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Antimicrobial Potential of *Azadirachta indica* Against Pathogenic Bacteria and Fungi

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Drugs from natural sources are used for treating various diseases since the ancient times. From the literature it is clear that various type of pharmacological and biological activities are associated with *Azadirachta indica*. The leave oil of *A. indica* is known to have good antimicrobial potential. The oil of *A. indica* leaves, was tested against the different infectious microorganisms [Gram positive bacteria and Gram-negative bacteria], such as bacterial strains; *S. aureus*, *E. coli*, *B. cerus*, *P. vulgaris*, *S. typhi*, *K. pneumoniae*, *S dysenteriae* and Fungal strains; *F. oxysporum*, *A. flavus*, *A. fumigates*, *A. niger*, *C. albicans*, *Cladosporium* sp., *M. canis*, *M. gypseum*, *T. rubrum*, *T. mentagrophytes*, *P. notatum* and *P. citrinum* etc. The results showed that level of antimicrobial activities of the *A. indica* oil depends on both the protein and carbohydrate contents. Generally, the high level of protein and carbohydrate contents of extract had better antimicrobial activities.

Keyword: *Azadirachta Indica*, Antimicrobial Activity, Antifungal Activity, Pathogenic.

1. Introduction

Nature has provided a complete store house of remedies to cure all ailments of mankind. The natural or herbal remedies are still the backbone of medicines. Phytotherapy is a medicinal practice based on the use of herbal plants and their extracts. These herbs or plants and their active ingredients are used in traditional herbal remedies. The easy availability, low cost and negligible side effects, natural products are popular in the nowadays in the world^[1-7]. All the herbs produced bewildering variety of phytochemicals like primary metabolites [carbohydrates, fats, proteins] and secondary metabolites [Alkaloids, flavonoids, steroids, saponins, polyphenols, etc.] for their normal metabolic activities^[8-10]. These secondary metabolites showed various biological activities

and act in plant defense mechanisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. The secondary metabolites have therapeutic actions, which produced drugs^[11].

1.1 *Azadirachta indica*: Neem tree [*Azadirachta indica*] is a tree in the mahogany family Meliaceae, is evergreen tree found in most tropical countries. It is one of two species in the genus *Azadirachta*, native to India and Burma, growing in tropical and semi-tropical regions. It is a fast growing tree, average height 15-20 m but rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. For thousands of years the beneficial properties of neem have been recognized in the Indian tradition^[12-14]. Each part of the neem tree

has some medicinal property^[15,16]. Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide^[17-20]. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children^[21]. The bark extract is also used as tonic, astringent and useful in relieving fever, thirst, nausea, vomiting and skin diseases [8]. The immunomodulatory activity of the neem-bark extract has also been reported^[22]. The medicinal and industrial uses of various parts of neem tree and the compounds isolated have been reviewed^[23]. The bark powder contains sugar, proteins, amino acids and oil [Subramanian and Lakshmanan. 1996]. Polysaccharides such as arabinogalactans and fucogalactoglucoarabinanes have also been isolated from neem bark^[24]. Flavonoids, flavone glycosides, dihydrochalcones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem^[25].

1.2 Medicinal Uses: European "Materia Medica" have acknowledged neem tree as "Panacea of all Disease". However in India it is famous with many other names like 'Divine Tree', "Heal All", "Nature's Drugstore", and "Village Dispensary". Traditional and Ayurvedic uses of neem include the treatment of fever, leprosy, malaria and tuberculosis. Various folk remedies use as an anthelmintic, antifeedant, antiseptic, diuretic, emmenagogue, contraceptive, febrifuge, parasiticide, pediculicide and insecticide^[26-30]. Traditional uses of twigs for brushing teeth as effective forms of dental care. Neem oil is useful for skin care such as acne, and keeping skin elasticity. Traditionally, patients suffering from Chicken Pox sleep on the leaves in India owing to its medicinal value^[17]. In Ayurvedic, Unani and folklore traditional medicine, different parts of neem were preferred in the treatment of a wide range of afflictions. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity. The extract or oil of neem is effective against inflammatory, analgesic, antipyretic activities, and immunomodulatory activities^[22]. It

also shown an immune-stimulant activity, anti-diabetic, antiulcer effect, *in-vitro* spermicidal. *In vivo* studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy. Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception. Oral administration of aqueous extract of neem leaf also shows antifertility effect in mice. The mechanism of action of neem oil appears to be non-hormonal, probably mediated through its spermicidal effect and may have less side effects than steroidal contraceptives. Neem extracts are effective against malarial parasites, both chloroquin-resistant and sensitive strains of malarial parasite. Recently, seed extract 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*. The oil possesses a wide spectrum of antibacterial action against various microorganisms, including *M. tuberculosis* and streptomycin resistant strains. Leaf extract offers antiviral activity against Vaccinia virus, Chikungunya and measles virus *in vitro*. The leaf extract effectively suppresses oral squamous cell carcinoma. The extract of neem leaf was found to offer protection against paracetamol induced liver necrosis. 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 aqueous leaf extract. The antioxidant activity of neem seed extract has been demonstrated *in vivo*, which is associated with low levels of lipoxxygenase activity and lipid peroxides. Varying degrees of central nervous system [CNS] depressant activity in mice was observed with the leaf extract. The extract of stem barks and root bark showed hypotensive, spasmolytic and diuretic activities^[12-16].

1.3 Phytochemicals: More than 135 compounds have been isolated from different parts of neem. The compounds have been divided into two major classes: isoprenoids [like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin

and its derivatives, vilasini type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin] and non-isoprenoids, which are proteins/amino acids and carbohydrates [polysaccharides], sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. Condensed tannins from the bark contain gallic acid, [+] gallo catechin, [-] epicatechin, [+] catechin and epigallocatechin, of which gallic acid, [-] epicatechin and catechin three tricyclic diterpenoids, margolone, margolonone and isomargolonone isolated from neem stem bark^[31,32]. A polysaccharide extracted from bark inhibits carrageenin-induced inflammation in mouse. Sulphur-containing compounds such as cyclic trisulphide and tetrasulphide isolated from the steam distillate of fresh, matured neem leaves have antifungal activity against *Trichophyton mentagrophytes*. 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 tetranortriterpenoids, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Neem oil also contains steroids [campesterol, beta-sitosterol, stigmasterol] and a plethora of triterpenoids of which Azadirachtin is the most well-known and studied. The Azadirachtin content of Neem Oil varies from 300 ppm to over 2000 ppm depending on the quality of the neem seeds crushed. The chemical composition of neem leaves was characterized by low values of lipid, respectively^[33,34]. A search for the biological activities of oily extracts, *in vitro*, done to evaluate them as antimicrobial agents.

Table1. Average composition of neem oil fatty acids

| Common Name | Acid Name | Composition range |
|------------------|----------------------|-------------------|
| Omega-6 | Linoleic acid | 6-16% |
| Omega-9 | Oleic acid | 25-54% |
| Palmitic acid | Hexadecanoic acid | 16-33% |
| Stearic acid | Octadecanoic acid | 9-24% |
| Omega-3 | Alpha-linolenic acid | ??% |
| Palmitoleic acid | 9-Hexadecenoic acid | ??% |

1.4 Antimicrobial Activity: An antimicrobial kills or inhibits the growth of microbes. They are used for cures of microbial infections. Various types of antimicrobials are used such as antibiotics, synthetic and natural compounds. However, prolonged use of mostly antibiotics can decrease the number of gut flora and causes resistance, which can have a negative impact on health. Prolonged courses of antibiotic also cause serious side effects. Antifungal agents work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes cells. These cells are similar at the molecular level. Consequently, there are often side-effects to some of these drugs. Some of these side-effects can be life-threatening if the drug is not used properly^[35-40].

1.5 Antibacterial and Anti-Fungal Activities:

The antibacterial activity of Neem leaves oil against various bacterial and fungal strains. The Neem oil showed considerably activity against bacterial [Gram-positive bacteria: example, *Staphylococcus species* and the Gram-negative bacteria: example *Escherichia coli*] and fungal strains. The antibacterial activity against microbial cultures namely: Bacterial Strain; *Escherichia coli*, *Bacillus cerus*, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Shigella dysenterae* and Fungal strain; *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Candida albicans*, *Cladosporium sp.*, *Microsporium canis*, *Microsporium gypseum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Penicillium notatum* etc. The oil was not able to inhibit *Proteus vulgaris*. It was observed that the oil exhibited inhibitor effects against most of the microorganisms tested. The antifungal activity of neem oil against above fungal strains showed considerably activity. Moreover, the aqueous extract of plant has been previously reported to show antifungal activity^[41,42]. In this study the antibacterial and antifungal activities of the extracts from leaves oil. The crude oil is generally active against bacteria and fungi. In the

light of these results we can conclude that level of antimicrobial activities of the Neem oil was compared with the chemical composition: activity relationship of extract^[43].

2. Discussion: Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. The search for eternal health and longevity and for remedies to relieve ailment or discomfort early to explore his immediate natural surroundings and led to the use of many plants and the development of a variety of therapeutic agents^[44,45]. Today, there is a renewed interest in herbal medicines and an increasing demand for more drugs from plant sources. The green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Nature has a very rich botanical wealth and a large number of diverse types of plants. The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times^[46-49].

Neem oils were screened for potential antibacterial activity against medically important bacterial strains, namely *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *B. cereus*, *E. coli* and *S. typhimurium* etc. *P. aeruginosa* and *S. typhimurium* were the most resistant strains while the most susceptible bacterial strains were *B. cereus* and *P. mirabilis*. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs [antimicrobials]. Natural products are known to play an important role in both drug discovery and chemical biology^[51-53]. Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents, to give its therapeutic value. A number of such studies have been done in various places of the world^[54-55]. There are several reports on the antimicrobial activity of different herbal extracts.

3. Conclusion: Neem oil has already been established to possess antimicrobial activity. In this study, Neem oil showed antimicrobial

activity revealed the significant antimicrobial potential of the oil against various strains of bacteria and fungi. However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms are becoming resistant more quickly than new drugs are being found. Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means. Many of plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. So, it is worthwhile to study plants and plant products for activity against resistant bacteria.

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