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Biological action and medicinal properties of various constituent of *Azadirachta indica* (Meliaceae)" an Overview

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ABSTRACT

Neem (Azadirachta indica A. Juss) is perhaps the most useful traditional medicinal plant in India. Biologically active ingredients of this plant have diverse applications. Each part of the neem tree has some medicinal property and is thus commercially exploitable. These compounds belong to the natural products called triterpenoids. (Limonoids). Alkali treated neem cake performed significantly better when incorporated into poultry feeds. Several animals and plant pathogenic fungi, bacteria, viral, protozoan and Helminthes are sensitive to neem preparations, with antiseptic properties. NSO and leaves extract significantly inhibited fertility in males, but not anti-ovulatory, hence "sensal" a contraceptive. This review gives a bird's eye view mainly on the biological activities of some of the neem compounds isolated, pharmacological actions of the neem extracts, clinical studies and plausible medicinal applications of neem along with their safety evaluation.

Key words: *Azadirachta indica*, pharmacological actions, medicinal applications, Limonoids.

INTRODUCTION

Neem (*Azadirachta indica)* is a member of the Meliaceae family. The former is popularly known as Indian neem (margosa tree) or Indian lilac, and the latter as the Persian lilac. Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity [1–6]. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. The sanskrit name of the neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as 'Sarbaroganibarini'. The

tree is still regarded as 'village dispensary' in India. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems'. Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles. Miliacin forms the bitter principles of neem seed oil (NSO), the seed also contain tignic acid (5-methyl- 2-butanic acid) responsible for the distinctive odor of the oil.

Table 1. Some bioactive compounds from neem

Neem compound	Source	Biological activity	Reference
Nimbidin		Anti-inflammatory	12
		Antiarthritic	13
		Antipyretic	14
		Hypoglycaemic	15
		Antigastric ulcer	16, 17
		Spermicidal	20
		Antifungal	21
		Antibacterial	21
		Diuretic	22
Sodium nimbidate		Anti-inflammatory	12, 13
Nimbin (1)	Seed oil	Spermicidal	19
Nimbolide (2)	Seed oil	Antibacterial	23
		Antimalarial	23,24
Gedunin (3)	Seed oil	Antifungal	26
		Antimalarial	24
Azadirachtin (4)	Seed	Antimalarial	28
Mahmoodin (5)	Seed oil	Antibacterial	09
Gallic acid (6), (–) epicatechin (7) and catechin (8)	Bark	Anti-inflammatory immunmodulatory	29
Margolone (9), margolonone (10) and isomargolonone (11)	Bark	Antibacterial	30
Cyclic trisulphide (12) and cyclic tetrasulphide (13)	Leaf	Antifungal	31
Polysaccharides		Anti-inflammatory	32
Polysaccharides GIa (14),	Bark	Antitumour	33
Polysaccharides GIIa (15),	Bark	Anti-inflammatory	34
NB-II peptidoglycan	Bark	Immunomodulatory	35,36

The compounds have been divided into two major classes: isoprenoids and others. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and csecomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins

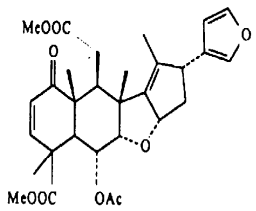
(amino acids) and carbohydrates (polysaccharides), sulphurous compounds,polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds,etc.

Biological activity of some neem compounds

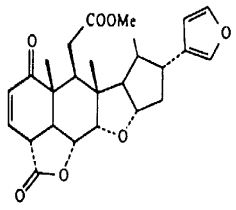
Although a large number of compounds have been isolated from various parts of neem, a few of them have been studied for biological activity as shown in **Table 1**. Nimbidin, a major crude bitter principle extracted from the oil of seed kernels of *A. indica* demonstrated several biological activities. From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated [7, 11].

Nimbidin and sodium nimbidate possess significant dose dependent anti-inflammatory activity against carrageen in induced acute paw oedema in rats and formalin-induced arthritis [12, 13]. Antipyretic activity has also been reported and confirmed in nimbidin [14]. Oral administration of nimbidin demonstrated significant hypoglycemic effect in fasting rabbits [15]. A significant antiulcer effect was observed with nimbidin in preventing acetylsalicylic acid, indomethacin, stress or serotonin-induced gastric lesions as well as histamine or cyst amine-induced duodenal Ulcers[16, 17]. Nimbidin can also suppress basal as well as histamine and carbachol-stimulated gastric acid output and may act as an antihistamine by blocking H2 receptors, thereby helping as an antiulcer agent [18]. Nimbidin also demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum [*21]. *In vitro*, it can completely inhibit the growth of *Mycobacterium tuberculosis* and was also found to be bactericidal[21]. Diuretic activity was also reported for sodium nimbidinate in dogs [22]. Nimbolide (**2**) has been shown to exert antimalarial activity by inhibiting the growth of *Plasmodium falciparum[*23, 24]. Nimbolide also shows antibacterial activity against *S. aureus* and *S. coagulase[*25]. Gedunin (**3**), isolated from neem seed oil has been reported to possess both antifungal[26] and antimalarial [24]activities. Azadirachtin (**4**), highly oxygenated C-secomeliacins isolated from neem seed and having strong antifeedant activity [8, 10,27] has been demonstrated to have antimalarial property as well. It is inhibitory to the development of malarial parasites [28]. Mahmoodin (**5**), a deoxygedunin isolated from seed oil, has been shown to possess moderate antibacterial action against some strains of human pathogenic bacteria[9]. Condensed tannins from thebark contain gallic(+) gallocatechin, (–) epicatechin, (+) catechin and epigallocatechin, of which gallic acid (**6**), (–) epicatechin (**7**) and catechin (**8**) are primarily responsible for inhibiting the generation of chemiluminescence by activated human polymorphonuclear neutrophil(PMN)[29], indicating that these compounds inhibit oxidativeburst of PMN during inflammation. Three tricyclic diterpenoids, margolone (**9**), margolonone (**10**) and isomargolonone (**11**) isolated from neem stem bark are active against *Klebsiella*, *Staphylococcus* and *Serratia* species [30]. Sulphur-containing compounds such as cyclic trisulphide (**12**) and tetrasulphide (**13**) isolated from the steam distillate of fresh, matured neem leaves have antifungal activity against *Trichophyton mentagrophytes [*31].Several polysaccharides from neem exhibit various biological effects. A polysaccharide extracted from bark inhibits carrageenin-induced inflammation in mouse [32]. Two water-soluble polysaccharides GIa (**14**) and GIb isolated from the bark of *Melia azadirachta*, demonstrated strong antitumour effect with complete regression of the tumors, when administered in mice at a daily dose of 50 mg/kg for four days from 24 h after subcutaneous inoculation of Sarcoma-180 cells [33]. Two more polysaccharides, GIIa (**15**) and GIIIa (**16**) isolated from *M.azadirachta* bark also showed significant anti-inflammatory effect on carrageen in induced oedema in

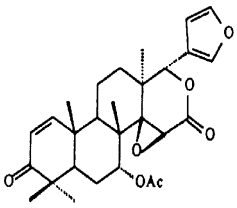
mice[34].Two polymers isolated from an aqueous extract of neem bark possess anticomplement activity, amongst which the compound NB-II, a peptidoglycan of lower molecular weight was found to be more potent[35,36]. Some active ingredients (phytosterol fraction) isolated from the lipid part of neem fruits, exhibit antiulcer activity in stress induced gastric lesions [37].



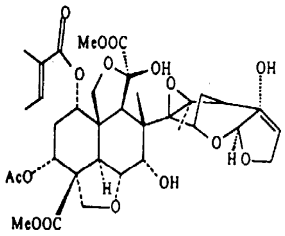
NIMBIN
(1)



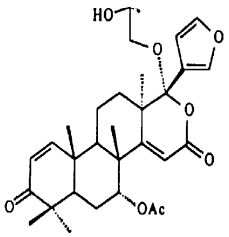
NIMBOLIDE
(2)



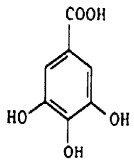
GEDUNIN
(3)



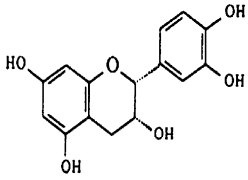
AZADIRACHTIN
(4)



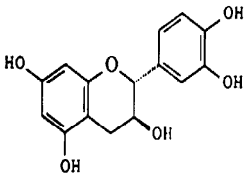
MAHMOODIN
(5)



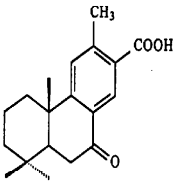
GALLIC ACID
(6)



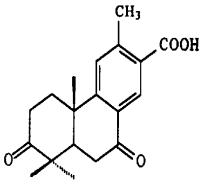
EPICATECHIN
(7)



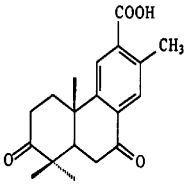
CATECHIN
(8)



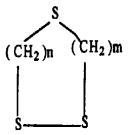
MARGOLONE
(9)



MARGOLONONE
(10)

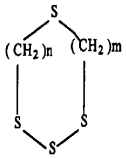


ISOMARGOLONONE
(11)

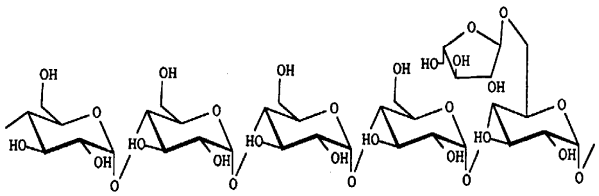


CYCLIC TRISULPHIDE
(12)

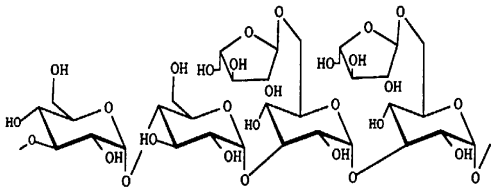
n = 2 - 3
m = 1 - 4



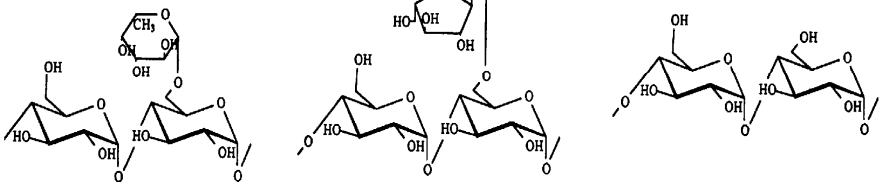
CYCLIC TETRASULPHIDE
(13)



GIa
(14)



GIIa
(15)



GIIIa
(16)

Pharmacological actions of neem extract

Several pharmacological activities and medicinal applications of various parts of neem are well known [38].Biological activity of neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. However, crude extract of different parts of neem have been used as traditional medicine for the treatment of various diseases.

Medicinal use of various parts of neem

Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial [39].The medicinal utilities have been described, especially for leaf, fruit and bark [4]. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation and also as a general health promoter [40]. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident [3]. Neem oil finds use to control various skin infections [1]. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. Some of the medicinal attributes of various parts of neem [41]]as mentioned in ayurveda [39] have been summarized in **Table 2.** However, apart from these uses, there are several reports on the biological activities and pharmacological actions of neem based on modern scientific investigations.

Anti-inflammatory, antipyretic and analgesic activities: The chloroform extract of stem bark is effective against carrageenin-induced paw oedema in rat and mouse ear inflammation [42]. Inflammatory stomatitis in children is cured by the bark extract [43]. Antipyretic activity has been reported in neem oil [21, 44]. A methanol extract of the leaves exerts antipyretic effect in male rabbits [45]. The plant also possesses analgesic activity mediated through opioid receptors in laboratory animals [46]. Anti-inflammatory and antipyretic activities in various extracts have been reviewed [47].

Immunostimulant activity: The aqueous extract of neem bark possesses anticomplement activity, acting both on the alternative as well as the classical pathway of complement activation in human serum [35]. Recently, an aqueous extract of stem bark has been shown to enhance the immune response of Balb-c mice to sheep red blood cells *in vivo [*48]. The aqueous extract of leaf also possesses potent immunostimulant activity as evidenced by both humoral and cell-mediated responses [49,50]. Leaf extract at 100 mg/kg after three weeks of oral administration causes higher IgM and IgG levels along with increased titer of antiovalbumin antibody [50]. Neem oil has been shown to possess immunostimulant activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge[51].

***Hypoglycemic activity*:** Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycemia [52]. The aqueous leaf extract when orally fed, also produces hypoglycemia in normal rats and decreased blood glucose levels in experimentally-induced diabetes in rats [53]. Aqueous leaf extract also reduces hyperglycemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, quercetin [54]. A significant hypoglycemic effect was also observed by feeding neem oil to fasting rabbits [15]. Recently, hypoglycemic effect was observed with leaf extract and seed oil, in normal as

well as alloxan-induced diabetic rabbits [55]. The possible mechanisms underlying the hypoglycemic activity of the aqueous leaf extract have also been discussed [56, 57].

Antiulcer effect: Neem leaf aqueous extract produces antiulcer effect in rats exposed to restraint cold stressor ethanol orally by preventing mucus depletion and mast cell degranulation [58]. An aqueous extract of neem bark has been shown from our laboratory to possess highly potent antacid secretory and antiulcer activity and the bioactive compound has been attributed to a glycoside [59].

***Antifertility effect*:** Neem oil proved spermicidal against rhesus monkey and human spermatozoa *in vitro [*60]. *In vivo* studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy [60]. Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception [61–63]. Oral administration of aqueous extract of neem leaf a antifertility effect in mice [64]. Purified neem seed extract (Praneem) has also been demonstrated to abrogate pregnancy in both baboons and bonnet monkeys, when administered orally [65]. From the hexane extract of neem seed, an active fraction containing six components has been found to completely abrogate pregnancy in rodents when given orally up to a concentration of 10%, with no apparent side effect [66]. The effect is possibly due to activation of cell-mediated immune reaction. The mechanism of action of neem oil appears to be non-hormonal, probably mediated through its spermicidal effect and may have fewer side effects than steroidal contraceptives.

Antimalarial activity: Neem seed and leaf extracts are effective against malarial parasites [24, 67]. Components of the alcoholic extracts of leaves and seeds are effective against both chloroquin-resistant and sensitive strains of malarial parasite [68]. Recently, neem seed extract and its purified fractions have been shown to inhibit growth and development of asexual and sexual stages of drug sensitive and resistant strains of the human malarial parasite *P. falciparum [*69].

Antifungal activity: Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi, including *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporon*, *Geotricum* and *Candida [*70]. High antimycotic activity with extracts of different parts of neem has already been reported [47].

Antibacterial activity: Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycin resistant strains [71]. *In vitro*, it inhibits *Vibrio cholerae, Klebsiella pneumoniae, M. tuberculosis* and *M. pyogenes [*72]. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis [*73]. NIM-76, a new vaginal contraceptive from neem oil showed inhibitory effect on the growth of various pathogens, including bacteria, fungi and virus [74]. Recently, the antibacterial activity of neem seed oil was assessed *in vitro* against 14 strains of pathogenic bacteria [75].

Antiviral activity: Aqueous leaf extract offers antiviral activity against Vaccinia virus [76], Chikungemya and measles virus *in vitro [*77]. The antiviral and virucidal effects of the methanol extract of neem leaves (NCL-11) have recently been demonstrated against group-B Coxsackie

viruses [78]. 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.

Anticarcinogenic activity: Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a]anthracene (DMBA), as revealed by reduced incidence of neoplasm[79]. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes. That neem leaf extract exerts its protective effect in *M*ethyl- *N*□-nitro-*N*-nitrosoguanidine (MNNG) (a carcinogenicmaterial)-induced oxidative stress has also been demonstrated by the reduced formation of lipid peroxides and enhanced level of antioxidants and detoxifying enzymes in the stomach, a primary target organ for MNNG as well as in the liver and in circulation[80,81].

Hepatoprotective activity: The aqueous extract of neem leaf was found to offer protection against paracetamol induced liver necrosis in rats[82]. The elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) indicative of liver damage were found to be significantly reduced on administration of the neem leaf aqueous extract.

Antioxidant activity: The antioxidant activity of neem seed extract has been demonstrated *in vivo* during horse grain germination, which is associated with low levels of lipooxygenase activity and lipid peroxides [83]. An antioxidant principle has also been isolated, which is a potent inhibitor of plant lipooxygenases.

Table 2. Some medicinal uses of neem as mentioned in ayurveda

Part	Medicinal use
Leaf	Leprosy, eye problem,epistaxis, intestinal worms, anorexia, biliousness, skin ulcers.
Bark	Analgesic, alternative and curative of fever.
Flower	Bile suppression, elimination of intestinal worms and phlegm.
Fruit	Relieves piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy.
Twig	Relieves cough, asthma, piles, phantom, tumour, intestinal worms, spermatorrhoea, Obstinate urinary disorder, diabetes.
Gum	Effective against skin diseases like ringworms, scabies, wounds and ulcers.
Seed pulp	Leprosy and intestinal worms.
Oil	Leprosy and intestinal worms.
Root, bark, leaf, Flower	Blood morbidity, biliary afflictions, itching, skin ulcer, burning sensation and fruit together leprosy.

Effect on central nervous system: Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the leaf extract[84]. Fractions of acetone extract of leaf

showed significant CNS depressant activity [85]. Leaf extract up to a dose of 200 mg/kg body weight produces significant anxiolytic activity in rats [86]. The crude ethanol extract of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities [87, 88].

CONCLUSION

Neem, the versatile medicinal plant is the unique source of various types of compounds having diverse chemical structure. A drug-development programme should be undertaken to develop modern drugs with the compounds isolated from neem. Although crude extracts from various parts of neem have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mechanism ofaction, pharmacotherapeutics, toxicity and after proper standardization and clinical trials.

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