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Research

Therapeutic Potentials of Azadirachta *Indica:* A Comprehensive Review on Its Antibacterial, Antiviral, Antifungal, Anti-inflammatory, and Anticancer Activities

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Abstract

Neem (*Azadirachta indica*), a cornerstone of traditional Indian medicine, has been extensively used in Ayurveda and Unani systems for its broad spectrum of therapeutic benefits. Among its various parts, neem leaves are particularly valued for their pharmacologically active compounds, including flavonoids, terpenoids, limonoids, and polyphenols. This study highlights the diverse pharmacological properties of neem leaf extracts, focusing on their antibacterial, antiviral, antifungal, anticancer, and anti-inflammatory effects. Neem leaves also exhibit notable antiviral effects, particularly against hepatitis C virus (HCV), through the action of 3-Deacetyl-3-cinnamoyl-azadirachtin, which inhibits the NS3 viral protease, reducing viral replication and improving liver function in treated subjects. Antifungal activity is evident against pathogens like *Aspergillus flavus*, attributed primarily to terpenoid constituents. In murine models, neem leaf extract has shown chemopreventive and anticancer potential by enhancing phase II detoxifying enzymes, elevating antioxidant levels, and suppressing cellular proliferation without causing observable toxicity. Additionally, anti-inflammatory assessments in rats have demonstrated moderate, dose-dependent suppression of edema, though the effects are generally milder than standard anti-inflammatory drugs. Collectively, these findings support the traditional use of neem leaves and highlight their potential as safe, plant-based alternatives for treating infectious and inflammatory diseases, as well as for chemoprevention. Further clinical studies are warranted to validate these effects and determine optimal formulations for therapeutic applications

Keywords: Azadirachta indica, Antibacterial activity, Antiviral activity, Antifungal activity, Anti-inflammatory activity, Anticancer activity, Traditional medicine, NS3 Protease Inhibitor

INTRODUCTION

The medical properties of Neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of Neem's fruits, seeds, oil, leaves, root and bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines and is now being used in the manufacture of modern day medicinal, cosmetics, toiletries and pharmaceuticals. The Neem tree has been known as the wonder tree for centuries in the Indian subcontinent. Neem has become important in the global context today for its variety of medicinal uses. Neem extract which have Nimbinin, nimbandiol as active constituents, alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect, which are very useful against diabetes. Neem is used in Dermatitis Eczema, Acne, Bacterial, Fungal infections and other skin disorders. It has demonstrated its effectiveness as a powerful antibiotic. Neem also has shown antiviral, anti-fungal and anti-bacterial properties. It helps support a strong immune system and is used in cases of inflammatory skin conditions. Traditionally Neem has been used for skin and blood purifying conditions. Perhaps Neem's most touted advantage is the effect it has upon the skin. Preparations from the leaves or oils of the tree are used as general antiseptics. Due to Neem's antibacterial properties, it is effective in fighting most epidermal dysfunction such as acne, psoriasis, and eczema. Ancient ayurvedic practitioners believed high sugar levels in the body caused skin disease; Neem's bitter quality was said to counteract the sweetness.

Traditionally, Indians bathed in Neem leaves steeped in hot water. Since there has never been a report of the topical application of Neem causing an adverse side effect, this is a common procedure to cure skin ailments or allergic reactions. Neem also may provide antiviral treatment for smallpox, chicken pox and warts--especially when applied directly to the skin. Its effectiveness is due in part to its ability to inhibit a virus from multiplying and spreading. Neem produces pain-relieving, anti-inflammatory and fever-reducing compounds that can aid in the healing of cuts, burns, sprains, earaches, and headaches, as well as fevers. Several studies of Neem extracts in suppressing malaria have been conducted, all supporting its use in treatment.

Neem has broad applications to human and animal health, as well as organic farming. Neem is a powerful antiviral and antibacterial. But, it has peculiarities that set it apart from other herbs in that class of broad antimicrobials. Neem oil is also commonly added to a variety of creams and salves. It is effective against a broad spectrum of skin diseases including eczema, psoriasis, dry skin, wrinkles, rashes and dandruff. A few drops can be added to hand healing salves and shampoo. Neem oil is highly effective as a mosquito repellent. Because of its unpleasant smell, it is best when it is added to a formula with other essential oils, such as citronella. Neem oil is an effective and environmentally safe pesticide when it is diluted and sprayed on crops through irrigation systems. It is a healthier alternative to artificial chemical pesticides. Neem oil does not harm the soil and it increases yields

Chemical constituents and properties

Neem contains a bitter fixed oil, nimbidin**,** nimbin, nimbinin and nimbidol, tannin and uses are:

- Anti-inflammatory (nimbidin, sodium nimbidate, gallic acid, catechin, polysachharides).
- Anti-arthritic, hypoglycemic, antipyretic, hypoglycemic, diuretic, anti-gastric ulcer (nimbidin)
- Anti-fungal (nimbidin, gedunin, cyclic trisulfide)
- Anti-bacterial (nimbidin, nimbolide, mahmoodin, margolone, margolonone, isomargolonone)
- Spermicidal (nimbin, nimbidin)
- Anti-malarial (nimbolidfe, gedunin, azadirachtin)
- Anti-tumor (polysaccharides)
- Immunomodulatory (NB-II peptoglycan, gallic acid, epicatechin, catechin)
- Hepatoprotective (aequeous extract of neem leaf)
- Anti-oxidant (neem seed extract)
- Anti-Viral (3-Deacetyl-3-cinnamoyl-azadirachtin) [3].

Taxonomical description of Neem [3]

Table 1 : Taxonomy of Neem

Kingdom	Plantae
Division	Magnoliophyta
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	*indica*

Botanical description

Azadirachta indica belongs to the Meliaceae family. With a trunk girth of 2.5 meters and a height of /U) meters, this broad-leaved evergreen tree has a lifespan of more than two centuries. Although its deep root system is highly sensitive to water logging, it is well adapted to extracting nutrients and water from the soil profile. Neem trees grow best in hot, dry regions with annual rainfall ranging from 400 to 1.200 mm and shade temperatures that frequently approach 5°C. The tree is resistant to numerous environmental stresses, including as drought and soils that are infertile, rocky, shallow, or acidic. Neem develops ellipsoidal drupes on axillary clusters that measure approximately two centimetres in length. High amounts of secondary metabolites are seen in the kernels of these fruits. Its many flowering panicles are mostly found in the axils of the leaves. The selel have ovoid, about centimetre-long petals that smell deliciously of white oblanceolate. It produces golden, ellipsoid, glabrous drupes that are 12–20 mm long. The green fruits eventually turn yellow as they ripen and have a strong, garlic-like smell. In March and April, flowers and new foliage appear. Fruits ripen between April and August, depending on the location [8].

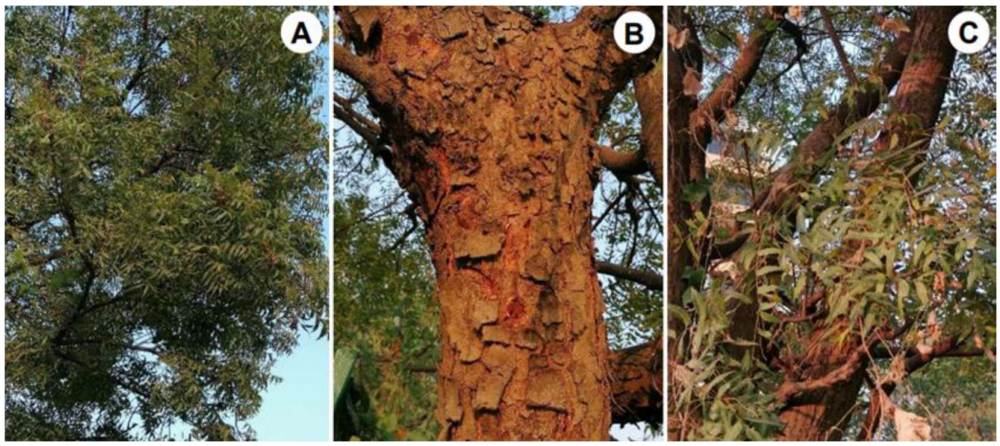

Fig 1 : Neem Tree, Barks and Branches

Medicinal properties

Neem leaves are valued for their potent medicinal properties, including antioxidant, antibacterial, antifungal, and anti-inflammatory effects. Traditionally used to treat skin conditions like acne and eczema, they also help purify the blood, boost immunity, and support liver health. Neem aids in diabetes management by regulating blood sugar and offers antiviral benefits to enhance overall health and wellness.

Anti bacterial activity

Biological evaluation of herbal products, based on their use in traditional folklore practice is the key step in the development of new potential antibacterial agents from plants. Several studies paving the way for new drug discoveries have found that the therapeutic agents derived from plants could be used as an important surrogate, alternative, or complementary treatment of infectious diseases. Such concerted efforts are required for the research and development of new treatment strategies for combating these notorious bugs.

Material and method

A cross-sectional analytical study was undertaken at a tertiary care teaching hospital in North India to evaluate the antibacterial activity of ethanolic extracts of Neem leaves against standard ATCC strains and the pathogens isolated from clinical specimens.

This cross-sectional analytical study was conducted at the Department of Microbiology of a tertiary care teaching hospital in North India for two months after obtaining clearance from institutional ethics clearance. *In vitro* antibacterial activity of different dilutions of Neem extract was studied against:

Three standard American Type Culture Collection (ATCC) strains

- ✓ E. coli (ATCC 25922)
- ✓ P. aeruginosa (ATCC 27853)
- ✓ S. aureus (ATCC 25923)

63 culture isolates obtained from clinical specimens sampled through convenience sampling[5]

Preparation of extracts by Cold extraction method

Leaves were cleaned, shade dried for one week, and pulverized to a coarse powder. About 25 g of powder was soaked in 100 mL of 95% ethanol and allowed to macerate at room temperature for seven days with intermittent shaking. This was followed by straining through sterile muslin cloth and filtering through sterile Whatman No. 1 filter paper. The filtrate was thereafter concentrated at 40°C. The solvent was completely evaporated to yield sticky black material and stored in sterile airtight containers at 4oC in the refrigerator. This material was reconstituted in 0.1% DMSO (dimethyl sulfoxide) prior to use to prepare five different concentrations of the extract: 200, 100, 50, 25, 12.5, and 6.25 mg/mL, which could be stored in separate sterile labelled aliquots in the refrigerator**.**

Antibacterial activity test

The antibacterial activity of the ethanolic Neem extract was assessed by two methods:
1) Disc diffusion method
2) Broth dilution method
Disk diffusion antimicrobial tests
Modified disc diffusion assay (DDA) on Mueller Hinton Agar plate

Preparation of dried filter paper discs

Whatman filter paper No. 1 was used to prepare discs approximately 6 mm in diameter, which were placed in a Petri dish and sterilized by autoclaving.

Inoculum preparation

At least three to five well isolated colonies of the same morphological type were selected from culture plates. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4 to 5 mL of a suitable broth medium. The broth culture was incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards(usually 2 to 6 hours). The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to the 0.5 McFarland standards. This results in a suspension containing approximately 1-2×108 CFU/mL bacteria. A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube to express the excess fluid. The surface of the Mueller-Hinton Agar plate was inoculated with the swab. A representative sample of each batch of the plate should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Inoculation of test plates

Optimally within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. To obtain a uniform and confluent growth, the dried surface of a Mueller-Hinton Agar plate was inoculated by streaking the swab over the entire sterile agar surface three times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the disks.

Application of disk to inoculated agar

The six Whatman filter paper No. 1 paper disks approximately 6 mm in diameter were placed peripherally and pressed onto the surface of the inoculated agar plate. The markings corresponding to different concentrations, including negative control (NC) and positive control (PC) were made on the culture plate. A gentamicin (20 µg, Himedia) disk placed in the center was used as the PC. Disks were distributed eventually so that they were no closer than 24 mm from center to center. One drop of different concentrations of the extract (50 µL) comprising the moist weight of 7.08 mcg was placed on the corresponding five disks as per the markings on the culture plate. One drop of plain DMSO was added to the disk marked as NC. The plate was inverted and placed in an incubator set to 37°C within 15 minutes after the application of disks.

Reading plates and interpreting result

Plates were incubated at 37°C for 24 hours, and then the antibacterial activity was assessed based on the measurement of the diameter of the inhibition zone formed around the disk. The diameter of zones of inhibition was measured, including the diameter of the disc. The zones were measured to the nearest whole mm using a zone size measuring scale, which is held on the back of the inverted Petri
plate [4,7,11,17-19].

Dilution methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobials in either agar or broth media[4,5,7,14,16].

Minimum inhibitory concentration (MIC)

Broth dilution method

The MIC of the extract for different test isolates was determined by the broth dilution method (Media used: Mueller-Hinton broth. Five millilitres of Mueller-Hinton Broth were transferred to seven test tubes each. One test tube was labelled as PC (growth control tube), one as NC, and the remaining five test tubes were labelled as per the concentration of the Neem extract to be added (as 200, 100, 50, 25, and 12.5 mg/mL). Then, 100 µL of bacterial suspension equivalent to 0.5 McFarland standards was added in each test tube except NC. Then, 100 µL of different concentrations of the extract was added to the corresponding labelled test tubes except for controls, which were instead loaded with 100 µL of DMSO solvent. The broths were incubated at 37°C for 24 hours. After incubation, tubes were observed for any visible growth. MIC was expressed as the lowest concentration of the extract showing visibly complete clearance. [4,5,7,14,16]

Anti viral acivity

Phytochemicals of leaves of neem shows antiviral activity against protease (NS3-4) of HCV. It is found that a component of leaves of neem known as 3-Deacetyl-3-cinnamoyl-azadirachtin have an ability to bind with NS3 protease of HCV and act as inhibitor of this protease.

Material and methods

Thirty five HCV patients and failure of conservative treatment were studied. Effect of neem leaves was seen blood of HCV patients. Extract of Azadirachta indica leaves was prepared and given orally to patients. The seropositivity of hepatitis C virus was estimated prior and after the usage of neem leaves extract by HCV RNA quantitative analysis by Polymerase chain reaction. Level of ALT. AST and total protein were estimated by standard kit methods.

Preparation of Extraction of Azadirachta indica leaf extract

Fresh leaves of Azadirachta indica were collected. About 10-12 leaves neem were soaked in a glass of water for whole night and extract was prepared by boiling water (neem extract) is only one table spoon. Extract was used by the patients for the period of 2.0 weeks in fasting condition. Seropositivity of HCV was estimated before & after the usage of leave extract by reverse transcriptase Polymerase chain reaction (RT-PCR). After using the leave extract of Azadirachta indica the HCV seropositivity was significantly decreased ($P<0.001$). ALT and AST were significantly decreased after taking neem extract. However, the level of serum protein was not changed [9].

Table 2 : HCV Seropositivity and liver enzymes in Hepatitis C positive patients before and after the leave extract of neem

Patient (35)	HCV-seropositivity (IU/ml)	ALT (U/L)	AST (U/L)	Serum protein (gm/l)
Before using extract	78.5±35.5	15.84±17.50	13.8±13.5	6.40±1.01
After using extract	26.5±14.8**	8.5±3.5*	7.5±3,9*	6.60±2.01

**P < 0.001 = highly significant; *P < 0.05 =significant

Anti fungal activity

According to Polaquini *et al*., terpenoids are known to be responsible for its fungicidal or fungistatic activity on several pathogenic fungi.[20]

The purpose of the study was to assess the antifungal activity of Neem leaves against pathogenic fungi like-

- ✓ Aspergillus flavus
- ✓ Alternaria solani
- ✓ Cladosporium

Materials and Methods

The plant of Neem (*Azadirachta indica*) was selected for study. Its leaves were collected from college campus. The collected leaves were identified with the help of taxonomic key available in departmental library and confirmed with departmental herbaria.

Leaf extract

The completely dried material was powdered and allowed for successive extraction, with concentration of methanol and ethanol (25%, 50% 75% and 100%). The obtained liquid extracts were stored at 4°C in air tight bottle.

Microorganism

The fungal strains - *Aspergillus flavus, Alternaria solani* and *Cladosporium sp.* were used. These strains were isolated from local area.

Disc diffusion method

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

Medium

Sabouraud dextrose agar was prepared, autoclaved at 121ºC for 15minutes at 15lbs and poured in sterile petri-plates up to a uniform thickness of approximately 5-6mm and the agar was allowed to set at ambient temperature and used.

Inoculums

The fungus were inoculated in sabouraud dextrose agar and incubated at 37o C and all three fungal species were used as inoculums. Point of inoculums was inoculated over the sabouraud dextrose agar medium, using sterile inoculums loop. After few minute, four disc loaded with 25%, 50%, 75% and 100% methanolic and ethanolic extract were kept at equal distance in each petri-plate. Ketoconazole solution (100% concentration) was added in another plate. Plates were incubated at 37°C for 24hrs. Anti-fungal activity was evaluated by measuring zone of inhibition by using Himedia zone scale[20].

Table 3 : Inhibition of mycelial growth at different concentration of Leaf Extracts

Solvent Used for Extraction	Fungal Species	Zone of Inhibition (mm)				
		Concentration of Extract				0% (Control)
		25%	50%	75%	100%	Positive Control
Methanol	*Aspergillus flavus*	1	1.4	2.0	4.0	0
	Alternaria solani	0.6	0.8	0.9	1.0	0
	Cladosporium	0.1	0.3	0.5	0.6	0
Ethanol	*Aspergillus flavus*	0.3	0.5	0.8	1.0	0
	Alternaria solani	0.2	0.3	0.5	0.6	0
	Cladosporium	0.1	0.2	0.5	0.6	0

Anti cancer activity

In recent years various dietary constituents have been found to provide protection against any disease including cancer. It is search and research that helps in the identification of such potential agents, which can either abolish or delay the development of carcinogenesis. The latter can be brought about either by preventing the activation of carcinogen or by increasing detoxification or by blocking the interaction of ultimate carcinogen with cellular macromolecules, or by suppress ing the clonal expansion of neoplastic cells Azadirachta indica (Indian Neem tree) traditionally employed intensively as folklore remedy for a wide spectrum of diseases in India. Azadirachta indica has a wider arrary of uses than any other herb The first recorded use of Neem is attributed to the ancient East Indian Harrappa Culture which added the plant to dozens of health and beauty aids 4500 years ago. The centuries old healing system, Ayurvedic medicine, has utilized these timeless Neem formulation as a mainstay of Ayurvedic pharmacy. Its medicinal qualities are outlined in the earliest 'Sanskrit writings that states uses of various parts of Azadirachta indica to treat bacterial, fungal, and viral infections and to boost the immune system also it's usefulness as a natural non-toxic insecticide. Practically every part of Azadirachta indica (leaves, bark, fruit, flowers, oil, and gum) have been reported to be associated with various remedial properties such as, antimicrobial effects, storage behaviour, reduction of paracetamol-induced liver damage, enhancer of hepatic glutathione and glutathione-dependent enzymes, in vitro antiviral activity , insecticidal activity, antibacterial agent etc. Chemo preventive potential of Azadirachta in dica has also been evaluated recently on 7,12-dimethy benz(a)anthracene (DMBA)-induced hamster buccal pouch and against IBD virus in broilers. In the present study 80% ethanolic extract of Neem leaf extract was used to evaluate the induction pattern of these enzymes in the

liver of mice, since greatest of the reactions are known to be carried in the liver. Oxidative stress implicates in all the stages of the development of cancer as well as in the genesis of other diseases so in addition hepatic antioxidant defence enzymes comprising of superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPX), glutathione reductase (GR), and reduced glutathione (GSH) have been evaluated as they protects the cellular macromolecules against oxidative damage.

Methods

Preparation of modulator

Fresh Azadirachta indica (Neem leaf) was obtained. The leaves were rinsed with water and blot dry. Material of known weight was soxheleted using 80% hydro-alcoholic solvent (80% ethanol, 20% double distilled water, v/v) three times. Finally the extract was lyophilized and stored at 4°C.)

Animals

Random-bred Swiss albino mice (7-8 weeks old) were used for this study. They were maintained in our air-conditioned animal facility (Jawaharlal Nehru University, New Delhi) with a 12-h light:12-h dark cycle, and provided (unless otherwise stated) with standard food pellets and tap water ad libitium All animals were cared for according to the "Principles of Laboratory Animal care" of the national Institute of Health (NIH, USA) and under strict adherence to Indian Animal Ethic Committee (IAEC).

Experiment

Modulation of hepatic and extrahepatic carcinogen metabolising and antioxidant enzymes. Animals were as-sorted in the following group:

Group I (n=8): Animals were put on a normal diet and treated with 50 µl of an emulsion made of peanut oil and double distilled water (ratio 4:1 ml), by oral gavage daily for 15 days; this group of animals served as negative control.

Group II (n=8): Animals were put on a normal diet and treated daily with 250 mg per kilogram body weight of lyophilized Azadirachta indica (Neem leaf) extract; which was suspended in the control vehicle and was given to the mice 50 µl per mouse per day by oral gavage for 15 days.

Group III (n=8): Animals were put on a normal diet and treated daily with 500 mg per kilogram body weight of lyophilized Azadirachta indica (Neem leaf) extract; which was suspended in the control vehicle and was given to the mice 50 µl per mouse per day by oral days.) gavage for 15 days.

Preparation of homogenates, cytosol, and microsome fractions

Animals were sacrificed by cervical dislocation and the entire liver was then perfused immediately with cold 0.9% NaCl and thereafter carefully removed, trimmed free of extraneous tissue and rinsed. The liver was then blotted dry, weighed quickly, and homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) to yield 10% (w/v) homogenate An aliquot of this homogenate (0.5 ml) was used for assaying reduced glutathione levels while the remainder was centrifuged at 10,000 rpm for 20 min. The resultant supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuged at 105,000 × g for 60 min in a Beck-man ultracentrifuge.

Extrahepatic organs

The lung, kidney, and fore-stomach were carefully removed, along with the liver, trimmed free of extraneous tissue and rinsed in chilled 0.15 M Tris-KCl (pH 7.4) (The lung was cut into small pieces. The stomach was opened longitudinally; the fore-stomach was separated from the glandular stomach and cleaned of all its contents by flush-ing with and changing the buffer five to six times. The lung, kidney, and fore-stomach were then blotted dry, weighed quickly and homogenized in ice-cold 0.15M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. 0.5 ml aliquot of this homogenate was used for assaying reduced glutathione The rest of the homogenate was centrifuged at 15,000 g for 30 min at 4°C; the resulting supernatant obtained was used for assaying glutathione S-transferase and DT-diaphorase enzymes[6].

Anti-inflammatory activity

Inflammation is fundamentally a protective response, ultimate goal of which is to get rid the noxious but sometimes it may be potentially harmful and needs pharmacological treatment to control its symptoms. Many anti-inflammatory drugs (both NSAIDs and corticosteroids) have been developed but their safety profile studies have shown that none of them is clearly safe. They show wide ranges of adverse effects. Due to adverse reactions of synthetic and chemical medicines being observed round the globe, herbal medicines have made a come back to improve our basic health needs. Many plants and herbs such as ginger, turmeric, olive oil, have been shown to exhibit potent anti-inflammatory effect. Neem is reported in ayurvedic, tibbi and homeopathic system of medicine to be useful in rheumatic disorders have also shown that different types of extracts from various parts of neem tree (bark, seed, leaf) have anti-inflammatory, anti-pyretic, analgesic, immunostimulant, hypoglycaemic,

antiulceur, anti-fertility anti-malarial, antibacterial, antifungal, anti-viral, anti-carcinogenic, anti-oxidant, hepatoprotective effects. More than 135 compounds have been isolated from different parts of neem. Some of them such as nimbin, nimbinin, nimbidinin, nimbolide and nimbidic, are biologically active. The chloroform extract of stem bark is effective against carrageenin-induced paw edema in rat and mouse ear inflammation. Neem leaf extract exerted significant anti-inflammatory effect in cotton pallet granuloma in rats[15].

MATERIALS AND METHODS

Preparation of Extracts

Dried materials (1kg) were extracted with 50% acetone in a Soxhlet. The extract was evaporated until a solid residue was obtained. The percentage yield was found to be 0.5.

Animals

Male albino (Swiss) weighing 80 -100g bred in king Institute Guindy, Chennai were selected for studies. The anti-inflammatory activity was studied by carrageenan induced rat hind paw oedema. The animals were kept in Microlon boxes and had access to water ad *libitum*.

Carrageenan induced rat paw oedema

The rats were divided into 11 groups, each consisting of six animals. One group served as a control (received normal saline only), the second group served as a positive control (received the various doses of the extracts suspended in 5% acacia solution and given intraperitoneally.

Group iii, iv, v – Received 150,100,150 mg/kg bark extract of *A. indica*

Group vi, vii, viii – Received 50,100,150 mg/kg bark extract of *A. indica*

Group ix, x, xi – Received 50,100,150 mg/kg bark extract of *A. indica*

The drugs and extracts were administered intraperitoneally.

Anti-inflammatory activity

Edema was produced b the method descried. The paw volume was measured 0 hr, 1hr, 2hr after the injection of carrageenan (0.1 ml of 1% solution injected in the sub plantar region). The apparatus used for the measurement of rat paw volume was that of Buttle et al 1996 modified by singh and gosh (8). This method is able to detect a minimal change of paw volume of 0.02 ml. Drug pretreatment was given 1 hr before the injection of carrageenan. The values are shown in Table 1.

Serial dilution technique

A nutrient broth medium of neutral pH containing peptone 1% (w/v), yeast extract 0.5% (w/v) was prepared in distilled water and sterilized by autoclaving for about 30 min (9). A standard volume (8ml) of nutrient broth medium that would support the growth of the test organisms was added to several labelled, sterile, stoppered and identical assay tubes. Solutions of each test compound at three different concentrations viz 50, 100 and 200 µg/ml and a control containing no drug were also prepared. One loopful of the inoculum (of suitable dilution) of over night broth culture of t test organism was added. All these experimental manipulations were carried out under absolute aseptic conditions, the assay tubes we are then incubated at 37 ± 1oC for 48 hrs and the resultant turbidities were measured with Nepheloturbidity meter. The percentage of bacterial growth inhibition produced by a particular growth inhibition produced by a particular concentration of the test compound was calculated from the measure of the turbidity of the control and the turbidity of the specific treatment by employing the following relationship

$$\%\text{Inhibition} = \frac{Tc-Tt}{Tc}$$

Where Tc is the turbidity of the control and Tt is the turbidity after the treatment. The results are listed in Table

Table 4 : Effect of Extracts on carrageenan induced rat paw edema

GROUP	DOSE mg/kg (B.W)	0hr		+1hr		+2hr	
		Edema Volume (ml)	% Of AIA	Edema Volume (ml)	% Of AIA	Edema Volume (ml)	% Of AIA
	-	0.55	-	0.55	-	0.56	-
CONTROL	50	0.53 (±0.004)	3.6	0.53(±0.002)	3.6	0.53(±0.005)	5.3
Leaves	100	0.52 (±0.002)	5.4	0.45 (±0.002)	18.1	0.46(± 0.001)	18.0
	150	0.51(±0.004)	7.2	0.44 (±0.004)	20.0	0.42(± 0.003)	25.0
Ketorolac -Trometh	10	0.49(± 0.004)	11.0	0.38 (± 0.002)	31.0	0.3 (± 0.001)	32.1

-Amine

Table 5 : Effect of Extracts on the growth of Bacteria

Drug	Concentration (Mg/Ml)	% Inhibition	
		E.coli	S.aureus
Leaves	50	23	30
	100	30	19
	150	37	19

Statistical analysis

The results were analysed by Analysis of variance (10). The significance of the differences between groups was determined by their P- values calculated by students't' test. Ten values are considered as significantly different from each other only when $P<0.05$ [10].

RESULTS

Anti bacteral activity

The ethanolic extract of *Azadirachta indica* leaves showed significant antibacterial activity when tested against both standard strains and clinical bacterial isolates. Using the disc diffusion method, *E. coli* (ATCC 25922) exhibited a zone of inhibition of 20.0 mm at the highest tested extract concentration (2000 mg/mL). Even at lower concentrations such as 50 mg/mL, inhibition zones of 13.0 mm were observed, indicating strong antibacterial potency. Comparatively, Gram-positive bacteria, including *Staphylococcus aureus* (ATCC 25923), displayed smaller inhibition zones ranging from 9 to 15 mm, requiring higher concentrations for a measurable effect.

The broth dilution method was employed to determine the Minimum Inhibitory Concentration (MIC) of the extracts. For Gram-negative clinical isolates, MIC values ranged from 18.4 to 70.1 ng/mL, demonstrating high sensitivity. In contrast, Gram-positive isolates required MIC values ≥100 ng/mL, indicating comparatively reduced susceptibility. For standard ATCC strains, MIC values correlated with disk diffusion results, with *E. coli* showing MICs between 5.3 and 9.0 mg/mL. These results were statistically significant ($p \leq 0.00001$) and supported the dose-dependent antibacterial activity of Neem[12].

Anti viral activity

It Is reported that Azadirachta indica have shown hepatotropic due to its anti-inflammatory and antioxidant properties. A study experimentally proved that a compound 3-Deacetyl-3-azadirachtin of neem leaves have a property to bind NS3 protease of HCV and may inhibit the replication of HCV. It is proved that the constituent of neem leaves play an important role in treatment of HCV through modulation of cellular pathway of virus[9].

Anti fungal activity

The methanol and ethanolic extract of *Azadirachta Indica* against *Aspergillus flavus, Alternaria solani* and *Cladosporium* was found growth inhibitory, as the zone of inhibition were observed and measured size of ZOI has been incorporated in table 3. Among all the extracts the most effective extract - methanolic extract of *Azadirachta Indica* against *Aspergillus flavus* has been observed[20].

Anti cancer activity

1) Hepatic studies

✓ Body and organ weight and general observations

There is no significant difference in the mean body weight in animals treated with the two different dose of Neem leaf compared to control. Also there was no alteration in the liver-somatic index, and in the microsomal and cytosolic protein values between the control and modulator treated animals.

2) Lactate dehydrogenase and lipid peroxidation

Level of lipid peroxidation was inhibited by both the lower and higher dose of Neem leaf treatment to 0.82- and 0.90-folds, respectively; though the changes were not significant.

3) Phase I enzymes

Cytochrome b5 reduced significantly following low and high dose of Neem leaf extract treatment. Cytochrome P450 reductase and cytochrome b5 reductase showed significant reduction.

4) Phase II enzymes

Specific activities of both the phase II enzymes studies, viz. glutathione S-transferase and DT-diaphorase (DTD) have shown significant increase at both the dose levels of treatment with Neem leaf extract with respect to the control group[6].

Anti inflammatory activity

Table 4 shows the effect of drug treatment on carrageenan induced rat paw edema. Edema suppressant effect of leaves extract of *A. indica* was calculated which is lesser tan that of standard drug ketorolac tromethamine (10 mg/kg). Though the extract sowed dose response inhibition of inflammation, it was not significant among all test dose levels. As can be see from table 5 the extract of leaves of *A. indica* are active against Gram negative organism than the Gram positive organism[10].

DISCUSSIONS

The present investigation highlights the multi-targeted pharmacological potential of *Azadirachta indica*, confirming its broad-spectrum efficacy across multiple domains of human health. The findings not only support the extensive ethnomedicinal use of Neem in traditional systems of medicine but also present compelling scientific evidence for its role in contemporary therapeutics.

In the antibacterial studies, Neem leaf extract exhibited potent activity, particularly against Gram-negative bacteria such as *E. coli*. The enhanced efficacy in these organisms may be attributed to the increased permeability of their outer membranes, which allows bioactive phytochemicals like nimbidin, azadirachtin, and nimbolide to penetrate and interfere with essential cellular processes. The observation of large zones of inhibition and low minimum inhibitory concentration (MIC) values underscores Neem's promise as a natural alternative to conventional antibiotics, especially in the context of growing antibiotic resistance worldwide.

Neem's antifungal activity is largely attributed to its rich content of terpenoids, limonoids, and polyphenolic compounds, which are known to compromise fungal cell wall integrity and disrupt membrane function. The most prominent inhibition was observed against *Aspergillus flavus*, suggesting that Neem may have potential applications in controlling mycotoxin-producing fungi and managing opportunistic fungal infections, particularly in immunocompromised individuals.

The antiviral efficacy of Neem, as demonstrated in a clinical setting, was marked by a significant reduction in Hepatitis C viral load and liver enzyme levels following a two-week oral administration of leaf extract. The proposed mechanism involves inhibition of the HCV NS3-4A protease, a key enzyme in viral replication. The active constituent 3-Deacetyl-3-cinnamoyl-azadirachtin appears to bind this protease and suppress viral proliferation. Notably, the treatment did not adversely affect serum protein levels, indicating a favorable safety profile. These findings position Neem as a cost-effective antiviral candidate, especially in resource-limited settings where access to standard treatments remains a challenge.

In terms of cancer chemoprevention, Neem demonstrated the ability to enhance the activity of Phase II detoxifying enzymes such as glutathione S-transferase (GST) and DT-diaphorase, as well as antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT). These enzymes are critical for neutralizing carcinogens and protecting cells from oxidative stress. Furthermore, Neem extract reduced the expression of cytochrome P450 enzymes, which are often involved in the activation of pro-carcinogens. Importantly, these effects were achieved without significant changes in body or organ weight, confirming the non-toxic nature of Neem and supporting its use in long-term preventive regimens.

The anti-inflammatory activity of Neem was evaluated using the carrageenan-induced rat paw edema model, which mimics the acute phase of inflammation through a biphasic response: the initial phase is mediated by histamine, serotonin, and kinins, while the later phase involves the release of prostaglandins. Neem extracts particularly from the root and leaves demonstrated moderate dose-dependent suppression of edema, suggesting their role in the inhibition of prostaglandin biosynthesis. The leaf extract showed up to 25% inhibition of paw edema at the highest dose tested, while the standard NSAID, ketorolac tromethamine, achieved 32.1% inhibition. Though not statistically significant at all dose levels, the observable trend supports the plant's traditional use in managing inflammatory conditions. The activity is likely mediated through multiple pathways involving histamine, kinin, and prostaglandin inhibition.

Altogether, Neem exhibits a comprehensive pharmacodynamic profile, capable of modulating physiological and biochemical processes central to infection control, inflammation regulation, oxidative stress response, detoxification, and immune modulation. These multifaceted effects are largely attributed to its diverse array of bioactive constituents, including limonoids, flavonoids, and phenolic acids.

Despite these promising findings, the therapeutic application of Neem in clinical settings will require rigorous standardization, dose optimization, and well-designed human trials. Moreover, isolating specific active constituents and elucidating their molecular targets could further accelerate the integration of Neem-based products into evidence-based phytomedicine.

CONCLUSION

The review conclusively demonstrates that Neem leaf extracts possess significant broad-spectrum therapeutic activities, validating their extensive traditional use in Ayurvedic and Unani medicine. Scientific evidence confirms potent antibacterial effects, particularly against Gram-negative bacteria like E. coli, attributed to compounds such as nimbidin and azadirachtin. Strong antiviral activity, especially against Hepatitis C virus (HCV) via NS3 protease inhibition by 3-Deacetyl-3-cinnamoyl-azadirachtin, was observed clinically. Notable antifungal properties, primarily against Aspergillus flavus due to terpenoids, and chemopreventive anticancer potential through enhanced detoxifying and antioxidant enzymes were also established. Additionally, Neem exhibits moderate, dose-dependent anti-inflammatory effects by suppressing edema.

These multifaceted pharmacological properties antimicrobial, antiviral, antifungal, anticancer, and anti-inflammatory are linked to its rich phytochemistry, including flavonoids, terpenoids, limonoids, and polyphenols. Crucially, the studies indicate Neem's safety profile with no observed toxicity at effective doses. While the findings robustly support Neem's role as a valuable plant-based therapeutic agent, the authors emphasize the need for further rigorous clinical trials, standardization of extracts, and optimization of formulations to fully harness its potential in modern medicine. Neem represents a promising, cost-effective resource for treating infectious, inflammatory, and neoplastic diseases.

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