WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Review on Anti-Bacterial Activity on Azadirachta Indica (Neem)

[1]Mr. Mahadik Suraj Santosh, [2]Mr. Phanse Milind D.

[1,2]MSS` College of Pharmacy, Medha.

ABSTRACT

Azadirachta indica, commonly known as neem, nimtree or Indian lilac is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to the Indian subcontinent. It is typically grown in tropical and semi-tropical regions. Neem trees also grow on islands in southern Iran. Its fruits and seeds are the source of neem oil. Different parts of this tree contain numerous types of ingredients such as azadirachtin and quercetin and limonoids such as nimbin, nimbidin, and nimbinin with diverse pharmacological activities. Neem tree parts have also been used as a general folk medicine, and more recently, its constituents have been purified and found to possess greater antioxidant, hepatoprotective, antimicrobial, and anticancerous activities. This review presents an overview of the health-promoting effects of neem and its ingredients through modulation of biological activities.

Keywords: Azaridichta indica, folk medicine, antibacterial activity, antitumor activity, Antioxidant activity, Antimicrobial activity, nimbinin, quercetin.

Introduction

- Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent earlier studies on Neem have showed that it contains active substances with multiple medicinal properties.
- Different phytochemicals such as quercetin and azadirachtin and liminoids such as nimbin, nimbinin, and nimbidin have been purified from the different parts of the plant. Moreover, the leaves also contain mixture of compounds such as nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, nimbiol, various amino acids, and several other types of ingredients.
- Neem tree extracts have been extensively used in health management since ancient times and have a variety of health-promoting properties.
- The purpose of the present study was to investigate the antimicrobial activity of Neem leaves against human pathogenic bacteria, including Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Bacillus pumilus.
- The leaf extract has been used therapeutically for its antimicrobial effects against dental pathogens. Due to neem's vast therapeutic applications, products from the plant were manufactured for various ailments such as cancer, digestive disorders, AIDS and skin diseases.

Objectives

1. To study chemical constituent present in Azadirachta indica.
2. To study antimicrobial activity of Azadirachta indica.
3. To study solvent used in Azadirachta indica.
4. To study the microorganisms which shows antimicrobial activity.

Plant Profile

- Neem trees can reach 15–30 metres (49–98 feet) in height and have attractive rounded crowns and thick furrowed bark. The compound leaves have toothed leaflets and are typically evergreen but do drop during periods of extreme drought. The small fragrant white flowers are bisexual or staminate (male) and are borne in clusters in the axils of the leaves. The fruit is a smooth yellow-green drupe and has a sweet-flavoured pulp.
- **Botanical Name:** Azadirachta indica.
- **Synonyms:** M**argosa, Indian Lilac.**

- **Common Name :** Nim, Margosa, Neem
- **Plant Family :** Meliaceae
- **Plant Kingdom :** Plantae
- **Plant Form :** Tree
- **Occurrence (Area):** Dry forest areas of South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia.
- **Habit:** A tall tree. Grow up to 30 m tall and 2.5 m in girth.
- **Leaves :** The compound (pinnate) leaves are alternate, 20–40 cm long, with 20–30 dark green, serrated leaflets, each about 3–8 cm long.

The terminal leaflet is often absent. Young leaves are reddish to purplish in colour.

- **Fruits:** The fruit is a smooth, ellipsoidal drupe, up to almost 2 cm long. When ripe, it is yellow or greenish yellow and comprises a sweet pulp enclosing a seed.

The seed is composed of a shell and a kernel (sometimes two or three kernels), each

about half of the seed's weight.

- **Flowers:** The small fragrant white flowers are bisexual or staminate (male) and are borne in clusters in the axils of the leaves.
- **Flowering and Fruiting Time:** Flowers from January to May & Ripening time of Fruits is from May to August.

Fig no 1

Fig no 2

Cultivation:

Neem grows on almost all kinds of soils including clayey, saline and alkaline soils but does well on black cotton soils. It thrives better than most other trees on dry stony saline soils with a waterless sub-soil or in places where there is a hard calcareous or clay pan near the surface. It does not tolerate inundation. It has a unique property of calcium mining which changes the acidic soil into neutral. *Neem* also grows well on some acidic soil. It is said that the fallen neem leaves which are slightly alkaline are good for neutralising acidity in the soil. It generally performs well on areas with annual rainfall varying from ***400 – 1200 mm***. It thrives under the hottest conditions where maximum day temperature reaches $\boldsymbol{50^0\ C}$. But it cannot withstand freezing or extended cold.

Pharmacological Activities:

Antimicrobial activity : Neem extracts are rich in antimicrobial compounds as some studies have clearly shown that neem extracts can be potentially useful to control some foodborne pathogens and other spoilage organisms. Antiviral activity of neem bark extract confirmed that bark extract extensively blocked HSV-1 entry into cells at 50–100µg/ml concentration. Antifungal activity of extracts of seed on Candida spp. has also been evaluated, and the finding of the study has concluded that neem seed extract appears to be hopeful anticandidal agents.

Antioxidant activity: Different parts of neem plants such as leaf, bark, root, seed, and flowers show role in disease management through modulation of various biological activities. A study was performed to evaluate the antioxidant activity of different extracts obtained from various parts of the neem tree. The results suggest that extracts from leaf, flower, and stem bark hold high antioxidant activity.

Anti-inflammatory effect: Anti-inflammatory effect of neem plants has been reported by various studies. In an experimental study based on rat models, nimbidin from neem trees was used orally to evaluate its anti-inflammatory response. It was confirmed that the phagocytosis was inhibited, and further, the migration of macrophages to their peritoneal cavities was significantly inhibited in response to inflammatory stimuli. The anti-inflammatory activities of neem fruit skin and its specific ingredient, azadiradione, have also been evaluated. The results have concluded that the animals treated with 100mg/kg dose of this fruit skin extract and azadiradione exhibited significant anti-inflammatory activities.

Wound-healing effect: The effects of neem oil in the treatment of chronic, nonhealing wounds were performed, and the results showed that after 8 weeks of treatment, 50% wound healing was observed in almost 44% patients. The aqueous extract of neem leaves was used to check the wound-healing activities, and a significant reduction in the longest diameter wounds has been observed. The wound-healing properties of the aqueous extracts of neem leaves are supposed to act biochemically through inflammatory response and neovascularization.

Immunomodulatory effect: Neem oil is also used as a nonspecific immunostimulant as it plays a role in the activation of cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogens.

Material and Method:

Selection of plant: The plant Azadirachta indica was selected for study.

Methanol Extract: 50g of dried leaf powder were taken in a separate container. To this 250ml of methanol was added and kept for 24 h with periodic shaking then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled.

Microorganism: The Pathogenic strains of Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus and Bacillus pumilus were used.

Leaf extract: The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with methanol and ethanol. The obtained liquid extracts were subjected to subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vaccum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

Antimicrobial screening

Agar disc diffusion method: This method is suitable for organism that grows rapidly over night at 35-37°C. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases. There is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined.

Method: A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube so as to express the excess fluid. The surface of Muller Hinton Agar plate was inoculated with the swab. To ensure that the growth is uniform and confluent (or semi confluent) the swab is passed three times over the entire surface, by repeating the procedure, taking care the second and third time to turn the plate through 60° leaf extract and which were prepared using Dimethylsulfoxide: Methanol (1:1) solvent to dissolve the plant extract and then placed on the inoculated agar surface using sterile forceps. Standard disc of Streptomycin (10µg/disc) and Tetracycline (30µg/disc) (Himedia), 6 mm in diameter were used as positive control and the solvent used for preparing extract was used as negative control. The plates were incubated overnight at 37° C for 18-24 hours. Antimicrobial activity was evaluated by measuring zone of inhibition by using Hi Media zone scale.

Medium : 3.8g of Muller Hinton Agar is added to 100 ml distilled water and autoclaved at 121° C for 15 minutes at 15 lbs and poured in sterile Petri plates up to a uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used.

Inoculums: The microorganisms were inoculated in peptone medium and incubated at 37° C for 3-4 hours and this was used as inoculums.

Minimum Inhibitory Concentration (MIC) Analysis: The minimum inhibitory concentration was determined after the essential oil displayed sensitivity against the growth of the isolates, the zone of inhibition of the essential oil was above 20 mm. A 38 g/500 ml medium of Mueller Hinton Agar solution was used. It was regulated and dispersed into McCartney bottles and sterilized in an autoclave at 121 ℃ for 15 min. the agar solution was cooled to 45 ℃ and each graded solution was poured into petri-dishes and let to solidify for 1 hr. Extract concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781 mg/ml were prepared by serial dilution. The plates were then divided into sections and labelled appropriately. The 5 mm diameter paper discs were placed into each labelled section of the plate aseptically with the aid of sterilized forceps. 0.1 ml of each isolate was injected into the labelled paper discs on the agar plates appropriately with the aid of an automatic micropipette. The plates were incubated for 24 hr at 37 ℃ after which they were observed to determine the growth or death of the test organism. The lowest concentration inhibiting growth was considered to be the minimum inhibitory concentration.

Minimum Bactericidal Concentration (MBC) Analysis: Determining the minimum bactericidal concentration is done using sterilized Mueller Hinton Agar. The paper discs used during the minimum inhibitory concentration tests were reactivated using a mixture of 0.5% egg lecithin and 3% Tween 80 solution. The isolates were uniformly streaked on labelled quadrants using a wire loop after the reactivated isolates were sub – cultured into the quadrants of the sterilized Mueller Hinton Agar plates. The isolates were incubated at 37 ℃ for 24 hr after which growth was observed and recorded, the minimum bactericidal concentration is the quadrant with the lowest concentration of the essential oil without growth.

Phytochemical Analysis:

The following methods were used for qualitative phytochemical analysis of oil extracts from neem leaves.

- **Test for Carbohydrates:** 1 ml of Molisch's reagent was added to 2 ml of the oil extract after which a few drops of concentrated sulphuric acid was added. A purple colouration depicts the presence of carbohydrates.
- **Test for Tannins:** 2 ml of 5% ferric chloride was added to 1 ml of oil extract. A greenish black colouration depicts that tannins are present.
- **Test for Saponins:** 2 ml of distilled water was added to 2 ml of oil extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.
- **Test for Flavonoids:** 5 ml of dilute NH3 solution was added to 1 ml of oil extract prior to the addition of concentrated sulphuric acid. A yellow colouration depicts that flavonoids are present.
- **Test for Anthocyanins and Betacyanins:** 1 ml of 2N NaOH was added to 2 ml of oil extract and then heated for 5 minutes at 100oC. A yellow colouration depicts their presence.
- **Test for Alkaloids**: 2 ml of concentrated HCl was added to 2 ml of oil extract before a few drops of Mayer's reagent were added. A greenish colouration depicts that alkaloids are present.
- **Test for Quinones:** 1 ml of concentrated H2SO4 was added to 1 ml of oil extract. A red colouration depicts that quinones are present.
- **Test for Cardiac Glycosides:** 2 ml of glacial acetic acid and a few drops of 5% ferric chloride was added to 0.5 ml of oil extract before 1 ml of concentrated sulphuric acid was added to the mixture. A brown ring formation at the interface depicts that cardiac glycosides are presence.
- **Test for Terpenoids**: 2 ml of chloroform and concentrated H2SO4 was added to 0.5 ml of oil extract. A red brown colouration at the interface depicts that terpenoids are present.
- **Test for Phenols:** 2 ml of distilled water and a few drops of 10% ferric chloride was added to 1 ml of oil extract. A green colouration depicts that phenols are present.
- **Test for Acids:** Sodium bicarbonate solution was added to 1 ml of oil extract. Formation of effervescence depicts the presence of acids.
- **Test for Glycosides:** 3 ml of chloroform and 10% NH3 solution was added to 2 ml of oil extract. A pink colouration depicts that glycosides are present.

Uses & Effectiveness

- Dental plaque.
- Ulcers.
- Psoriasis.

- Fever.
- Upset stomach.
- Breathing condition.
- Malaria.
- Head lice.
- Skin conditions and diseases
- Heart diseases.
- Diabetes.
- Birth control (contraception)
- Other condition

Special Precautions & Warnings

- **Pregnancy and breast-feeding**: Neem oil and neem bark are likely unsafe when taken by mouth during pregnancy. They can cause a miscarriage. Not enough is known about the safety of need during breast-feeding. Stay on the safe side and avoid use.
- **Organ transplant**: There is a concern that neem might decrease the effectiveness of medications that are used to prevent organ rejection. Do not use neem if you have had an organ transplant.
- **Surgery**: Neem might lower blood sugar levels. There is a concern that it might interfere with blood sugar control during and after surgery. Stop using neem at least 2 weeks before a scheduled surgery.
- **Children**: Taking neem seeds or oil by mouth is likely unsafe for children. Serious side effects in infants and small children can happen within hours after taking neem oil. These serious side effects include vomiting, diarrhea, drowsiness, blood disorders, seizures, loss of consciousness, coma, brain disorders, and death.
- **"Auto-immune diseases" such as multiple sclerosis (MS), lupus (systemic lupus erythematosus, SLE), rheumatoid arthritis (RA), or other conditions**: Neem might cause the immune system to become more active. This could increase the symptoms of auto-immune diseases. If you have one of these conditions, it's best to avoid using neem.
- **Diabetes**: There is some evidence that neem can lower blood sugar levels and might cause blood sugar to go too low. If you have diabetes and use neem, monitor your blood sugar carefully. It might be necessary to change the dose of your diabetes medication.
- **Reduced ability to have children (infertility)**: There is some evidence that neem can harm sperm. It might also reduce fertility in other ways. If you are trying to have children, avoid using neem.

Interactions

- **Medications for diabetes (Antidiabetes drugs) Interaction Rating**: Moderate Be cautious with this combination. Talk with your health provider.

Neem might decrease blood sugar. Diabetes medications are also used to lower blood sugar. Taking neem along with diabetes medications might cause your blood sugar to go too low. Monitor your blood sugar closely. The dose of your diabetes medication might need to be changed.

Some medications used for diabetes include glimepiride (Amaryl), glyburide (DiaBeta, Glynase PresTab, Micronase), insulin, pioglitazone (Actos), rosiglitazone (Avandia), chlorpropamide (Diabinese), glipizide (Glucotrol), tolbutamide (Orinase), and others.

- **Medications that decrease the immune system (Immunosuppressants) Interaction Rating**: Moderate Be cautious with this combination. Talk with your health provider.

Neem might increase the immune system. By increasing the immune system, neem might decrease the effectiveness of medications that decrease the immune system.

Some medications that decrease the immune system include azathioprine (Imuran), basiliximab (Simulect), cyclosporine (Neoral, Sandimmune), daclizumab (Zenapax), muromonab-CD3 (OKT3, Orthoclone OKT3), mycophenolate (CellCept), tacrolimus (FK506, Prograf), sirolimus (Rapamune), prednisone (Deltasone, Orasone), corticosteroids (glucocorticoids), and others.

- **Lithium Interaction Rating**: Moderate be cautious with this combination. Talk with your health provider.

Neem might have an effect like a water pill or "diuretic." Taking neem might decrease how well the body gets rid of lithium. This could increase how much lithium is in the body and result in serious side effects. Talk with your healthcare provider before using this product if you are taking lithium. Your lithium dose might need to be changed.

Discussion:

Azadirachta indica leaves possessed good anti-bacterial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens. The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium, S. sobrinus.

Conclusion:

Neem is a powerful medicinal plant endowed with potential therapeutic benefits, mainly for antibacterial and antifungal properties. In addition, industrial and medicinal sectors prepare countless formulations of neem through potent clinical uses in developing novel drugs to treat different ailments due to their effectiveness against pathogens. Thus, these past few decades have shown a progressively growing interest in neem research in chemistry exploration and therapeutic discovery.

References:

1. Abu. Syed Md. Mosaddek and Md. Mamun Ur Rashid (2008) A comparative study of Anti-inflammatory effect of aqueous extract of Neem leaf and dexamethasone. Bangladesh J Pharmacol 3: 44-47.
2. Almas, K. Ansal lafi, T.R. (1995) The natural toothbrush. World health Forum 16: 206-210.
3. Almas, K. Ansal lafi, T.R. (1995) The natural toothbrush. World health Forum 16: 206-210.
4. Sonia Bajaj, Srinivasan B.P. (1999) Investigation into the Anti diabetic activity of Azadirachta indica. Indian journal of pharmacology 31:138-141.
5. Saseed A. Khan and Junaid Aslam (2008) Study on the effect of Neem (Azhadirachta indica) leaves smoke in controlling airborne Bacteria in Residential premises. Current research in Bacteriology1 (2): 64-66.
6. A.M. EI- Mahmood, O.B Ogbonna and M.Raji (2010) The antibacterial activity of Azadirachta indica (Neem) associated with eye and ear infections. Journal of medicinal plant Research,. 4(14).1414-1421.
7. Gbotolorun S.C. Osinubi A.A., Noronha C.C., Okanlawon A.O (2008) Antifertility potential of Neem flower extract on adult female Sprague Dawley rats. African Health Science 8(3): 168-173.
8. Hassan Amer, Wafaa A. Helmy, Hanan A.A Taie (2010) invitro Antitumour activities of seeds and leaves Neem (Azadirachta indica) extracts. International journal of Academic research. 2(2), 165-171.
9. Md Mohashine Bhuiyan, Michiko Nishimura seishi matsumura and Tsutomu shimono (1997) Antibacterial effects of the crude Azadirachta indica Neem bark extract on Streptococcus sobrinus, Pediatric dental journal 7(1): 61-64.
10. Srivastava A Shukla Kumar YN (2000) Recent development in plant derived antimicrobial constituents A Review. J Med Arom Pl. Sci.20: 717-72.
11. Saradhajyothi Koona, Subbarao Budida (2011) Antimicrobial potential of the extracts of the leave of Azadirachta indica , Linn. Nat Sci Biol, 3(1) 65-69.
12. Hafiza, R.E. (2000) Peptides antibiotics, Lancet 349: 418- 422.
13. Faiza Aslam, Khalil.Ur. Rehman, Mohammad Asghar and Muhammed Sarwar (2009) Antibacterial activity of various Phytoconstituents of Neem. Pak. J.Agri. Sci., Vol. 46(3), 456-463.
14. Ali A. Textbook of Pharmacognosy. New Delhi, India: Publication and Information Directorate; 1993.
15. Hossain MA, Shah MD, Sakari M. Gas chromatography-mass spectrometry analysis of various organic extracts of Merremia borneensis from Sabah. Asian Pac J Trop Med 2011;4:637-41.
16. Kokate C, Purohit AP, Gokhale SB. Pharmacognosy. Maharashtra, India: Nirali Prakashan; 2010.
17. Sunarwidhi AL, Sudarsono S, Nugroho AE. Hypoglycemic effect of combination of Azadirachta indica A. Juss. and Gynura procumbens (Lour.) merr. ethanolic extracts standardized by rutin and quercetin in Alloxan-induced hyperglycemic rats. Adv Pharm Bull 2014;4:613-8.

18. Kumar VS, Navaratnam V. Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pac J Trop Biomed 2013;3:505-14.

19. Koul A, Mohan V, Bharati S. Azadirachta indica mitigates DMBA-induced hepatotoxicity: A biochemical and radiometric study. Indian J Biochem Biophys 2014;51:37-45

20. http://www.agrilearner.com/neem-cultivation/

21. Mahfuzul Hoque MD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S. Antibacterial activity of guava (Psidium guajava L.) and neem (Azadirachta indica A. Juss.) extracts against foodborne pathogens and spoilage bacteria. Foodborne Pathog Dis 2007;4:481-8.

22. Tiwari V, Darmani NA, Yue BY, Shukla D. In vitro antiviral activity of neem (Azardirachta indica L.) bark extract against herpes simplex virus type-1 infection. Phytother Res 2010;24:1132-40

23. Lloyd CA, Menon T, Umamaheshwari K. Anticandidal activity of Azadirachta indica. Indian J Pharmacol 2005;37:386-9.

24. Sithisarn P, Supabphol R, Gritsanapan W. Antioxidant activity of Siamese neem tree (VP1209). J Ethnopharmacol 2005;99:109-12.

25. Kaur G, Sarwar Alam M, Athar M. Nimbidin suppresses functions of macrophages and neutrophils: Relevance to its antiinflammatory mechanisms. Phytother Res 2004;18:419-24.

26. Ilango K, Maharajan G, Narasimhan S. Anti-nociceptive and anti-inflammatory activities of Azadirachta indica fruit skin extract and its isolated constituent azadiradione. Nat Prod Res 2013;27:1463-7

27. Singh A, Singh AK, Narayan G, Singh TB, Shukla VK. Effect of neem oil and Haridra on non-healing wounds. Ayu 2014;35:398-403.

28. Chundran NV, Husen IR, Rubianti I. Effect of neem leaves extract (Azadirachta indica) on Wound Healing. AMJ 2015;2:199-207

29. Osunwoke EA, Olotu EJ, Allison TA, Onyekwere JC. The wound healing effects of aqueous leave extracts of Azadirachta indicaon wistar rats. J Nat Sci Res 2013;3:181-6.

30. Upadhyay SN, Dhawan S, Garg S, Talwar GP. Immunomodulatory effects of neem (Azadirachta indica) oil. Int J Immunopharmacol 1992;14:1187-93.

31. Durrani FR, Chand N, Jan M, Sultan A, Durrani Z, Akhtar S. Immunomodulatory and growth promoting effects of neem leaves infusion in broiler chicks. Sarhad J Agric 2008;24:655-9.

32. https://www.rxlist.com/neem/supplements.htm

33. E. A. Adebayo, O. R. Ishola, O. S. Taiwo, O. N. Majolagbe, and B. T. Adekeye, "Evaluations of the methanol extract of Ficus exasperata stem bark, leaf and root for phytochemical analysis and antimicrobial activities," African J. Plant Sci., vol. 3, no. 12, pp. 283–287, 2009.

34. B. Q. Guevara, A guidebook to plant screening: phytochemical and biological. University of Santo Tomas Publishing House, 2005.

35. A. J. Harborne, Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media, 1998.