

Chapter 43

Exploring Neem (*Azadirachta indica*) for Antidermatophytic Activity

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Key Points

- *Azadirachta indica* is a well-known medicinal plant in the Indian system of medicine for treatment of various skin diseases.
- The therapeutic action is due to the presence of several antimicrobial phytoconstituents such as azadirachtin, nimbin, nimbolide, gedunin and mahmoodin.
- *A. indica* is found to be active in prevention and treatment of dandruff.
- Neem does not have any negative health effects for humans because it is gentle.

Keywords *Azadirachta indica* • Antidermatophytic • Antifungal • Antidandruff

Abbreviations

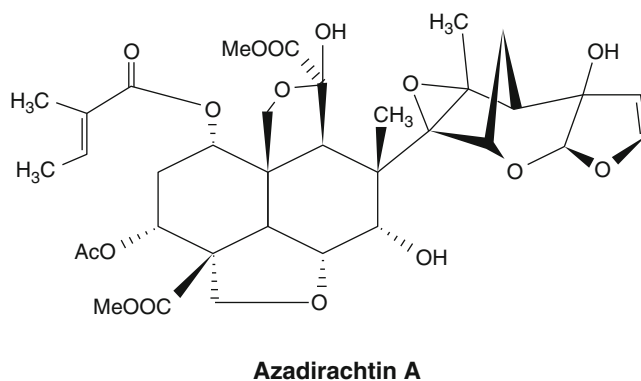
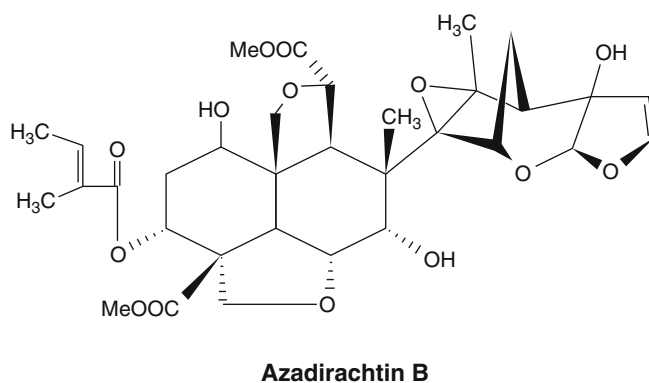
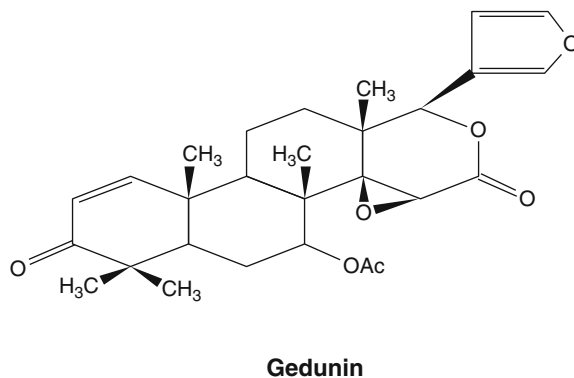
MFC Minimum fungicidal concentration
MIC Minimum inhibitory concentration
SD Sabouraud dextrose

Introduction

Azadirachta indica A. Juss. (Neem) is a large evergreen tree that usually attains a height of 18–20 m. The plant belongs to mahogany family Meliaceae. Neem is indigenous to Indian subcontinent. The plant flourishes in tropical or subtropical regions with semiarid to humid climate. The plant is widely spread in Pakistan, Malaysia, Australia and Africa. Neem plant was used in ancient Indian medicinal

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Fig. 43.1 Azadirachtin A**Fig. 43.2** Azadirachtin B**Fig. 43.3** Gedunin

practices for treatment of various skin ailments. Azadirachtin A (Fig. 43.1), azadirachtin B (Fig. 43.2), gedunin (Fig. 43.3), gallic acid (Fig. 43.4), isomargolonone (Fig. 43.5), mahmoodin (Fig. 43.6), margolone (Fig. 43.7), margolonone (Fig. 43.8), nimbin (Fig. 43.9), nimbolide (Fig. 43.10) and sodium nimbidate are the phytoconstituents responsible for antifungal, antiviral, antibacterial and antiseptic properties of the plant [1]. Hair care and treatment of conjunctivitis, psoriasis, pruritis, urticaria, chicken pox and measles are the major benefits of neem plant [2].

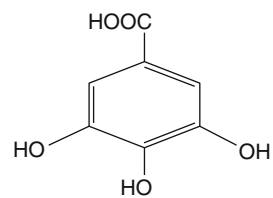
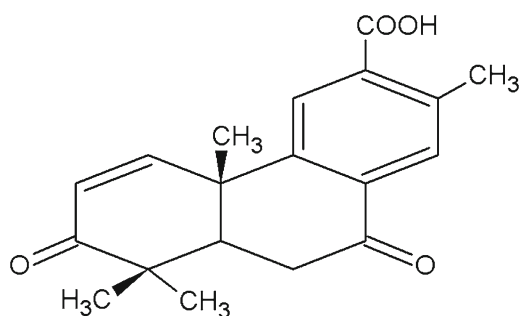
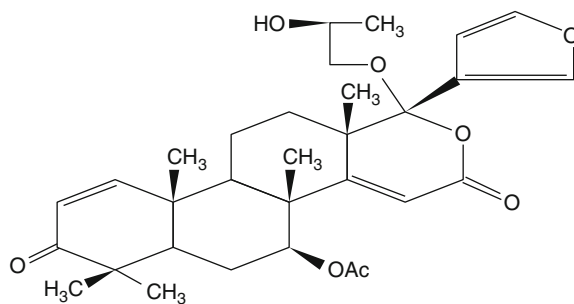
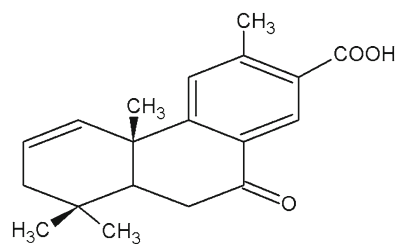
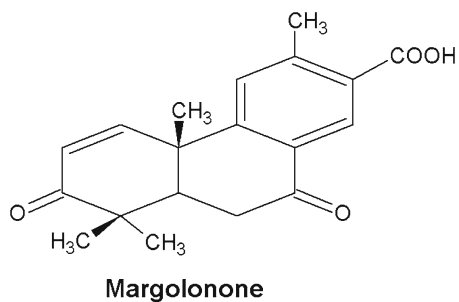
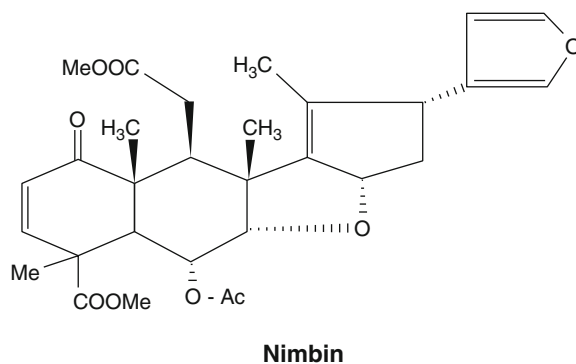
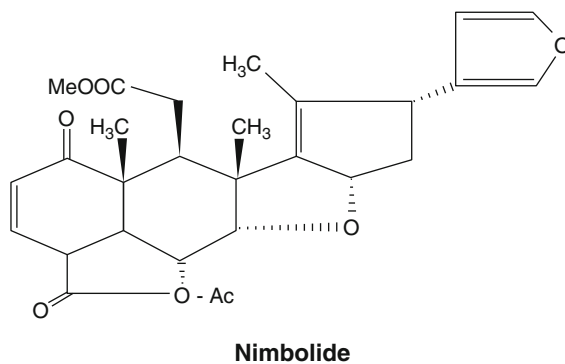
Fig. 43.4 Gallic acid**Gallic Acid****Fig. 43.5** Isomargolonone**Isomargolonone****Fig. 43.6** Mahmoodin**Mahmoodin****Fig. 43.7** Margolone**Margolone**

Fig. 43.8 Margolonone**Fig. 43.9** Nimbin**Fig. 43.10** Nimbolide

Scientific Classification of *A. indica* A. Juss

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Super division: Spermatophyta
 Division: Mangnoliophyta
 Class: Mangnoliopsida
 Subclass: Rosidae
 Order: Sapindales
 Family: Meliaceae

Genus: *Azadirachta*

Species: *Azadirachta indica* A. Juss.

Source: [1]

- Synonym: *Melia indica* (A. Juss.) Brand., *Melia parviflora* Moon
- Common name: Neem, margosa, Indian lilac [3]
- Vernacular names:
 - Sanskrit—Nimbah, prabhadrah
 - Hindi—Nim
 - Nepali and Urdu—Neem
 - Persian—Azad dirakt
 - Nigerian—Dongoyaro
 - Arabic—Margosa, neeb
 - Spanish—Paraiso
 - English—Indian lilac

Botanical Description

The genus *Azadirachta* (family Meliaceae) comprises two species: *A. indica* A. Juss syn. *Melia azadirachta* Linn. and *Azadirachta excelsa* (Jack) Jacobs syn. *Azadirachta integrifolia* Mers.

Plant. The tree is a hardy medium to large, mostly evergreen attaining 20 m height and 2.5 m girth. The branches are wide spreading and with glabrous twigs forming a round to oval crown. Neem is a fast-growing tree and can reach a height of 15–20 m, rarely to 35–40 m. It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are widespread. The fairly dense crown is roundish or oval and may reach the diameter of 15–20 m in old, free-standing specimens.

Bark. The bark is thick and woody. Externally it is dark-gray with numerous longitudinal furrows and transverse cracks. Internally the bark is reddish brown with few furrows. The bark is normally colonised by mites and insects (Fig. 43.11).



Fig. 43.11 Neem leaves

Leaves. Leaves are imparipinnately compound, alternate, exstipulate and 20–38 cm long. The leaves 20–31 medium to dark green leaflets about 3–8 cm (1–3 in.) long. The terminal leaflet is often missing. The petioles are short (Fig. 43.11).

Flowers. Flowers are white or pale yellow, small, bisexual, pentamerous and bracteate. Inflorescence is long, slender, axillary, or terminal panicle. Stamens ten; filaments unite to form a moniliform tube. Gynoecium is tricarpeal and syncarpous, ovary superior, trilocular. Each carpel bears two collateral ovules on parietal placentation.

Fruits. Fruit is one-seeded drupe with thin exocarp, bitter sweet pulpy mesocarp and woody endocarp. The ripe fruits are greenish yellow and are 1.4–2.8 × 1.0–1.5 cm in dimensions.

Seeds. Seed is ellipsoid, cotyledons thick, fleshy and oily. Neem has chromosome number $2n=28$. Neem trees tend to become deciduous for a brief period in dry ecology. Ecotypes, exhibiting morphological variation in root growth, leaf size, contents, bole length, canopy, inflorescence, fruit bearing, seed size, shape and quality, exist in natural populations [3].

Chemical Compounds

Neem plant is chemically rich and has over 300 secondary compounds. Most of the active compounds are terpenoids, found in the fruit, seeds, twigs, stem and root bark. A tetranortriterpenoid azadirachtin (a liminoid) is the chief constituent in the seed kernels.

The seed oil is yellowish in color, malodorous and has an unpleasant taste due to the sulphur compounds. It chiefly comprises glycerides of oleic and stearic acids, at 50% and 20%, respectively. But, the azadirachtins are the most bioactive and popular commercially. In addition to the azadirachtins, other major liminoids are the salannins (Fig. 43.12). Three bitter compounds were extracted from neem oil, which were named nimbin, nimbinin and nimbidin [1]. Dry neem leaves contain nimboesterol (b-sitosterol), kaempferol and myricetin. Seed and oil contains desacetylnimbin, azadirachtin, nimbidol, meliantriol and tannic acid. Neem cake contains the highest sulphur content of 1.07% among all the oil cakes. Trunk bark contains nimbin 0.04%, nimbinin 0.001%, nimbidin 0.4%, nimboesterol 0.03%, essential oil 0.02%, tannins 6.0%, margsosine and desacetylnimbin [3]. The antidermatophytic phytoconstituents are briefly summarised in Table 43.1.

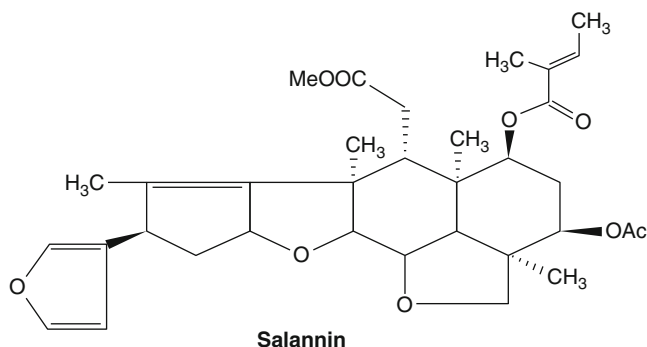


Fig. 43.12 Salannin

Table 43.1 Antidermatophytic neem compounds

Part	Major compound(s)	Medicinal activity
Leaf	Cyclic trisulphide (Fig. 43.13), Cyclic tetrasulphide (Fig. 43.14)	Antifungal
Seed	Azadirachtin, nimbin, nimbolide, gedunin, mahmoodin	Antifungal, antimalarial
Fruit	Nimbidin	Antifungal, antibacterial
Stem Bark	Gallic acid, margolone, margolonone, isomargolonone	Antibacterial
Roots	Azadirachtin	Antibacterial

Source: [1]

Pharmacological Activities

Antidermatophytic Activity of Neem: A. indica A. Juss

Traditional medicinal systems of India treat neem as “Panacea for all diseases.” Products made from neem tree have been used in India for over two millennia for their medicinal properties. Neem has potent antifungal, antibacterial and antiviral property. It is considered a major component in ayurvedic and Unani medicine and is particularly prescribed for skin disease. Certain neem-based ayurvedic formulations prescribed for skin diseases are listed in Table 43.2.

Antifungal Activity

The DMSO extract of neem seeds and *n*-hexane, ethyl acetate and ethanol extract of the leaves were separately studied for their antifungal activity. The dermatophytic fungal cultures of *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum nanum* were used for the study. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were estimated by incorporating different concentrations of extracts in Sabouraud dextrose (SD) broth, and 20 µl of standard fungal inoculum was added to each tube and incubated at room temperature for 21 days. Suitable controls were also included. SD broth with 20 µl of inoculum served as positive control. SD broth alone served as negative control. The ethanol extracts of neem leaves showed MIC and MFC at 250 µg/ml concentrations for all the strains of *T. rubrum* and *M. nanum* tested. MIC and MFC recorded for stains of *T. mentagrophytes* was 125 µg/ml. The ethyl acetate extract of neem leaf showed MIC and MFC at 125 µg/ml for all the stains of *T. rubrum* and *T. mentagrophytes* and 250 µg/ml for *M. nanum*. Hexane extracts of neem leaf showed MIC and MFC at 500 µg/ml for all the strains of *T. rubrum*, *T. mentagrophytes* and *M. nanum*. The neem seed extract showed MIC and MFC at 31 µg/ml for all the dermatophytes tested.

The MIC and MFC of the neem seed extract were similar, which shows that MIC is sufficient for measuring fungicidal activity. Neem seed extract has high antidermatophytic properties. Neem seed extract at a concentration of 15 µg/ml (below MIC) was observed to distort the growth pattern of the *T. rubrum*, *T. mentagrophytes* and *M. nanum*. This finding supports the use of neem oil in the treatment of various skin infections by alternative systems of medicine [5].

Table 43.2 List of neem-based official ayurvedic formulations for skin disorders

Formulation	Part used	Indications	Ayurvedic formulary of India
Punarnavasava	Stem bark	Inflammation, dermatitis	[4]
Tiktaka Ghrta-A	Stem bark	Burning sensation, skin diseases, itching, cervical lymphadenitis, blisterous eruptions, localised hyperpigmentation of skin, abscess	[4]
Tiktaka Ghrta-B	Stem bark	Burning sensation, skin diseases, itching, cervical lymphadenitis, localised hyperpigmentation of skin, abscess	[4]
Pancatikta Guggulu Ghrta	Stem bark	Leprosy, cervical lymphadenitis, abscess	[4]

Source: [4]

Antidandruff Property

Pityrosporum ovale is one of the fungi that cause dandruff. Ethanol extract of neem leaves was evaluated for the antifungal activity on *P. ovale*. Various concentrations of neem extract (25, 50, 75 and 100%) were used for the study. The inhibiting capacity of each level on the fungus was tested using agar cup method.

The results showed that 50% and above level of concentration had optimal level of inhibition on the dandruff growth. The higher the concentration, the higher was the inhibition on the growth of dandruff. The measurement of antifungal activity was done by calculating the zone of inhibition (diameter). The 100% extract of neem leaves produced the widest zone of inhibition (18 mm), which was found statistically highest than the other concentration levels. The sample treated with 75% neem extract showed the second widest zone of inhibition (11.33 mm) but was found at par with the 50% concentration, which produced about 9.33 mm zone of inhibition. The sample treated with the lowest concentration of 25% extract produced the smallest diameter of 6.67 mm. The study revealed that ethanol extract of neem leaves can inhibit the growth of *P. ovale* fungus, which is the main cause of dandruff [6].

Treatment of Scabies

A paste prepared by combination of neem leaves and *Curcuma longa* (turmeric) was used to treat scabies in 814 people. About 97% of them were cured within 3–15 days of application, and no adverse reactions were observed [7].

Antibacterial Activity

Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycobacterium tuberculosis* and streptomycin-resistant strains. A study on agar plates indicated that neem seed oil at concentration of 0.3 and 0.4% was active against *Staphylococcus aureus* and *Salmonella typhosa*, respectively. The seed oil was found

to be inactive against *Pseudomonas aeruginosa*, but was active against *E. coli* and *Proteus* species at a concentration of 3%, and active against *Klebsiella pneumoniae*, at a concentration of 6%.

In vitro, it inhibits *Vibrio cholerae*, *K. pneumoniae*, *M. tuberculosis* and *Streptococcus pyogenes*. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *Streptococcus faecalis*. NIM-76, a new vaginal contraceptive from neem oil, showed inhibitory effect on the growth of various pathogens, including bacteria, fungi and virus. Recently, the antibacterial activity of neem seed oil was assessed in vitro against 14 strains of pathogenic bacteria [1].

Antiviral Activity

Aqueous leaf extract offers antiviral activity against *vaccinia* virus, *chikungemya* and measles virus in vitro. The antiviral and virucidal effects of the methanolic extract of neem leaves (NCL-11) have recently been demonstrated against group-B coxsackie viruses. NCL-11 inhibits plaque formation in different antigenic types of coxsackie virus B at a concentration of 1 mg/ml at 96 h in vitro. Further studies indicated that NCL-11 is most effective in *coxsackie* virus B-4 as a virusidal agent, in addition to its interference at the early events of its replication [1].

Effect on Head Lice

A shampoo containing neem leaf extract (identified as Type AP30) has been proven to be highly effective against all stages of head lice for 66 children (4–15 years old) with significant head lice infestations, even after only 10 min of exposure time. The percentages of effectiveness ranged from 86 to 97% after a single application of the shampoo; only a second treatment was needed for most children to remain lice-free. No adverse effects were observed [8].

Mosquito Repellent Effect

Neem leaf paste is applied to the skin to treat acne, and in a similar vein is used for measles and chicken pox sufferers. Extract of neem leaves is thought to be helpful as malaria prophylaxis. Neem products are good mosquito repellents showing 90–100% protection against malaria vectors and about 70% against *Culex quinquefasciatus*. One controlled study evaluated the efficacy of a cream formulation containing 5% neem oil against *C. quinquefasciatus* and *Anopheles culicifacies*. Neem oil has been found to be an effective mosquito repellent. Neem oil cakes and karanja (*Pongamia glabra*) oil cake were used in combination against three mosquito species (*C. quinquefasciatus* (say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.)); the efficacy increased from four- to tenfold for the LC 50 and two- to sixfold for the LC 95 over individual applications.

About 4–5 g of the cream was applied to the exposed skin areas of human volunteers in Ghaziabad, India, in the summer months of May/June and the monsoon months of August/September. Neem cream was found to offer 82% protection against *Culex* bites and 100% protection against *Anopheles* bites, as compared to untreated controls [9].

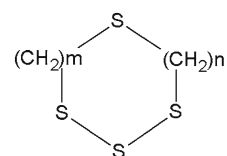
Cosmetics

Neem oil is used for preparing cosmetics (toilet soaps, shampoos, sunscreen lotions, balms and creams), and is useful for skin care such as acne treatment, and maintaining the skin elasticity.

Safety and Toxicity

Neem does not have any negative health effects for humans because it is gentle. As with any bioactive compound, azadirachtin still needs to be treated with care because poisonings have been reported.

Neem seed oil can cause toxic encephalopathy, especially in infants and young children. The symptoms of poisoning are vomiting, drowsiness, tachypnea (abnormally fast breathing), recurrent generalised seizures (that may lead to coma or cardiopulmonary arrest), leucocytosis and metabolic acidosis. Treatment is primarily supportive and directed toward controlling convulsions. In addition, postmortem autopsies of children who died after ingesting margosa oil showed swelling of hepatocytes, fatty metamorphosis of liver, depletion of glycogen, mitochondrial pyknosis (cell degeneration), more peroxisomes and significantly more smooth endoplasmic reticulum (indicating increased detoxification activity). Neem oil is not approved by FDA for internal human use, but an estimated safe daily dose of azadirachtin is 15 mg/kg body weight [10] (Figs. 43.13 and 43.14).

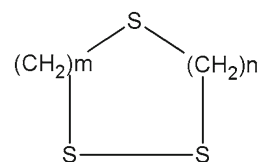


$$m = 1 - 4$$

$$n = 2 - 3$$

Cyclic Tetrasulphide

Fig. 43.13 Cyclic tetrasulphide



$$m = 1 - 4$$

$$n = 2 - 3$$

Cyclic Trisulphide

Fig. 43.14 Cyclic trisulphide

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