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Therapeutic activity study in fungal disease by *Azadirachta indica*.

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Abstract

The aim to present that fungal infection is one of the most common skin problems throughout the world. There are broad choices of treatment from liquid to semi-solid dosage forms. Preparation has more than two or two herbal ingredients called polyherbal preparation. As compared to synthetically produced drugs, phytochemicals are a better source of medicine. Drug originated from herbal origin used in traditional system of medicine such as Unani, Siddha, tribal and Ayurveda medicines since pristine times, Ayurveda is most broadly accepted system of medicine.

For few studies shows antifungal and antimicrobial effect on fungal and microbial growth Azadirachtin that is a tetranortriterpenoid present in seeds of plants. The extract of neem leaves gives antibacterial activity. Due to antioxidant properties, neem plays an important role as free radical scavenging.

This can be extract by following method-infusion, maceration, percolation and decoction which shows best effect in antimalarial activity, antiviral, antifungal, anti-diabetics, anticancer, anti-inflammatory, and antioxidant and immuno modulator and growth etc. There is a need of reduction in the use of chemical substance as anti-fungal agents to fight infections caused by fungal that are resistant to the use of synthetic anti-fungal agent.

Keywords: Fungal, Polyherbal, Antimalarial activity, Anti-viral, Antifungal.

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Introduction

Fungal infection is one of the most common skin problems throughout the world. There are broad choices of treatment from liquid to solid dosage forms. Preparation has more than two or two herbal ingredients called polyherbal preparation [1]. Among the topical preparation, gels have broadly recognized. Due to its high effectiveness against fungal infection, the polyhedral formulation is popularly known's [2]. Many effective drugs originated from traditional medicinal practice, it is necessary to study the herbal medicinal plant for their effect [3]. Fungal infection is a skin infection caused by Candida is more popular than *Pseudomonas species*, *Escherichia coli*, and *aspergillosis* species. It is the most common infection worldwide. This infection causes on the nails, hair, skin, and other body parts, and is difficult to treat. As compared to synthetically produced drugs, phytochemicals are a better source of medicine [4]. Drugs originated from herbal origin used in traditional system of medicine such as Unani, Siddha, tribal and Ayurveda medicines since pristine times, Ayurveda is most broadly accepted system of medicine [5].

Hydrogels are hydrophilic and polymeric in form, having the capacity of absorbing a large volume of water or biological fluids. It is available in dry or as a hydrated gel in DDS for single use. It is soluble in water. The leaf extract of *Azadirachta indica* has also been used as the biological activity.

1. Anti-fungal
2. Anti-cancer
3. Anti-diabetic
4. Anti-ulcer
5. Anti-fertility

Use of azadirachta

1. Neem's small branches used as a toothbrush.
2. Oil of Neem is utilized in herbal gel, soap, shampoo, and toothpaste.
3. Neem oil is used in acne treatment and keeping skin healthy.
4. Neem oil is also used in the manufacturing of mosquito repellent.
5. All parts of the Neem tree are utilized in pharmacy companies for making various useful formulations or drugs.

6. Neem is a fast-growing tree that can reach a height of 15 to 20 m, rarely to 35 to 40 m, it is evergreen [6].

Biological sources of Azadirachta indica L

Neem tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical areas like India, Bangladesh, Pakistan, and Nepal. It's far a match is a quick-growing tree with a trunk diameter of four-five toes and a peak between 20 and 23 m. As a quick-developing tree, it is 20-23 m tall with an immediately trunk and a diameter around 4-5 feet. The leaves are compound, imparipinnate, with 5-15 feet. Its end result is inexperienced drupes which flip golden yellow on ripening in the months of June-August [7] (Tables 1 and 2).

Table 1. *Synonyms of neem tree.*

S.no	Language	Synonyms
1	Sanskrit	Nimbakavrksa
2	Hindi	Neem
3	English	Neem tree
4	Tamil	Neem
5	Malayalam	Neem
6	Punjabi	Neem
7	Marathi	Kadunimbaci

Table 2. *Scientific classification of the neem tree.*

S.no	Classification	Name
1	Kingdom	Plantae
2	Sub-kingdom	Tracheobionta
3	Domain	Eukaryota
4	Class	Magnoliopsida
5	Order	Sapindales
6	Phylum	Spermatophyta
7	Subphylum	Angiospermae
8	Family	Meliaceae
9	Genus	Azadirachta A. Juss. (azadirachta)
10	Species	*Azadirachta indica*

Botanical description of neem

Neem tree has a place in the family meliaceae that is found in plenitude in tropical and semitropical areas like India, Nepal, Bangladesh and Pakistan. It may be a fast-growing tree 20-23 m tall and the trunk is straight and encompasses a diameter around 4 to 5 ft. The clears out are compound, imparipinnate, with each comprising 5-15 pamphlets. Its natural products are green drupes which turn brilliant yellow on maturing within June-August [8].

Active constituents of Azadirachta indica L

Azadirachtin is a tetranortri terpenoid of the elegance of limonoids that provides the chemical method C35H44O16 and a molecular weight of 720.71 g mol-1 with other compounds obtained from the seeds of Neem, specifically Nimbin and Salanin. The currently recognized chemical shape of AZA become first defined in 1976 [9] (Figure 1).

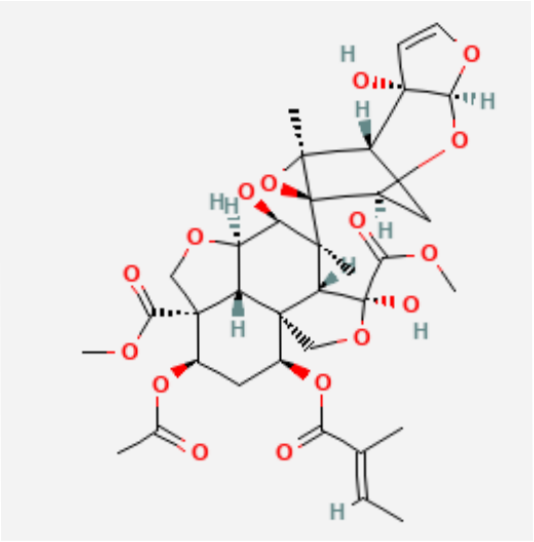

Figure 1*: Structure Azadirachta.*

It changed into the application of NMR and X-ray crystallographic evaluation that authorized to affirm and recognize the proposed chemical structure for AZA. AZA is especially oxidized compound that gives an extremely complicated shape with a rigid conformation due to the presence of intermolecular hydrogen bonds and a large wide variety of reactive useful businesses in extremely near positions. Similarly, to an inflexible conformation, AZA has a specific set of oxygenated functionalities [10]. The chemical structure of AZA incorporates 16 stereo genic centers, seven of which can be tetra substituted carbon atoms and nine are disubstituted carbon atoms. Moreover, the shape of AZA incorporates 16 oxygen atoms organized in 4 ester companies, one hemiacetal institution a done epoxide institution, two hydroxyl agencies (secondary and tertiary) anode dihydrofuran group [11,12].

Mechanism of action azadirachta

Azadirachta indica plants parts gives Antifungal and antimicrobial effect on fungal and microbial growth Azadirachtin is a tetranortriterpenoid present in seeds of plants, is the important Substance responsible for both antifeedant and toxic effects in the insects. Results suggest that the extract of neem leaves gives antibacterial activity. Due to antioxidant properties, neem plays an important role as free radical scavenging. Neem ingredient plays an important role in the management of cancer through the cell signaling pathways. Neem harmonizes the activity

of various tumor suppressor genes. Neem also shows anti-inflammatory activity *via* regulation of enzymatic activities including cyclooxygenase and lipoxygenase enzyme [13].

Impacts on marked production have been blocked vitellogenesis and slowing the development of testes. Azadirachtin, salanin and other limonoids from seeds inhibit ecdysone 20-mono-oxygenase, an enzyme that makes a final step away. Ecdysone in the active hormone 20-hydroxyecdysone. The results obtained are very important in explaining the obvious visual effects. In the whole animal in preventing growth, growing disability and infertility, however your results are probably secondary caused by the main mode or modes of action in cell division and microtubule formation in cells. All the effects of azadirachtin on recorded physiology of insects, perhaps the most important effect on actively reproducing cells, for example, wing discs in developing larvae, body fat, ovaries (Figure 2).

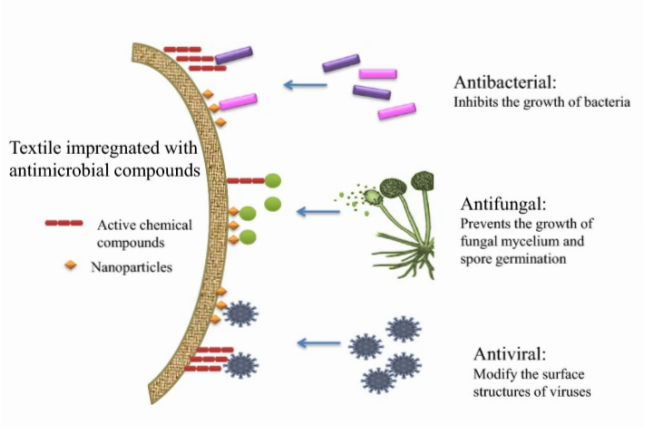

Figure 2*: Textile impregnated with antimicrobial compounds.*

Extraction Methods

Infusion

The *Azadirachta indica* leaves have been used to treat fungal infections. The beakers made using wood are filled with water and kept overnight to give 'Beeja Wood Water'. Prepared the aqueous extract of *Azadirachta indica* leaves by keeping them for about 12 hours in a beaker containing 100 ml. distilled water. The brown-colored aqueous extract with a light blue shade on the surface was collected in the morning and concentrated on the water bath. The product was dried using a rotary evaporator, finally dried in sunlight, and powdered. The aqueous extract was prepared by using 24 hours infusion with the strength of 1:8 which was prepared daily and consumed. By 7 days infusion in the same strength, they were also prepared. Prepared the aqueous infusion of *Azadirachta indica* by soaking 50 grams leaves in 500 ml. distilled water and allow to stand overnight at room temperature. The liquid was collected and the final volume was adjusted so that 10 ml of infusion represented 1 gram of the crude drug. The infusion was stored in a tightly sealed container in the refrigerator at 4°C for subsequent use [14].

Ground the leaves into a fine paste and soak in an equal volume of water, stirring occasionally and allowing to overnight. The obtained pulp was filtered and then the filtrate dried at reduced temperature after that lyophilization, the aqueous extract was collected.

Grinded the leaves of *Azadirachta indica* in an equal amount of water, stirred intermittently, and then allow to overnight. The pulp was then filtered by using a coarse sieve and the filtrate was dried at reduced temperature. Prepared the aqueous extract by grinding the leaves of *Azadirachta indica* to a fine paste, then paste obtained was soaked in 1500 ml of distilled water and stirred occasionally and then left stationary. After allowing standing for 36 hours, Filtered the resultant mixture by using a sieve with muslin cloth and obtained filtrate was completely lyophilized to dry powder. The resultant dry powder was stored at a low temperature [15].

Decoction

50 to 100 gram drug for decoction preparation recommended by the Ayurveda Pharmacopoeia 1990. In one study, leaves of *Azadirachta indica* were pulverized, boiled with water for 15 minutes until the volume was reduced to less than 100 ml, and filtered, and volume was adjusted to 100 ml to obtain an extract, 1 ml of which corresponds to 1 gram of the drug. Prepared the decoction of *Azadirachta indica* leaves in boiling water and then spray after drying it [16].

Maceration

Chopped the leaves of *Azadirachta indica* into small pieces and extracted them in absolute ethanol for 1 week. Collected the leaves and cut them into small pieces. Maceration was done with methanol for 7 days. The extract was dried by using vacuum drying and stored in a refrigerator until further use [17].

Percolation

As the term shown, percolation is a continuous flow process of the solvent through the crude drug material to obtained extract. In this process, first the powdered the drug is treated with sufficient amount of menstruum to make uniformly wet. Damp material is allowed to stand for 15 minutes, and then material transferred to a percolator, which is generally a V-shaped vessel open at both ends. To add sufficient menstruum to saturate the drug. The lid is placed on the top. When the liquid starts dripping from outlet of percolator, the lower opening is closed. The drug material is allowed to macerate for 24 hours and then percolation process is continued using sufficient menstruum to produce 1,000 ml of solution. The percolation process is flow dependent of solvent by the powered drug, and it result the product of more concentration than maceration process [18].

Therapeutic role of azadirachta indica on Health care management

Active ingredients of neem play a role as chemo preventive *via* regulation of cellular pathways and play an important role in the diseases curing through activation of an anti-oxidative enzyme, rupture the cell wall of bacteria and. Pharmacological activities of neem are given below [19] (Figure 3).

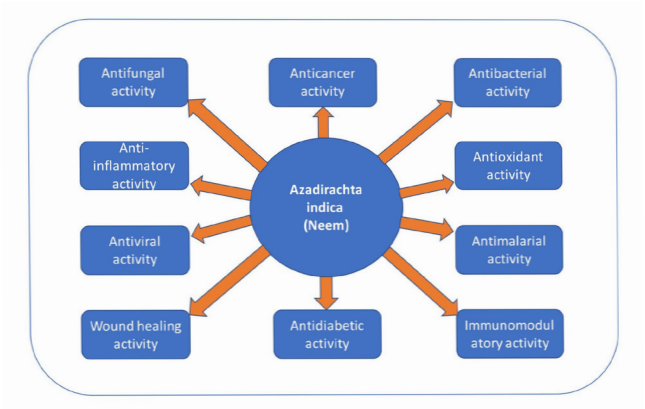

***Figure 3**: Therapeutic activities of Azadirachta indica L. (neem) in diseases prevention and treatment through the modulation of various activities.*

Antifungal activity

An experiment was performed to evaluate the action of *A. indica* leafs extracts on seed-borne fungi rhizopus and aspergillus and results cleared that growth of both fungal species was inhibited with both water and alcoholic extract. Further high alcoholic extract of neem leafs was highly effective if compared to aqueous extract for the growth of both fungal species. A result of the study inform that methanol and ethanol extract of A.indica showed growth inhibition against Aspergillus flavus. Aqueous extracts of parts of A.indica such as neem oil, have antifungal activities, and have been confirmed by earlier evaluation. A study was performed to investigate the antifungal efficacy of *A. indica* against alternaria solani sorauer (fungal pathogen) [20].

Anticancer activity

Cancer is a multifactorial disease and a major health problem all over the world. The modification in genetic pathways plays a role in the development of cancer. The allopathic treatment module is effective on one side but gives an adverse effect on a normal cell. Earlier studies show that plants constituents show inhibitory effects on the growth of malignant cells through modulation of cellular proliferation, tumor suppressor gene, apoptosis, and various other molecular pathways. *Azadirachta indica* contains flavonoids and other ingredients that play the most important role in the inhibition of cancer growth. A large number of epidemiological studies proposed that a high intake of flavonoids might be correlated with decreased risk of cancer [21].The part of the neem have been effectively givento cause cancer cells death by promoting autophagy and inducing apoptosis. Leaf extracts evaluated for their increased apoptotic effects in various human cancers such as leukemia, prostate, cervical and breast cancer.Another study was conducted by Morris J et al. demonstrated that a highly pure neem leaf extract effectively decreased the pro-cancer inflammatory cytokines and caused disruption of cell signalling and cell migration [22].

Antibacterial activity

An experiment was performed to formulations and control (base) was tested for their antibacterial activity by Disc plate method. It was tested on nutrient medium against *B. subtilis*, *S. aureus, A. niger* and *E. coli* which are representative types of Gram-negative organisms and Gram-positive organisms. The activity was detected by measuring the diameter of zone of inhibition recorded. The test strains of *A. Niger, B. subtilis, S. aureus*, and *E. coli* were collected from Dept. of Microbiology, YC College, Karad (415124), and Maharashtra, India. The plates were incubated at 37 ± 1ºC for 24 h and were inoculated with test cultures. Next day, the wells (6 mm diameter) were made with help of 6 mm diameter cork borer and the wells were loaded with prepared formulations namely A, B and C along with base as a control [23].

After 24 h of incubation, the test determined the efficacy of the product in terms of zone of inhibition of the organism. A study was also conducted to investigate the antimicrobial activity of herbal alternatives as endodontic irrigants and compared with the standard irrigants sodium hypochlorite and confirmed findings that extract of leaf and grape seed constituents (extracts) represented zones of inhibition that they show antimicrobial activity. Furthermore, leaf extracts represent increased greater zones of inhibition than 3% sodium hypochlorite [24].

Anti-inflammatory activity

Plants or their extracted constituents act as anti-inflammatory agents. A result of the study has represented that extract of *Azadirachta indica* leaves at a dose of 200 mg/kg, showed significant anti-inflammatory action in cotton pellet granuloma assay in rats. Another study results show that *Azadirachta indica* leaf extract gives significant anti-inflammatory action, but it is less effective than dexamethasone and the result of the study suggests that nimbin suppresses the functions of neutrophils and macrophages relevant to inflammation. The earlier studies showed the anti-inflammatory and immuno modulatory action of leave and bark extracts and anti-inflammatory and antipyretic activities of oilseeds. The experiment was conducted to evaluate the analgesic action of neem seed oil on albino rats and the results of the study represented that the analgesic activity of *Azadirachta indica* seeds oil showed, an analgesic effect in the dose of 2 mL/kg and oil has dose-dependent analgesic activity [25]. Another study was conducted to check the anti-inflammatory effect of neem seed oil on albino rats using carrageenan-induced paw edema and results revealed that Neem seeds oil gives

increased inhibition of paw edema with the increase in dose from 0.25 mL to 2 milliliter/kilogram body weight. At the dose of 2 milliliter/kilogram body weight, neem seeds oil gives maximum (53.14%) inhibition of edema at the 4th hour of carrageenan injection. studies result concluded that the treated animals with 100 mg/kg, a dose of carbon tetrachloride extract of *Azadirachta indica* fruit skin and isolated and extracted ingredient azadiradione showed anti-inflammatory activities [26].

Antioxidant activity

Reactive oxygen species in the genesis of various diseases are the main culprits. However, neutralization of the activity of reactive oxygen is an important step in diseases prevention. Antioxidants deactivates the free radicals, before they attack targets in biological cells, and play important role in antioxidative enzyme activation that plays a role in the control of damage caused by reactive oxygen species. Medicinal plants have been investigated to have antioxidant activity. Plants seeds, fruits, leaves, oil, roots, and bark show a role in diseases prevention due to their rich source of antioxidants. Leaf extracts of *Azadirachta indica* have been studied for antioxidant activity and results of the study clearly represented that the tested leaf extracts of neem have significant antioxidant properties. Another study was conducted based on fruits, leaves, flowers, and stem bark extracts from the *A. indica* tree to assess the antioxidant property and results suggest that extracts of stem bark, flower, and leaf have strong antioxidant efficiency [27].

Antiviral activity

Studies results represented that *A. indica* bark extract blocked herpes simplex virus (HSV)-1 entry into cells at concentrations ranging from 50-100 μg/milliliter.Leaves extract of *A. indica* has shown virucidal activity against coxsackie virus B-4 as suggested *via* virus inactivation and yield reduction assay besides interfering an early event of its replication cycle [28].

Antimalarial activity

The study was conducted to evaluate the antimalarial activity of extracts using Plasmodium berghei infected albino mice model and results disclose that A.indica leaf and stem, bark extracts reduced the parasitemia in infected mice model by 51%-80% and 56%-87%, respectively, and one other studies showed that limonoids and other azadirachtins present in A.indica extracts are effective on malaria vectors [29].

Wound healing effect

Various plants and their active constituents play an important role in wound healing. A study was conducted to investigate the wound healing effect of the extracts of *Azadirachta indica* using incision wound models in Sprague Dawley rats and results showed that extract of plants raises the wound healing effect in incision wound models [30]. Furthermore, in incision wounds, the strength of the wound healing tissue of plants treated groups was found to be importantly higher as compared to the control group. Other results displays that extract of leaves of neem provide wound healing action *via* increased inflammatory action and neovascularization [31].

Anti-diabetic activity

Diabetes or the less of control over glucose level in the blood is rapidly rising as one of the major chronic degenerative dis-orders. Conservatively by 2030, there is expectancy for diabetes to be the 11th leading cause of death throughout the world. As the disease progresses, it becomes a chronic condition to the patient, therefore low cost treatments become necessary. Among the various methods and pharmacotherapies being developed, the use of Neem extracts has steadily grown in interest briefly; there are two types of diabetes. On both types of diabetes, there have been studies of Neem extracts for their effects, we caution the direct use of Neem extracts as they continue being researched for both effects and toxicity. Type I diabetes have an early onset, due to the lack of come from the pancreatic β-cell to produce insulin. While a combination of an excessive caloric intake in genetically and sedentary lifestyle recognizable individuals leads to cause of diabetes type II, in which insulin resistance is the principal sinner of glucose intake by fat and muscle cells [32].

As a final opinion, now we can say that use of Neem leaf extract have shown positive results in the reduction of glucose and overall pancreatic healthy condition as mentioned by McCalla and Patil et al, as well as a obstacle of liver and kidney damage, and recovery in antioxidant system given by Basir et al, in murine models, giving an important details in the possible use of these compounds [33].

Immuno modulatory and growth

A study was conducted to evaluate the immuno modulatory, growth-promoting effect of *A. indica* leaves infusion on broiler chicks, and results represent that *A. indica* infusion successfully increases growth performance, antibody titer, and gross return at the level of 50 milliliter/liter of fresh drinking water. Another study evaluated the effects of the feeding of neem leave dry powder on cell-mediated immune responses, humoral and in broilers and results represent that *A. indica* (2 gram/kg) treatment improved the antibody titers against New Castle Disease Virus (NCDV) antigen [34]. *In vivo* activity of neem (*Azadirachtaindica*) fungal biofilms are resistant to varying degrees in both AMBs and triazole antifungal. Extracellular (1→3)-β-D-glucans makes a large part of the biofilm matrix directly bind again sequester AMB [35,36].

AMIC MICs are 4 to 8 times higher ifthe drug is tested *in vitro* against Candida spp. or as per gillusspp. Grown

in biofilm vs planktonic conditions. However, LAmB and ABLC retain much of the antifungal activity against biofilm embedded organisms, suggesting that lipids can protect AMB from glucans biofilm matrix, and thus have a better *in vitro* function than AMB against a fungus embedded in biofilm. In an *in vitro* model modified *in vivo* catheter lock, 4 hours LAMB exposure to a combination of only 0.2 mg/mL reduced metabolic activity [37]. *C. albicans*, *C. glabrata*, and *C. parapsilosis* at least 75% in 12-hour biofilms. In comparison, the same yeast in 5-day-old biofilms is also easily absorbed LAMB but only 1.0 mg/mL. In an *in vivo* study of rabbits with catheters that live indoors with 3 days of *C. albicans* biofilms, LAmB at 10 mg/mL closed catheter 8 hours a day for 7 days. By at the end of the study, liposome-treated catheters were not available biofilms and all catheter cultures were bad, while controlling. The catheters contained many biofilm patches and all parts of the catheter reduce good yeast cultures.

Given the frequency of Candida infections in four patient surine catheter, internal LAMB use has been the norm tested in a preclinical model of C-climb, Albicans urinary tract infection. Management of 200 μgL AmB transurethrally (drug washing) daily for 5 to 24 days hours after the yeast challenge to reduce the yeast so that it is not visible bladder levels compared to untreated mice about 1000 units form colonies per gram.

Conclusion

A. indica (Neem) and its active constituents have therapeutics action and have been traditionally used throughout the world especially in Indian regions since ancient time. Clinical studies confirmed that *A. indica* plays an important role in fungal disease prevention and treatment. The phyto constituents of the indica plant had major pharmacological action. Herbal formulations demand increasing in the world market due to their safety or less side effect. Popularity of natural plant products in diseases shows prevention and treatment. Phytochemicals of various plant species has indicated that the phytochemicals could be a better source of medicine as compared to synthetically produced drugs for the treatment of infection. There is a need of reduction in the use of chemical substance as anti-fungal agents to fight infections caused by fungal that are resistant to the use of synthetic anti-fungal agent. The substances derived from plants extracts or essential oils, can play a fundamental important role.

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