**Azadirachta indica**: Antibacterial Activity of Neem Against Different Strains of Bacteria and their Active Constituents as Preventive in Various Diseases

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**ABSTRACT**

Neem has become valuable plant in the world which shows the solutions for hundreds to thousands problems. *Azadirachta indica* (Neem) is a rapidly growing evergreen well known tree found Pada generally in various regions of world like America, Africa and India. It has been widely used in Chinese, Ayurveda and Unani medicines across the world especially in Asians countries for the prevention and treatment of diseases. The different parts of neem plant contain biological compounds responsible for antibacterial, antiviral and antifungal activities. It is considered as safe medicinal plants and modulates the numerous biological processes without any adverse effect. Neem tree produces some active compounds which contain biological activities, parts of neem tree such as Root, bark, leaf, flower, seed and fruit together possesses biological activities. Various compounds have been obtained from various parts of neem. Biological activities of few of them have been studied. Hence, the article is aims to utilize the medicinal properties of whole neem plant in various disorders of mankind.

**Key words:** *Azadirachta indica*, Antibacterial, Antifungal, Natural products, Natural antibiotics.

**INTRODUCTION**

Plants have many biologically active compounds which have latent for development as medicinal agents. Herbal medicines already form the basis of beneficial use in the developing countries, but of recent, there has been rise in the use of herbal medicines in the developed world. Plants provide an alternate approach in search for new treatments. There is a plenty of plants reputed in traditional medicine to hold protective and therapeutic properties. It is likely that plants will continue to be a valued source of new molecules which may, after possible chemical manipulation, provide new and improved drugs.

Bacterial resistance to antibiotics represents a serious problem for clinicians and the therapeutic industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases.

Each part of Neem tree has various medicinal properties. Non woody part of Neem such as leaf, bark, oil, flowers, fruits and seed show great properties that is Antiallergic, Antifungal, Antibiotic, Antidermatic, Antibacterial, Anti-inflammatory, Insecticidal, Larvicidal, Antimalarial, Antiucler and other biological activities. Some water soluble extract of *Azadirachta Indica* have great importance in hyperglycemia, hypolipidemia and hypotensive activities.

**ANTIMICROBIAL ACTIVITY OF NEEM**

Neem has great Antimicrobial activity it contains 35 biological active compounds. Neem leaf juice and twigs are used to clean teeth and used as a tonic and people of India used to place Neem leaves in their beds, books and cupboards to prevent bugs. A number of potent pharmaceutical compounds limnooids and triterpenoids have been isolated from the fruits and bark of neem tree.

Neem extracts and its different constituents play essential role in the inhibition of several microbes which includes viruses, fungi and bacteria. The extracts of methanol and hexane chloroform of *Azadirachta indica* were selected against antibacterial activity on Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Bacillus subtilis, Micrococcus luteus, Streptococcus faecalis and Enterococcus faecalis. It was revealed that methanol extract was the most effective, chloroform reasonably effective and hexane extract showed little antibacterial activity. The antibacterial activity of neem describes as fellow.

**Antibacterial action of neem**

Neem usually used in medicine and pharmaceutics. The stem and bark of Neem has great antibacterial activity against Klebsiella, Serratia species and Streptococcus. The methanolic extracts of Neem has antibacterial activity against Vibrio cholera and chloroform extracts against E. coli, Bacillus subtilis, Enterococcus faecalis and Streptococcus faecalis.
The extraction of neem oil has strong activity against Gram positive and Gram negative bacteria like Mycobacterium tuberculosis and streptomycin-resistant strains. Mahmood et al. (2013) determined in his study that the crude extraction of Neem has antibacterial activity against infection of eyes and ear. The petroleum ether and methanol extract has highest effect against Candida albicans.

The antibacterial action of neem and guava samples against 21 strains of food endured pathogens was assessed and results of this examination recommended that neem and guava extracts have compounds holding antibacterial functions that can be beneficial to resist food endured pathogens and decomposer organisms. Another research was carried out to assess the antibacterial action of the leaf, seed, bark and fruit extracts of Azadirachta Indica (neem) on microbes obtain from mouth of adult and effects revealed that leaf and bark extracts possess antibacterial action against all the tested bacteria. Moreover, fruit and seed extract presented antibacterial action at greater concentration only.

Oil extracted from leaves, bark and seeds hold a broad range antibacterial activity against gram positive and gram negative microbes containing streptomycin-resistant mechanisms and Mycobacterium tuberculosis strains. The phytoconstituents like alkaloids, saponins, steroids, tannins, crude glycosides and flavonoids (Figure 1) was used for sterile action against strains of Escherichia coli, Corynebacterium bovis and Staphylococcus aureus.

When microorganisms exposed to neem extracts their susceptibility compared with particular antibacterial. Methanol extract of neem have

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**Figure 1:** Chemical compounds found in different parts of Azadirachta indica.
definite antibacterial activity against *Bacillus subtilis* (28 mm). Neem oil is also found active against wide range of bacteria *Bacillus pumilus*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Pseudomonas vulgaris*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterococcus faecalis*, *Streptococcus dysenteriae*, *Streptococcus salivarius*, *Streptococcus mutans* and *Streptococcus mitis*. Azadirachta indica leaf extracts have a best anti-bacterial activity which confirmed the bioactive compounds strength and proved the usage of neem plant in major health maintenance.15

*Khan et al.*, (1997) investigated antibacterial activity of impure neem bark extract (*Azadirachta indica*) tested to streptococcus sobrinus. The aqueous and acetone extracts of bark studied on agar dishes by streptococcus sobrinus strains.20

*Asthana et al.*, (2006) carried out antimicrobial activity against cyano bacterium *Fischerella spp* isolate from neem bark. Methanolic bark extract of neem (*Azadirachta indica*) initiate to be active against *Enterobacter aerogenes*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial activity was assessed by a little altered Disk Diffusion Kirby Bauer method of susceptibility.21

*Barua et al.*, (1999) stated neem (*Azadirachta Indica*) as antibacterial agent against pathogenic bacteria present in fish. Aqua-neem arranged from neem kernels (*Azadirachta indica*) which was used against four fish bacteria (*Pseudomonas fluorescens*, *Aeromonas hydrophilia*, *Escherichia coli* and *Myco bacteria spp*.) For the effectiveness of neem among all the bacteria tested *A. hydrophila*, *Mycobacteria spp* and *P. fluorescens*. Results showed that it holds maximum sensitivity to Aqua-neem (*Azadricht a aindica*).22

### Antifungal action of neem

*Azadirachta indica* leaves ethanol and aqueous extracts have exhibited anti-dermatophytic activity in contrast to dermatophytes from 88 clinical separates by agar dilution technique. These studies revealed that ethanolic extracts showed more visible activity as compared to aqueous extracts.23 *Azadirachta indica* methanolic and acetone extracts were tested for antifungal activities against two fungal strains i.e. *Aspergillus fumigatus* and *Aspergillus niger*. Results showed that methanol extracts of plant provide supreme antifungal activity as compared to acetone extracts. The leaf and seed extracts of neem screened for antifungal activity against dermatophytes. *Azadirachta indica* seed extracts Minimum inhibitory concentration (MIC) was lower than that of neem leaf extracts when tested against various species of trichophyton and *Epidermatophyton floccosum*.24

Antifungal activity of *Azadirachta indica* tested on different pathogens through different neem extracts it includes ethanolic, aqueous and ethyl acetone extracts on growth of pathogens i.e. *Microsporum gypseum*, *Aspergillus terreus*, *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus*, by using different concentrations and results showed that these leaves extracts stopped the growth of the tested pathogens (Table 1). This effect increased by increasing the concentration.25-31

### THERAPEUTIC EFFECTS OF NEEM AND ITS VARIOUS COMPONENTS IN HEALTH MANAGEMENT

The Phyto-constituents of neem is Alkaloids, Glicosides, Flavonoids and Saponins that showed antibacterial activity against different pathogen. Some biological compounds of Neem are very effective against various insects, mites, nematodes and other plants pathogens including 25 species of Pleopetera (beetles), 10 species of Diptera (flies), 25 species of Lepidoptera (moths) and 9 species of Orthoptera (locusts). Leaf and fruits extracts have been tested on different pest with desire results some parts of neem tree are use as physical ailments that are heart disease, Diabetes, Blood disorder, Digestive and nervous disorder, parasites and possibly cancer. Particular study on Neem bark extract concluded that it helps to reduce and heal acid reflux and Gastric ulcer.26

Active components play role in cure of diseases through activation of antioxidants enzymes, by breaking the cell wall of bacteria and act as chemo preventive through regulating cellular pathways. Different pharmacological activities of neem are discussed in detail below.

### Antimalarial activity

Ball shaped wood scrapes that is saturated in 5 percent oil of neem (*Azadirachta indica*) diluted with acetone and in 45 days the propagation of *Anopheles stephensi* and *Aedes aegypti* were organized, when it is placed in water storage overhead containers.33 Growth of plasmodium falciparum inhibited by the compound nimbo liole isolated from neem extracts that shows the antimalarial activity.34 Neem seeds oil possess gedunin which also showed antimalarial activity.35 Leaves and bark aqueous and alcohol extracts are very effective antimalarial agents, especially on resistant strains chloroquione.36

### Antitumor and antiviral activity

Europe, Japan and Indian scientist found the compounds limonoids and polysaccharides present in neem seed oil. Neem leaves and bark reduced cancers and tumors and also very effective against lymphocytic leukemia. Leaf extracts mitotic inhibition activity was detected. Several results have also showed the significant antiviral effect of neem leaf aqueous extracts against Small Pox, Fowl Pox, Polio and HIV as evaluated by virus inhibition assay (98100). Neem Aqueous leaf extracts and some oils of neem fraction (Nim-76) showed antiviral action against Polio Viruses and HIV.37

### Table 1: Antibacterial activity in vitro of Azadirachta indica reported in PUBMED CENTRAL.

| Azadirachta indica | Microorganism                      | MIC         | MBC         | Reference |
|--------------------|-----------------------------------|-------------|-------------|-----------|
|                    | *Helicobacter pylori*              | 25 - 51 µg/mL | 43 – 68 µg/mL | [26]      |
| Leaves ethanolic extract | Methicillin-resistant *Staphylococcus aureus* | 31.25- 125 mg/mL | 250-500 mg/mL | [27]      |
|                    | *Staphylococcus aureus*            |             |             |           |
|                    | *Enterococcus faecalis*            |             |             |           |
|                    | *Pseudomonas aeruginosa*           |             |             |           |
|                    | *Pseudomonas mirabilis*            |             |             |           |
|                    | *Vibrium vulnificus*               | 6 mg/mL     | n.d.        | [28]      |
|                    | *Streptococcus mutans*             | 6.25 mg/mL  | n.d.        | [29]      |
|                    | *Streptococcus mutans*             | 125 µg      | 250 µg      | [30]      |
|                    | *Enterococcus faecalis*            | 500 µg      | 1 mg        | [31]      |
|                    | *Staphylococcus aureus*            | 250 µg      | 500 µg      |           |
|                    | *Candida albicans*                 | n.a.        | n.a.        |           |

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; n.d.: not determined; n.a: no activity
Anti-inflammatory, antipyretic and analgesic activities

The stem bark chloroform extracts of neem show efficiency against carrageenan—which induced paw edema in rats and ear inflammation in mice. Bark extracts is also used to treat inflammatory stomatitis in children. Neem oil holds antipyretic activity, its leaf extracts showed antipyretic effect when injected into male rabbits. Various extract effects as Antipyretic and Anti-inflammatory have been studied.39

Male antifertility activity

Neem leaf extracts and seed oil (NSO) used as a great spermicide and also prevented spermatogenesis, lowered sperm mobility, number and pausing of fertility. Further, NSO keeps abortifacient and anti-implantation properties. The neem leaf extracts carry the spermicidal activity against rhesus monkey and humans. Biopsy of vagina reveals no side effects, while radio isotopes examination showed non-antiiovulatory and non-absorption in vagina. These all findings supported neem oil formulation "sensal" which is used as strong contraceptive in India.39

Antiucler activity

Nimbiden was known to be responsible for antiucler effect which prevents acetylsalicylic acid, omethacin, serotonin-induced gastric lesions and duodenal ulcers or histamine.40 Azadirachta indica (neem) leaf extracts shows antiucler effect and mucus depletion inhibition and most cell defragmentation as possible mechanism. Researcher isolated the phenolic glycoside as an active constituent. Furthermore, Azadirachta indica offers good option for active antiucler and harmless drug.41

Antioxidant compounds

The process, by which free radicals are formed, is a normal task of body but the molecules formed in a result are not stable and cause damage of other cells. A number of disorders like cardiovascular disease, health of eye and muscular degeneration and even cancer occurrence due to presence of free radicals’ high quantity in a body. Neem boosts the antioxidant level and protects against chemically induced carcinogens and liver damage.

Diabetes: Neem with its extremely bitter taste and properties has been used in disorders caused due to overeating sweets. The studies on neem focused on its hypoglycemic effects.

Liver functions: It protects the liver damage, liver in turn help in cleaning the blood. Neem leaf reduces chemically induced liver damage by regulating level of serum marker enzymes and boosts count of antioxidants, like some of them present in vitamin C and E. which prevent damage by neutralizing free radicals.

Strength: Neem leaf extracts low doses have relaxing effects. The effect fades at high doses, 400 or 800 milligrams per kilograms of body weights. It’s also lesser anxiety and stress.

Vitiligo: It’s an autoimmune disorder that causes skin patches to lose its color. Neem leaves dosage is of four grams for three times per day, mostly taken before the meal. Neem oil applied to the affected area for the aid in setback of discoloration.

AIDS: Neem may help in search for preventing and therapy of AIDS. It may probable to treat by intake of neem leaf extracts or the complete leaf or by drinking a neem tea.42

Anticancer action: Cancer is a main health issue across the world and multifactorial disease. The change in molecular pathways shows role in the improvement and advancement of cancer. The handling part based on allopatic which is operative on one side but also have some adverse effects on normal cell. Previous studies stated that plants different constituents inhibit growth of malignant cells through variation of cellular propagation, apoptosis, suppression of tumor and other molecular pathways. Neem oil have different components limonoids, which inhibit mutagenic effect of 7, 12-dimethylbenz(a)anthracene.43 A research was performed to evaluate the cytotoxic effect of nimbidol present in leaves and flowers on human choriocarcinoma (BeWo) cells and results revealed that nimbidol treatment resulted in dose and growth inhibition of BeWo cells with 2.01 IC50 values and 1.19μM for7-24h, correspondingly.44-48

Biological compound of Azadirachta indica (Neem)

Neem contain different biological compound that act against different activities (Table 2).

Medicinal properties of Neem (Azadirachta indica)

Neem show great properties of medicine. Their non woody parts like flowers, leaves, bark, fruits and seeds perform different functions. Neem has antibacterial, Antifungal and different others properties.49

Some medicinal properties of Neem tree as given below (Table 3).

Mechanism of action of active compounds

Mechanism of action of active compounds of Azadirachta indica is given as,

Neem (Azadirachta indica) different parts show activity against microbes through inhibiting the growth of microbes by breakdown of cell wall. Azadirachtin, which is present in seeds of neem, is the basic constituent responsible for antifeedant activity and harmful effects in pests. Results revealed that the ethanol leaves extracts indicated antibacterial action against S. aureus with highest inhibition zones occurred at concentration of 100%.51

Neem character as free radical hunting action due to presence of Azadirachtin and nimbidol which showed concentration reliant antiradical hunting activity in the given order nimbidol > azadirachtin > ascorbate.52

Table 2: Bioactive compounds of Azadirachta indica reported in scientific literatures.

| No. | Compound Name     | Source  | Biological activity     | References |
|-----|-------------------|---------|-------------------------|------------|
| 1   | Nimbidin          | Seed oil| Anti-inflammatory        | [45]       |
| 2   | Azadirachtin      | Seed oil| Antimalarial             | [46]       |
| 3   | Nimbin            | Seed oil| Spermicidal.             | [47]       |
| 4   | Mahmoodin         | Seed oil| Antibacterial            | [47]       |
| 6   | Margolone, mergolone and isomargolone | Bark | Antibacterial            | [48]       |
| 7   | Cyclic trisulphide and cyclic tetrasulphide | Leaf | Antifungal               | [48]       |
| 8   | Gedunine          | Seed oil| Antifungal               | [48]       |
| 9   | Polysaccharides   | Bark    | Anti-inflammatory.       | [48]       |
| 10  | NB-2 peptidoglucan | Bark | Immunomodulatory.        | [48]       |
CONCLUSION

Azadirachta indica (Neem) plant acts as a medicinal plant have been found effective in the treatment of bacterial, fungal, viral and other diseases and revealed the antibacterial, antifungal, antiviral, antimalarial, antilucre and other biological activities. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics neem plant are now growing popularity in the treatment of many infections. Neem plant is considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of neem plant is essential so that better, safer and cost effective drugs for curing various diseases and infections can be developed.

DISCLOSURE

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