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Antibacterial and antifungal activities of *Azadirachta indica* A. Juss (Neem): A review

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ABSTRACT

The role of natural products in pharmaceuticals is undisputedly very significant. In this vista, *Azadirachta indica* is becoming the cynosure of modern medical science. Traditionally, this plant has been widely utilized for a diverse variety of ailments in the animal world as well as in the plant forum. An array of phytochemicals present in neem plants has been attributed to their pharmacological activities. This review attempts to summarise the antibacterial and antifungal properties of the plant and emphasises the need for further analytical research for new drug discoveries.

Keywords: *Azadirachta indica; Neem; Antibacterial; Antifungal; Azadirachtin*

INTRODUCTION

Azadirachta indica, commonly known as "Neem", belongs to the family Meliaceae under the order Rutales. It is native to the Indian sub-continent and is also commonly found in other tropical and subtropical regions of the world. This is a fast growing tree that reaches over 20 metres in height and has an erect trunk. It has pinnately compound leaves, bears axillary panicle inflorescences, and fruits as drupes.

This valuable species has been used in traditional remedies since a long time ago and has been documented in various literature related to Ayurveda, Homeopathy, and Unani medicine. The multifaceted pharmacological attributes of this plant have attracted worldwide attention and it is now extensively utilized in modern medicine.

The virtue of the medicinal properties of the neem plant is attributed to the presence of a diverse and complex range of bioactive compounds. Each specific part of the plant is a significant source of important phytochemicals, belonging to the classes of terpenes and limonoids. More than 140 biological compounds have been identified and isolated to analyse the properties they possess. Azadirachtin is the most important limonoid or teranotriterpenoid. Nimbidin is the crude principle that is extracted from the seed kernels of neem and from this crude base, some more teranotriterpenes like nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid have been reported to be isolated (Kudom, Mensah & Botchey, 2011; Ojha, 2016). Gedunin and mahmoodin are also isolated from seed oil. Other compounds extracted from the fresh leaves of neem are quercetin and ß-sitosterol.

These various bioactive constituents isolated and identified from the different parts of the plant are responsible for the wide range of therapeutic properties like anti-inflammatory, immunomodulatory, anti-diabetic, hepatoprotective, neuroprotective, antiulcer, antimicrobial, antioxidant, antimutagenic, anticarcinogenic, and wound healing (Alzohairy, 2016). A large number of workers have contributed to review and research of the bioconstituents and the biological activities of the plant for decades together (Biswas *et al.*, 2002; Subapriya & Nagini, 2005; Arora, Singh & Sharma, 2008; Atawodi & Atawodi 2009; Nishan & Subramaniam, 2014; Kumar, Mehta & Pathak, 2018).

This review concentrates and enumerates the reported evidence of the antibacterial and antifungal activities of *Azadirachta*.

LITERATURE REVIEW

Antibacterial Activity:

Several researchers have long demonstrated the antimicrobial properties of neem extract.Aqueous and alcoholic extracts of the plant parts were subjected to different assays to understand their activity against bacteria.

Khan and Wassilew (1987) documented the antibacterial activity of the extracts of the leaves, seeds, and bark of neem. The extracts exhibited inhibition against a broad spectrum of gram-negative and gram-positive microorganisms, which included *Mycobacterium tuberculosis* and strains that are resistant to streptomycin. The extracts also showed inhibition against *Vibrio cholera, Klebsiella pneumoniae, Mycobacterium tuberculosis* and *M. pyogenes* in *in vitro* conditions.

Reports of nimbolide showing effectiveness against *Staphylococcus aureus* and *S. coagulase* have been given by Schmutterer (1995).

Govindachari *et al.* (1998) first reported about the polyphenolic flavonoids - Quercetin and ß-sitosterol, which were purified and isolated from fresh neem leaf extract and known to have antifungal and antibacterial properties.

Various extracts of neem were subjected to show antibacterial activity against certain bacteria, like *Aeromonas hydrophila, Pseudomonas fluorescens, Escherichia coli* and *Myxobacteria*, which are pathogenic to fishes.

Alzoreky and Nakahara (2003) reported the antibacterial action of neem extract against *Bacillus cereus, Escherichia coli* and *Salmonella infantis*.

A number of reports on the efficacy of the mouth rinses with the formulations of the extracts of different parts of the neem plant have been put forward by many workers. These neem extract-based preparations are observed to have positive results in treating chronic gingivitis and dental plaque (Botelho *et al.*, 2008; Chatterjee *et al.*, 2011).

Aslam *et al.* (2009) showed that the phytoconstituents in neem could stop the growth of *Staphylococcus aureus*. The crude flavanoids were found to be the most effective phytoconstituents.

Susmitha *et al.* (2013) reported the antibacterial activity of aqueous leaf extracts of neem against the two human pathogens - *Escherichia coli* and *Salmonella sp*. According to Farzana, Zerin, and Kabir (2014), methanolic extract of neem showed inhibition against *Vibrio cholera* and *Klebsiella*, and only the boiled water extract of the leaves could inhibit *Klebsiella* and *Escherichia coli*.

Raut, Sawant and Jamge (2014) worked on the antibacterial activity of neem on the gram negative bacteria of *Salmonella typhii*, *Vibrio cholera* and *Escherichia coli* and gram positive bacteria like *Bacillus subtilis*. The agar diffusion well method was used to determine the activity of the extracts of different plant parts. It was reported that the leaf and bark extracts worked effectively against *Vibrio cholera* and *Bacillus subtilis*.

Standardized disc diffusion and microdilution tests were subjected to assaying the biological activity of neem oil against forty-eight isolates of *Escherichia coli*. This assay performed by Del Serrone, Toniolo and Nicoletti (2015) showed that the neem oil extract showed antibacterial activity against all the isolates.

Ethanolic extract of the neem leaves was shown to exhibit growth-inhibitory effects on Methicillin-resistant *Staphylococcus aureus* biofilm and the formation of planktonic aggregation as well as anthelmintic activity against Schistosoma mansoni worms (Quelemes *et al.*, 2015).

Mandal and Mandal (2011) reported the bactericidal activity of neem seed extract against a wide spectrum of gram-negative as well as gram-positive pathogens. This property has been reported to be due to the crude principal nimbidin present in the seed oil.

Similar effects of crude seed extracts were observed against the pathogenic bacteria that cause human eye and ear infections, including *Staphylococcus aureus*, *S. pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* (El-Mahmood, Ogbonna & Raji, 2013).

Aqueous extract of the leaves of *Azadirachta indica* along with silver salt has been used to prepare silver nanoparticles, and these nanoparticles are subjected to test the antimicrobial efficiency of the neem extract. Different concentrations of sliver nanaoparticles—ranging from 0, 2, 4, 8, 10 and 12 µg/ml—were added to the agar substrate plates with the bacterial colonies of *E.coli* and *S. aureus*, as obtained from the soil samples. The antibacterial activity induced by these particles of neem extract is by changing the permeability of the cell membrane and degrading the enzymes in the bacteria (Verma & Mehata 2016). These silver nanoparticles can be prepared by altering certain environmental parameters such as temperature and pH, as well as the concentration of reactants and reaction time during the process of production. These altered green silver nanoparticles of neem extract showed elevated levels of growth inhibition against the soil-borne bacterial colonies.

The most commonly found microorganism in the infected root canals of both primary and permanent teeth is *Enterococcus faecalis*, a facultative anaerobic gram-positive coccus. *E. faecalis* is very resistant and survives even in its stages of starvation (Bhardwaj, Ballal & Velmurugan, 2012). Among many irrigants, chlorhexidine is found to be very effective as a broad-spectrum antimicrobial agent and can remove both gram-positive and gram-negative bacteria from inaccessible areas of teeth. Chlorhexidine gluconate is also equally efficient in disinfecting the teeth. But there are a number of undesired side effects like staining of teeth, alteration of taste and even developing microbial resistance after continuous use. Due to these effects, there is a need for herbal alternatives without creating ill effects with their usage. Chandrappa *et al.* (2015) made an ethanolic extract of mature neem leaves and demonstrated its activity against *Enterococcus faecalis*. Considerably better inhibitory action against the bacteria was reported by the neem extract as compared to 2% chlorhexidine solution.

Similar results were earlier reported by Vinothkumar *et al*. (2013), who found the high efficacy of neem extract against *E. faecalis* in comparison to 5.25% sodium hypochlorite. Ghonmode *et al.* (2013) also found that neem showed high effectiveness against *E. faecalis* compared to 3% sodium hypochlorite.

Ghosh *et al.* (2016) also reported the phytochemicals derived from neem to be potential contributors to several biological activities of the plant. The plant's essential oil has strong antibacterial properties against *Aeromonas hydrophila, Alcaligenes faecalis, Pseudomonas aeroginosa*, and *P. putida*, which can cause food to go bad.

The compound, mahmoodin, a deoxygedunin which is purified and isolated from the oil of neem seed, is shown to exhibit moderate antimicrobial action against certain strains of human pathogenic bacteria (Alzohairy, 2016).

The other compounds that are reported to have contributed to the antibacterial property of neem are nimbidin, nimbin, nimbolide, gedunin, margolone, and cyclic trisulfide (Al Akeel *et al.*, 2017).

One of the most rampant oral diseases is periodontitis, which is a polymicrobial infection caused by anaerobic bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Heyman *et al*. (2017) investigated and reported a persisting bacteriostatic action of the neem extract against these two species, and the antioxidant activity was also observed to be in play in the region of inflammation.

Evident bactericidal activity against *Helicobacter pylori* is established by the usage of the neem oil extract, where it exhibited properties like increasing acidity at low pH and the ability to kill non-growing bacteria. *H. pylori*, a potent pathogen that causes gastric disorders, can be prevented from infecting by administering a formulated dose of neem extract (Blum, Singh, & Merrell, 2019).

Antifungal activity:

The effectiveness of neem against certain phytopathogenic and human infecting-fungi has been

reported and proved by various workers. Several reports documented the effective use of neem extract preparations in controlling the growth of fungi causing athlete's foot, ringworm, and those occurring in the bronchi, lungs, mucous membrane in the case of mouth cavity thrush, intestinal cavity, etc.

The fungistatic and fungicidal properties of neem stem bark extract against *Aspergillus spp*. and *Candida* spp was investigated by Fabry, Okemo and Ansorg (1996).

Sphaerotheca fuliginea (*Podosphaera fuliginea*) is the causal organism of the powdery mildew on cucurbits. Reduction of conidial germination of the pathogen by 11% was reported when subjected to neem seed extracts; thereby establishing the antifungal activity of neem (Coventry & Allan 2001).

Neem seed and leaf extracts were administered to dermatophytes such as *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum nanum*. At 15µg/mL concentration, the seed extract was found to be efficient in disrupting the growth pattern of the fungi tested. This was reported by Natarajan, Venugopal and Menon (2003).

Mondall *et al.* (2009) investigated the antifungal activity of neem extract on the two saprophytic fungi, *Rhizopus* and *Aspergillus*. The study showed growth inhibition of both the fungi with the effect of crude aqueous and alcoholic extracts of neem leaves of varying ages. *In vitro*, it was also found that the ethanolic extract of the leaves stopped the growth of both types of fungi better, and that this was true even for the extract of older leaves.

Reports of antifungal activity against phytopathogens such as *Sclerotium rolfsii* by the usage of neem cake extract and against powdery mildew of balsam by using foliar spray of neem cake extract were given by Singh *et al.* (2010). Bhonde, Deshpande and Sharma (1999) documented the other pathogenic fungi whose growth is effected by the extract of neem cake are *Fusarium oxysporum, Alternaria solani,Curvularia lunata* and *Helminthosporium sp*. Absolute growth inhibition of *H. pennisetti* by using neem cake extract at 0.6% drug concentration. This is achieved by inhibiting spore germination of the sporulating fungus. It inhibited *Colletotrichum gloeosporioides* f. sp. *mangiferae* as well (Kumari *et al.*, 2013).

Candida albicans is the most commonly persisting fungal species in root canal infections and other dental infections like candidiasis. For successful pathogenesis by the fungus, adhesion to the target tissue is the most important prerequisite for colonization. The neem extract has been tested and found to be a promising candidate for inhibiting fungal colonisation in the substratum.The neem leaf extract works in an antiadhesive mode for the fungus, with effects on its hydrophobicity and biofilm formation (Polaquini *et al.*, 2003).

Gas Chromatography and Mass Spectroscopy analysis was performed for n-Hexane extract of *Azadirachta indica* leaves. 45 biocompounds were identified and 33 of them were reported to have antifungal activity. The study was observed to demonstrate the activity against *Candida albicans* (Akpuaka *et al*., 2013).

Rhizoctonia solani and *Sclerotium rolfsii* are phytopathogenic fungi which were subjected to nano emulsions of neem oil along with citronella essential oil. The nano emulsions were developed and characterised using Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM). The standardization of composition of the nano emulsions with their antifungal action against the two fungi was a pioneering report (Ali *et al.*, 2017).

All these reports of the antibacterial and antifungal properties of *Azadirachta indica* dictate the medicinal novelty of the plant in its entirety, where each part of the plant is a reservoir for novel compounds possessing immense biological significance.

CONCLUSION

For the enormous benefits that the neem plant caters to in various areas of health, this plant has rightly been labelled as' Sarvarogharini 'in the Charak Sanhita of Ayurvedic literature. This implies

that, traditionally, the plant has been hailed as the complete and perdurable component for curing any sickness. The variety of phytoconstituents present in the plant has rendered significant therapeutic values like antibacterial and antifungal properties. Scientific advances in the field of modern medicine have augmented the plant's utility in different pharmacological areas. *Azadirachta indica* is now the epicentre of medical research, and more upgraded exploration and analysis of its natural biocompounds would significantly benefit humankind therapeutically.

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