]

The administration of NMLE shielded the liver from the hepato- and nephrotoxic effects of cisplatin.^[62]

Azadirachta-A pretreatment decreased hepatocellular necrosis.^[61]

Neuroprotective Activity: It is used to treat peripheral neuropathy caused by partial sciatic nerve ligation, hyperalgesia, and allodynia.^[63]

Because of its direct, significant CNS action, neem leaf extract is effective in treating depression.^[64]

It is also employed to lessen the death of neurons.

Nephroprotective Activity: The methanolic leaf extract of neem has demonstrated noteworthy protective effects against nephrotoxicity induced by cisplatin, as this extract displays anti-inflammatory, antioxidant, and other free activities that scavenge radicals.^[65]

Neem leaf ethanol extract provides nephroprotection in cases of kidney damage.^[66]

Immunomodulatory effect: When applied at a concentration of 50 ml/l of fresh drinking water, neem infusion has been found to effectively enhance the antibody titer growth performance.^[67]

It strengthens your immune system by removing pollutants and toxins from your blood.

It is an all-natural immune stimulant and growth booster that raises body weight, death rates, and antibody titers against infectious bursal illnesses.

Cardio-protective effect: Neem leaf extracts have a cardio-protective effect: they lower blood pressure, may lessen clotting and the strain on the circulatory system, may ease irregular heartbeats, and may even lower harmful cholesterol levels.^[68,69] Thus, neem leaves' ability to reduce bad cholesterol is highly advantageous for heart health.

In diabetics, neem leaf extract dilates blood vessels.^[70]

Antimicrobial Activity: Due to their antimicrobial (antibacterial, antifungal, and antiviral) qualities, neem leaves, which are abundant in these compounds, have long been used in India.^[72]

Neem extracts may be helpful in the management of other spoiling organisms and foodborne pathogens.^[71]

Neem leaf extracts in both methanol and ethanol demonstrated antimicrobial properties against human pathogenic bacteria, including *Bacillus pumillus*, *Staphylococcus aureus*, and *Pseudomonas areuginosa*.^[73,74]

Antitumour: Neem leaves exhibit antitumor properties. It plays a crucial part in controlling cell signaling pathways to inhibit the growth of tumors.^[75,76,77,78]

A recent study found that azadiramide-A, an alkaloid-derived limonoid that primarily occurs in ethanolic extracts of Neem leaves, can inhibit cell growth.^[79]

Table: Pharmacological Activity of Neem leaves.

Activities	Findings of the Study	References
Antioxidant	It often prevents free radicals from attacking targets in biological cells.	[45]
Wound healing	Azadirachta indica leaf extract stimulates wound healing activity by increasing neovascularization and inflammatory response.	[52]
Antihypertensive	Neem leaf extract in alcoholic form causes a notable and dose-related drop in blood pressure.	[50]
Antihyperglycaemic	It has been discovered that aqueous neem leaf extract lowers blood glucose levels and guards against hyperglycemia brought on by glucose and adrenaline.	[51]
Hepatoprotective	Antitubercular medication-induced hepatotoxic damage is prevented and reversed by leaf extract.	[60]
Neuroprotective	Neem leaf extract has a direct, significant CNS action, it can be used to treat depressive activity.	[64]
Nephroprotective	Neem leaf ethanol extract functioned as a nephroprotection in cases of kidney damage.	[66]
Immunomodulatory	It aids in the removal of pollutants and poisons from the blood, boosting your immunity.	
Cardio-protective	In diabetics, neem leaf extract widens blood vessels.	[70]
Antimicrobial	Neem extracts may be helpful in the management of other spoiling organisms and foodborne pathogens.	[71]
Antitumour	Neem leaf modulates cell signaling pathways, which play a crucial role in inhibiting tumor growth.	[75-78]

CHROMATOGRAPHIC EVALUATION

Chromatography: One technique used in labs to break down a mixture into its parts is chromatography. The mixture passes through a system on which fluid solvent known as the mobile phase dissolves it. The stationary phase material is fixed.^[80]

The various forms of chromatography are as follows:

- Paper chromatography,
- Thin layer chromatography(TLC),
- Gel electrophoresis,
- Chromatography in columns,
- Chromatography with ion exchange,
- Chromatography with gel filtration,
- Gas-liquid chromatography &
- Affinity chromatography.^[81]

THIN LAYER CHROMATOGRAPHY (TLC)

To separate components in non-volatile mixtures, chromatography techniques such as thin-layer chromatography are applied.^[82]

It is carried out on a TLC plate composed of an adsorbent material thinly coated over a non-reactive solid.^[83] This is called the stationary phase.^[83]

The sample is eluted using the mobile phase, which is a solvent or solvent mixture, once it has been put on the plate.^[84]

Applications of TLC

- TLC aids in determining the sample's purity.
- In the test, TLC can isolate, purify, and identify natural products such as waxes, alkaloids, glycosides, volatile or essential oils, fixed oils, etc. samples.
- TLC can be used to isolate biochemical metabolites from bodily fluids such as blood plasma, serum, urine, etc.
- The food and cosmetic industries use TLC.
- For qualitative analysis, TLC is employed.

Advantages of TLC

- This method of separating the components is quite simple.
- Using this method, the non-volatile compounds can be separated.
- It is also possible to separate a microliter quantity of sample using TLC.
- Visualizing every aspect of UV light is possible.
- Due to the components' rapid elution, the separation is completed in a short amount of time.

Disadvantages of TLC

- Only applicable to components of soluble mixtures.
- Not a robotic procedure.
- Qualitative analysis, not quantitative analysis.
- Temperature and humidity can have an impact on the outcomes because TLC operates in an open framework.
- The separation process is limited in length because the plate length is restricted.

Procedure for TLC of Neem Leaves

- **Selection of solvent**

Several factors influence the choice of solvent, such as the

- Character of the material
- The stationary phase's nature
- Example – Methanol: Toluene (8:2) utilized as a solvent in neem TLC extract from leaves.

- **Selection of adsorbent**

- The process of choosing an adsorbent involves mixing it with water or another solvent to create a slurry.
- Example – A slurry made of silica gel G and water.

- **Selection of TLC plate**

- The TLC plate is chosen based on certain dimensions: a glass slide measuring 20 cm by 20 cm is chosen.
- A high-quality plate that can withstand high temperatures is required.

- **Pouring**

- Pouring the slurry onto the TLC plate is the next step.

- **Activation of plate**

- Plate activation involves either air drying the plate for 5-10 minutes or heating it to approximately 100° C in the oven for half an hour.

- **Sample spotting**

- Using a micropipette or capillary tube, make sure that the spotting area is not submerged in the mobile phase.



- **Place into TLC chamber**

- Place the TLC plate inside the chamber to begin the eluting process.

- **Remove the TLC plate**

- Take the TLC plate out of the chamber.



- **Agent for Detection or visualization**

- Place the slide on the iodine chamber and observe the compound's color.

- **Rf values**

- The positions of each molecule in the mixture can be ascertained by calculating the ratio between the solute and solvent travel times.^[85]
- Value serves as a qualitative means of characterizing the molecules.
- The value of Rf is between 0-1.

➤ $R_f = \frac{\text{The Solute's travel distance}}{\text{The Solvent's travel distance}}$

The Solvent's travel distance

The R_f value of neem leaf extract

Where, The solute's travel distance = 3

The solvent's travel distance = 4.2

$$R_f = 3/4.2$$

$R_f = 0.71$

CONCLUSION

The benefits of using natural products-based medicines are numerous, and our understanding of them is growing. Allopathic medicine is frequently used as a treatment method, however, these treatments can be quick but have negative health side effects. Among the best nontoxic is neem, the biological source used in medication development. This review article illustrates some benefits to general health that neem and its active ingredient have through anti-inflammatory, antioxidant, antimicrobial, and antineoplastic properties.

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