Azadirachta Indica (Neem) in Traditional Indian Medicine: Indigenous Knowledge & Scientific Validation

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DOI: https://doi.org/10.51244/IJRSI.2025.120500127

Received: 02 June 2025; Accepted: 06 June 2025; Published: 16 June 2025

ABSTRACT

Neem, scientifically known as Azadirachta indica, has been an integral part of human history for millennia, especially in the Indian subcontinent. Its significance in ancient times can be seen in various aspects such as traditional, medicinal and cultural. Neem's versatility and wide range of usage made it an invaluable resource since ancient time. Its legacy continues to be celebrated in modern times for its numerous benefits. Bark, leaves, and roots contain Antimicrobial, Antifungal, Insecticidal, Antiviral, Anti-Malarial, Antiperiodic, Mosquito Larvicidal, Anti-Inflammatory, Antifertility, Spermicidal, and Hypoglycaemic properties. In modern time the medicinal and healing properties of Azadirachta indica can be interpretate by using the chemical analysis technique i.e. GC-MS. The chemical and biological analysis of Azadirachta indica (Neem) discovered the existence of many bioactive substances in various plant parts, including limonoids (Azadirachtin, nimbin and nimbidin), flavonoids (Quercetin, catechin), phenolic compound (Gallotannins and Ellagic Acid), alkaloids and sulfur compounds (Margolone and Margolonone). These bioactive compounds are responsible for bioactivity of various part of Azadirachta indica . Hence, this article is aimed highlighting the therapeutical potential of Neem plant, and bringing together traditional practices and modern sciences with a scientific validation through GC-MS Analysis.

Keywords: Azadirachta indica, Neem, Traditional medicine, Phytochemicals, Scientific validation, Ayurveda, Antimicrobial, Anticancer, Antioxidant. Gas Chromatography–Mass Spectrometry (GC-MS)

INTRODUCTION

Since from ancient period human depended on nature for their survival and lives strictly connected with nature. Human depends upon surrounding environment for their livelihood, healthcare, and sustenance and for basic needs (food, Fibers, shelter, clothing and gum). Plants play a crucial role in human culture as they are recommended for various therapeutic properties. Neem is one of the plants that has been used traditionally to treat varieties of human diseases. Neem is Azadirachta indica (in Persian, Azadi = free, diracht = tree), literally meaning “the free tree of India”, is an alliteration for its being intrinsically free from insect and disease problems. This tree is considered as a ‘Sarvaroga Nivarini’ which means “The panacea for all diseases” and has also been hailed as ‘heal all’, ‘divine tree’, ‘village dispensary’ and ‘nature’s drugstore’’ (Nagini & Subapriya, 2005). It is an evergreen plant found in Pakistan, India, Bangladesh, Burma, Thailand, Indonesia, Malaysia and Sri Lanka.

According to ayurveda neem adjusts the pitta and kapha. It’s cold, light, and dry characteristics will in general aggravate vata. It is regularly prescribed in mix with different herbs that assist in stifle of its vata inciting nature (Giri et al., 2019). During chemical and biological analysis. These analysis were carried out by using GC-MS (Gas Chromatography-Mass Spectrometry) equipment , which isolate the various substances within a given sample, and then it is used to retrieve the accessible compounds from the plant extract. The active compounds of neem show anti-inflammatory, antibacterial, anti-fungal, anti-cancerous etc. properties.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

The naturally occurring low molecular weight secondary metabolites are called phytochemicals. Till date over 4,500 phytochemicals have been reported and are classified based on their protective functions, and physical and chemical characteristics, amongst these about 350 phytochemicals have been studied in detail (Koche et al., 2016). These have evolved from a natural interaction between plants and environment and are biologically active molecules being considered for a long time as natural toxicants and harmful for human race. As growing epidemiological evidence has been found showing protective effect of plants and vegetables against chronic diseases a revival of interest in secondary metabolites have been developed. However, in view of the current comprehensive understanding of food, phytochemicals as well as dietary fibers could be considered as nutrients (Waltz & Leitzmann, 2005). The primary and secondary metabolites can be distinguished on the basis of their divergent functions. The primary and secondary metabolites can be distinguished on the basis of their divergent function. Proteins, fats, and carbohydrates including dietary fibre contribute to energy metabolism and to the structure of the plant cell are called the primary plant substances. Secondary plant metabolites are non-nutritive dietary components that are essential for the interactions of the plant with its environment. They serve as defence against insects, fungi, and other; as growth regulators, pigments, and flavours, while the diverse chemical compounds that are present only in very small amounts are called Secondary plant metabolites. Since they have potential pharmacological effects on humans, scientists are systematically investigating the health-promoting effects of phytochemicals (Leitzmann, 2016). Most phytochemicals that were formerly regarded as harmful have a variety of health-promoting effects (Watzl & Leitzmann, 2012). Primary metabolites are the essential compounds naturally produced by living organisms that help them grow and function. These include common substances like sugars, amino acids, proteins, the building blocks of DNA and RNA (purines and pyrimidines), and pigments like chlorophyll that are crucial for photosynthesis. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics and glucosides (Koche et al., 2016).

GC- MS ANALYSIS OF DIFFERENT PARTS OF *AZADIRACHTA INDICA* (NEEM)

One of the most popular methods for identifying and quantifying phytochemical components of plant extract is gas chromatography-mass spectrometry (GC–MS), which is very well-suited and the most used method (Verma et al., 2024). GC-MS is an instrument that combines the features of gas-chromatography and mass spectrometry to identify different organic compounds presents in the organic matter, which includes Alkanes, Fatty acids, Alkenones, Sterols etc. It is becoming the tool of choice for tracking organic compounds derived from variety of plants hence nowadays it is widely used for screening of phytochemical components of compound. Different part of neem (Leaves, bark and flower etc.) consists of various type phytochemical which can be acknowledged by this analysis.

Leaves:

The GC-MS analysis of chloroform fraction of methanolic extract of Neem leaves sample was carried out to isolate the substances in which seven major compounds is detected and their nature and properties given in Table I (Khan & Javaid, 2021). Nonacosane and tetratriacontane were the most abundant phytochemicals in this analysis.

Table I. Nature and Properties of compound from chloroform extract of Neem leaves (Khan & Javaid, 2021)

No, of compound	Names of compounds	Formula	Biological Activities	Peak area (%)
1.	2-Pentanol, acetate	$C_7H_{14}O_2$	-	9.72
2.	Decane	$C_{10}H_{22}$	Antimicrobial, antifungal, antibacterial	8.96
3.	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	$C_{10}H_{16}O_2$	Antimicrobial	6.56

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

4.	Nonanoic acid, 9-(3 hexenylidenecyclopropylidene),2-hydroxy-1 (hydroxymethyl)ethyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	Antioxidant, antimicrobial, therapeutic agent	7.13
5.	Quinoline-4-carboxamide, 2-phenyl-N-n.-octyl-	$C_{24}H_{28}N_{2O}$	-	9.79
6.	Nonacosane	$C_{29}H_{60}$	Antibacterial, Antimicrobial, Anti-inflammatory and Antidiabetic	44.27
7.	Tetratriacontane	$C_{34}H_{70}$	Antimicrobial, antibacterial, anticancer, antioxidant, hypoglycaemic	13.43

Bark:

The GC-MS study of ethanolic extract of Azadirachta indica bark confirms the presence of multiple bioactive phytochemicals with medicinal significance as given in the Table-II (Akeem et al., 2020). Here 9-Octadecenoic acid, methyl ester (E), cis-13-Octadecenoic acid methyl ester and 11-Octadecenoic acid methyl ester was the most abundant composition.

Table II. Components Detected in Azadirachta Indica Ethanolic Stem Bark Extract and Their Properties (Akeem et al., 2020), (Reza et al., 2021)

No. of compound	**Name of compound**	**Molecular formula**	**Biological Activities**	**Peak area (%)**
1.	Hexadecanoic acid, methyl ester.	$C_{17}H_{34}O_2$	Antioxidant, Hypocholesterolaemia, Nematicide, Pesticide, Antiandrogenic flavour Hemolytic,5-Alpha reductase inhibitor.	10.943
2.	Pentadecanoic acid, 14-ethyl ester	$C_{17}H_{34}O_2$	Anti-oxidant, antifungal and antimicrobial activities	1.695
3.	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	Antioxidant, Antiviral, Anticancer	25.022
4.	cis-13-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	Antinflammatory, Hypocholesterolemic, cancer preventive, hepatoprotective,	25.022
5.	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	Antioxidant, Antiviral, Anticancer	25.022
6.	Methyl stearate	$C_{19}H_{38}O_2$	Anti-diarrheal, cytotoxic and antiproliferative activity.	6.509
7.	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	Antioxidant, anti- inflammatory activities	0.84
8.	Ethyl Oleate	$C_{20}H_{38}O_2$	-	0.823
9.	9,17-Octadecadienal, (Z)	$C_{18}H_{32}O$	Antimicrobial, Anti-inflammatory	0.788
10.	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	Antioxidant, Pesticide, Flavour, 5-Alpha Reductase inhibitor, Antifibrinolytic, Haemolytic, Lubricant, Nematicide, Antialopecic	2.226
11.	Methyl 9-eicosenoate	$C_{21}H_{40}O_2$	-	1.113

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Seeds (Unripe):

In the GCMS study of methanolic extract of unripe seeds of Azadirachta indica seven major compounds were detected as summarized in Table-III (Guchhait et al., 2022) .4-Ethyl-2-hydroxy-2-cyclopentene-1-one, phthalic acid, and 2-hexyl-tetrahydothiophane were the most abundant phytochemicals.

Table III : GC-MS Profile of Methanolic Extract of Unripe Neem Seed (Guchhait et al., 2022)

No. of compound	Compound Name	Molecular Formula	M.wt (Da)	% Peak Area	Chemical Class	Biological Activities
1.	4-Aminopyrimidine	$C_4H_5N_3$	95	3.48	Pyrimidine alkaloid	Antibacterial
2.	2-Oxo-2,3-dihydro-1H-imidazole-4-carbonitrile	$C_4H_3N_3O$	109	1.05	Imidazole derivative	Antimicrobial, Antioxidant
3.	1-Naphthyl acetoxy acetate	$C_{14}H_{12}O_4$	244	4.61	Ester compound	Antibacterial
4.	4-Ethyl-2-hydroxy-2-cyclopentene-1-one	$C_7H_{10}O_2$	126	8.65	Carbonyl compound	Antibacterial
5.	Phthalic acid	$C_{21}H_{28}O_4$	344	11.73	Aromatic dicarboxylic acid	Antimicrobial
6.	3,7-Dimethyl-triazolo-triazine	$C_6H_7N_5$	149	3.41	Nitrogen heterocycle	Anticancer
7.	2-Hexyl-tetrahydrothiophane	$C_{10}H_{20}S$	172	13.55	Sulphur-containing heterocycle	Antimicrobial, Anticancer, Antifungal

Neem Seeds (Ripe):

There were four major phytochemicals found in the GCMS analysis of methanolic extract of ripe seeds of Azadirachta indica exhibiting the biological activities given in Table-IV (Guchhait et al., 2022)

Table IV : GC-MS Profile of Ripe Neem Seed Methanolic Extract (Guchhait et al., 2022)

No. of compound	Name of the Compound	Molecular Formula	M.wt (Da)	% Peak Area	Chemical Class	Biological Activities
1	1-Methylpyrrol	C_5H_7N	81	2.18	Heterocyclic compound	Antimicrobial, Anticancer
2	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	144	11.41	Flavonoid	Antibacterial, Antioxidant, Anticancer
3	2-Hydroxy-3,3-trimethylbutanamide	$C_7H_{15}NO_2$	145	1.80	n-Acyl amide	Not reported
4	4-Ethylbenzamide	$C_9H_{11}NO$	149	4.75	Phenolic amide	Antitumor, Antimicrobial

The GCMS Analysis of unripe and ripe seeds in the methanolic extract revealed significant differences in their phytochemical profiles, which are closely linked to variations in their biological activities such as antibiofilm, antibacterial, anticancer effects and consists more potent secondary metabolites than unripe seeds.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

MODERN USAGE OF *AZADIRACHTA INDICA* (NEEM)

In the pharmaceutical industries natural products play an important role in drug development programs, therefore, over 50% of all modern clinical drugs are of natural product origin. In the addition of the importance of synthetic medicinal chemistry, there is huge interest in herbal medicine, there has been a revival of interest in herbal medicines to control utmost diseases and to discover new molecular structures as lead compounds from the plant kingdom. From thousands of year nature has been a source of herbal medicine. Human society used Azadirachta indica (neem) as medicinal plants to cure diseases since ancient time. Different parts of neem contain different type of bioactive phytochemical compound which is used to control various type of human disease with less side effect.

Neem leaves contain very powerful phytochemicals like quercetin, Nimbolide, Nimbin etc. which possess various biological activities ranging from antioxidants, anti-inflammatory, to anticancer which are useful in the treatment of diseases like cardiovascular diseases, cancer and inflammatory conditions. Similarly, bark is rich in Nimbidin, Nimbin, Nimbidol which possess various biological activities like antiseptic, anti-fungal, and antimaterial used to cure skin infections like eczema, leprosy etc. also useful for arthritis, and microbial infections. The seeds harbour azadirachtin, Salannin, Gedunnin possess insecticidal, antiparasitic and anti-malarial properties potent medicine for malaria, pest control and parasitic infections.

Overall, the phytochemicals present in leaves, bark, and seeds of this plant contain significant high medicinal, and agricultural values. From combating diseases like malaria and skin disorders to pest control these bioactive agents present contribute to various therapeutic and environmental applications, making the plant useful in many key sectors like agricultural, environment, cosmetics, personal care, industrial and medicinal. (Guchhait et al., 2022)

Due to its rich phytochemical composition, it has diverse range of biologically activity that offer numerous applications in different area. Some of these are following

Agricultural and Environmental

Neem (Azadirachta indica) is a powerhouse in agriculture, offering a natural and eco-friendly alternative to synthetic pesticides and fertilizers. Extract of neem is rich in Azadirachtin which is capable of being used as insecticide (Isman & B., 2020). Bitter compounds of Neem tree act as a natural defense mechanism, making it an invaluable asset in organic pest control (Van & Paul, 2023). Other phytochemicals such as Nimbin, Gedunin, Quercitin are responsible for properties like insecticidal, Insecticidal antibacterial and antifungal , Hypoglycaemic (Adusei & Azupio, 2022). In addition, the neem compounds Curcumin, 1-Cysteine, Nemicidine, Nemol And Vimicidin have been proved to be antifungal and strong insect repellents (Brahmachari, 2004). Fumigant of Neem has also been used as pesticide and disinfectant in the form of gaseous state. Being 100% natural, it is nontoxic and does not affect environment. The main composition azadirachtin is responsible for stopping the growth cycle of insects and pests (Lokanadhan et al., 2012).

Cosmetic and personal care

It is containing the bioactive compound that allows it to be proposed as raw material for the soap, body lotion, shampoo etc. Neem bark and Neem oil are utilized for dental cleaning, for preventing and curing gum disease such as gingivitis, for bleeding gums, and for reducing bacterial plaque. In the cosmetics industry, it is utilized for facial masks; face powders, lotions, and sunscreen creams (Aurora Martínez-Romero, 2016). In addition, the face pack of neem leaves has high amount of Vitamin E, will guarantee the skin to hold the moisture and does not dry out (Arora et al., 2019).

Industrial Application

In recent years neem has found vital compound in the industrial sectors due to its biodegradable, non-toxic, and sustainable nature hence it also plays a crucial role in supporting in green chemistry and sustainable

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

development. Developing the biodegradable plastic based on the neem leaf extract could be the best alternative of plastic (Ashwini Shellikeri, 2018). Wood and timber can be sustained from termites, fungi and insects by using neem oil possessing the bioactive compound azadirachtin which is highly oxidized tetranortriterpenoid that has antifeedant and growth regulating effect on insects and fungi (Gilmara de Oliveira Machado Laurie J. Cookson, 2013). Neem produces several useful fuels its oil is burned in lamp and its wood has long been used for fire wood , the charcoal made from neem wood is of excellent quality (B et al., 1992)

DISCUSSION

Neem (Azadirachta indica) has been widely used in traditional practices for thousands of years in India. Its extensive use is rooted in ancient texts, Ayurveda, folk traditions, and spiritual practices. The tree's healing, purifying, and protective properties have been recognized for millennia, making it one of the most versatile plants in Indian culture. since Each part of the neem tree contributes uniquely to its medicinal value Here, we have validated them by using GC-MS analysis which bridge the gap between the indigenous knowledge and folk usage with modern analytical science and uses of neem.

Neem leaves have been used for managing acne and skin infection related problems, blood purification, wound healing as home remedy. GC-MS of Neem Leaves from **Table-I** reveals the seven compounds out of which Nonacosane (peak area 44.27%) and Tetratriacontane (Peak Area 13.43%) appearing in significant quantities. These compound exhibit various Antibacterial, Antimicrobial, Anti-inflammatory and anticancer, antioxidant and hypoglycaemic properties confirming neem's effectiveness from skincare to systemic detoxification. Antibacterial and Antifungal activity supports traditional use in acne, eczema, and wound healing, Antioxidant and anti-inflammatory effects correlates with folk use in fever, swelling, in the same context antibacterial and antifungal properties supports blood purifying activity. It also contains Nimbolide and Nimbidin which show antibacterial property, a noticeable improvement was observed when a paste of crushed neem leaves was applied on the part of face affected by acne and leprosy (Pratibha Nand, 2012), (Bhowmik et al., 2010).

Neem bark has been widely applied to promote oral hygiene and treat ulcers. The ethanolic extract of neem bark GCMS from **Table-II** revealed that it contains 9-Octadecenoic acid, methyl ester (E) (Peak Area 25.022%), cis-13-Octadecenoic acid methyl ester (Peak Area 25.022%), 11-Octadecenoic acid methyl ester (Peak Area 25.022%), Hexadecanoic acid, methyl ester (Peak Area 10.943%) which are present in high quantity and exhibit the bioactivities like Absorption and distribution in human plasma and lipoprotein lipids, Antioxidant, Pesticide,. In India the twigs neem tree branches are used to scrub the tooth as one of the most effective forms of dental care in traditional medicines due to presence of antibacterial and antimicrobial compounds by inhibiting the growth of Streptococcus mutans and significant reductions in bacterial adhesion in vitro, suggesting that it can reduce the ability of some streptococci to colonize on tooth surface to maintain oral hygine and dental health (Prashant et al., 2007), (Atul Kaushik, 2012). Hypersecretion of acid in the parietal cells of the stomach lining is One of the most critical contributors in ulcers formation, Nimbidin, the bitter principle of neem, has been shown to have antigastric ulcer activity by reducing the hypersecretion of gestric acid and inhibiting the stimulation of parietal cells (Bandyopadhyay et al., 1998).

Neem seeds, in both ripe and unripe forms, have been utilized in expressed neem oil known for its effectiveness in alleviating muscular pain, joint inflammation, and skin detoxification. GC-MS analysis (Tables 3 and 4) reveals distinct variations in their phytochemical composition, highlighting the impact of seed maturity on medicinal potency. GCMS analysis of NEEM SEEDS revealed that it contained 2-Hexyl-tetrahydrothiophane (Peak Area 13.55%), Phthalic acid (Peak Area 11.73%) in unripe seeds and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (Peak Area 11.41%), 4-Ethylbenzamide (Peak Area 4.75%) in ripe seeds containing properties like antimicrobial, anticancer, antifungal, anti-bacterial, anti-oxidant properties in them **(Table III and IV).** These findings emphasize that the stage of seed maturity plays a vital role in determining the chemical composition and pharmacological properties of neem. The maturation process appears to facilitate biochemical changes in secondary metabolites, potentially increasing

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

their therapeutic efficacy, particularly in targeting microbial biofilms and cancer cells. The GCMS Analysis of unripe and ripe seeds in the methanolic extract revealed significant differences in their phytochemical profiles, which are closely linked to variations in their biological activities such as antibiofilm, antibacterial, anticancer effects and consists more potent secondary metabolites than unripe seeds (Guchhait et al., 2022). Thiophene derivative compounds are known to have potential remedial properties to certain biofilm-related bacterial infections, GC-MS analyses revealed the presence of 2-hexyl-tetrahydrothiophane in unripe methanolic neem seed extract, considered to be responsible for antibiofilm activity towards the bacteria. Ripe neem seed extracts containing highest percentage of 3,5-dihydroxy-6-methyl-2,3-dihydro-4 H-pyran-4 one and 4-ethylbenzamide showed significantly higher antibiofilm activity, as revealed through the MBIC, MBEC and fluorescence studies, than the unripe neem seed extract. 3,5-dihydroxy-6-methyl-2,3-dihy dro-4 H-pyran-4-one has been reported to have potent antioxidant and antimicrobial activities. Ripe seed extract possessed significantly greater killing potency against the cancer cell line than unripe extract 4-ethylbenzamide, derivatives of 4-thiazolidinone conferred their anticancer potency (Guchhait et al., 2022).

A comparative study of the traditional uses of Neem and the phytochemicals identified through GC-MS analysis strongly supports its long-standing role in Indian traditional medicine. The leaves, bark, and seeds each possess unique bioactive compounds that align closely with their traditional therapeutic applications. These insights substantiate traditional medicinal practices through phytochemical validation and underscore Neem's promise as a reservoir of novel bioactive constituents, warranting comprehensive pharmacodynamic and clinical evaluations to explore its full therapeutic potential.

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ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

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