

# Azadirachta Indica (Neem) Leaf

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## **Abstract:**

NEEM is a tree in the mahogany family Meliaceae. It is one of two species in the genus AZADIRACHTA, and is native to India and Burma, growing in topical and semi-tropical regions. It is a fast growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. For thousands of years the beneficial properties of Neem have been recognized in the India tradition Each part of the neem tree has some medicinal property The taxonomical classification of neem is, Rurales (order), Rutina (Suborder), Meliaceae (Family), Melioidea (Subfamily), Melieae (Tibe), Azadirachta (Genus), and indica (Species)

## **INTRODUCTION:**

NEEM is a tree in the mahogany family Meliaceae. It is one of two species in the genus AZADIRACHTA, and is native to India and Burma, growing in topical and semi-tropical regions. It is a fast growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. For thousands of years the beneficial properties of Neem have been recognized in the India tradition Each part of the neem tree has some medicinal property The taxonomical classification of neem is, Rurales (order), Rutina (Suborder), Meliaceae (Family), Melioidea (Subfamily), Melieae (Tibe), Azadirachta (Genus), and indica (Species).

## **TAXONOMY OF AZADIRACHTA INDICA :**

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Dipsacales
Order	-	Rurales
Sub-order	-	Rutinae
Genus	-	Azadirachta
Species	-	Indica

## **HISTROY:**

Along with Ayurveda most of world's other medicinal system like Unani, Chinese, and European "Materia Medica" have announced and acknowledged neem tree as "Panacea of all Disese" However in India it is famous with many other name like 'Divine Tree' "Heal All" "Nature's Drugstor" and "village Dispensary" Traditional Ayurvedic uses of neem include the treatment of fever, leprosy, Malaria, ophthalima and tuberculosis. Various folk remedies for neem included oral, vaginal and topical use. It is honoured colloquially in these circles as "The village Pharmacy' millions with exposure to the tree brush

their teeth with its twigs, use its juice on their skin disorders and place its leaves throughout their homes to keep away insects. Few most important traditional uses of the different parts of plants are below stated.

- Neem twigs are used for brushing teeth in India and Pakistan. This practice is perhaps one of the earliest and most effective forms of dental care.

### **DESCRIPTION :**

Leaves – Compound, alternate, rachis 15-25 cm long, 0.1 cm thick; leaflets with oblique base, opposite, exstipulate, lanceolate, acute, serrate, 7-8.5 cm long and 1.0-1.7 cm wide, slightly yellowish-green; odour, indistinct; taste, bitter.

### **MICROSCOPIC DESCRIPTION**

**LEAF MIDRIB :** Leaflet through midrib shows a biconvex outline; epidermis on either side covered externally with thick cuticle; below epidermis 4-5 layered collenchymas present; stele composed of one crescent shaped vascular bundle towards lower and two to three smaller bundle towards upper surface; rest of tissues composed of thin – walled, parenchyma Matos cells having secretary cells and rosette, crystals of calcium oxalate; phloem surrounded by non lignified fibre strand; crystals also present in phloem region.

**LAMIN:** Shows dorsiventral structure; epidermis on either surface, composed of thin walled, tangentially elongated cells, covered externally with thick cuticle; anomocytic stomata present on lower surface only; palisade single layered; spongy parenchyma composed of 5-6 layered, thin walled cells, traversed by a number of veins; rosette crystals of calcium oxalate present in a few cells; palisade ratio 3.0-4.5; stomata index 13.0-14.5 on lower surface and 8.0-11.5 on upper surface.

**STEM BARK:** Shows outer exfoliation pieces hard, woody, considerably thick in older barks; almost dead elements of secondary phloem, alternating with discontinuous tangential bands of compressed cork tissue, former composed of several layers of stone cells occurring in regularly arranged groups together with collapsed phloem elements filled with brown contents; in between the successive zones of cork tissue 3.5 layers of fibre groups with intervening thin walled and often collapsed phloem element; each zone of cork tissue consists of several layers of regular, thin walled cells occasionally with a few compressed rows of thick walled cells towards.

### **ORIGN AND DISTRIBUTION:**

The neem tree occurs throughout India. According to an estimate, there are about 20 million trees in the country. The neem tree is noted for its drought resistance. Normally it thrives in areas with sub-arid to sub-humid conditions. With an annual rainfall between 400 and 1200 mm. It can grow in regions with an annual rainfall below 400 mm but in such cases it depends largely on the ground water levels. Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils temperature between 21-30 c it can tolerate high to very high temperature. It does not tolerate temperature below 4 c ( leaf shedding and death may ensure. )

**LEAF:** Sulphur containing compounds such as cyclic trisulphide (10) and tetra sulphide (11) isolated from the steam distillate of fresh, matured neem leaves have antifungal activity trichophyton mentagrophytes. [1]

### REVIEW OF LITERATURE:

To investigate the possibility of producing dental antimicrobial toothpaste from *Allium cepa* L skin chaff, *Azadirachta indica* A seed, and *tetrapleura* pad extracts.

Methods : Ethanolic extracts of the three plant materials were obtained. These were subjected to phytochemical and GC-MS analyses. The different extract combinations were used for the production of various toothpaste. The toothpaste's physical, organoleptic, and antimicrobial properties were determined.

From the phytochemical analysis, *Allium cepa* has the highest phenolic (1.20 mgGAE/g), Saponic ( 14.80 % ) tannin ( 0.11 mg/g) and DPPH ( 82.80 % ), *Tetrapleura tetraptera* has the highest flavonoid ( 0.33 mg RE/g) The GC-MS revealed significant chemical components of *Allium cepa* as 1-heptatriacotanol, germacra 1 (10), 4,11 (13) trien – 12 oic acid, 6 alpha hydroxy – gamma lactone, (E,E) 11 H-Indeno ( 1,2b) quinoxaline, 2 methyl while *Azadirachta indica* have butyl benzoate, benzoic acid, hexyl, ester, hexadecanoic acid, methyl ester and *tetrapleura tetraptera* have the following 15-hydroxypentadecanoic acid, cis 9 hexadecenal, and 11,13 dimethyl 1-12 tetradecen1 -01 acetate. All the produced toothpaste has a brown colour and a pleasant smell, with pH from 7.30 to 8.10 and foamability from 19.23% of stand alone toothpaste to 44.40 % of *Allium cepa* based Amongst the produced toothpaste *Allium cepa* based toothpaste has the best antimicrobial activities against the tested bacteria ( *pseudomonas aeruginosa*, *escherichai* ) *coli*, *klebsiella pneumonide*, *Klebsiella oxytoca*, ) and fungi ( *candida albicans* and *candida parapsilosis* ). The stand-alone toothpaste has the lowest minimum inhibitory concentration of 1.25 mg/ml against bacteria and fungi.

Clinical significance the study provides information on the production of human health friendly dental antimicrobial toothpaste from plant materials.

### AIMS AND OBJECTION

Oral diseases are prevalent world wide and range from dental caries, periodontal disease oral mucosal lesions, mouth odour, endodontic ( root canal ) infections, periodontal ( gum) disease and oral cancers [2]

Microbes have been implicated as the root cause of these diseases. These microbes convert starchy food materials to acid leading to the leaching of teeth components [3] wearing of enamel, gum, and exposure to opportunistic human pathogens [4]. The vitality of oral health to the overall wellness of humans cannot be over emphasised because various sicknesses, such as diabetes, high blood pressure, and heart – related diseases, are associated with oral challenges. [3]

Different toothpaste has been produced using chemicals, such as fluoride, peroxide, chlorhexidine amine fluorides, cetypyridinium chloride, to combat various oral diseases. Unfortunately, these materials have drawbacks; for example, peroxide compounds used as whitewashing agents can cause sensational

mouth burning. Preservative agents used in toothpaste, such as ethylene diamine tetraacetic acid and formaldehyde, are unsafe for human health [5] Fluoride based-toothpaste, though highly effective against oral pathogens, fluoride is reported to have caused dental stains, nausea, and oral cancer, while chlorhexidine amine fluorides, and cetylpyridinium chloride have been reported for their adverse effects [2].

Researchers are searching for safe, effective and cheap raw materials to produce toothpaste to manage oral diseases and their associated sicknesses. Consequently, multiple extracts from plants such as *A. Indica* ( *A indica* ) *Aloe barbadensis*, *Hibiscus sabdariffa*, *punica granatum* Linn, *Astragalus membranaceus*, *Rehmannia glutinosa*, and others have been employed in the production of toothpaste, because of their anti-inflammatory, antibiotic, analgesic, and antibacterial potency [6].

*Aloe vera* L and *Fragaria vesca* L ( wild strawberry ) based toothpaste [7] ( and neem twig extract formulated toothpastes [8] with good antimicrobial activity against *Streptococcus mutans*. And *Streptococcus* has been produced. Bacterial plaque and gingival bleeding have been treated with *Rosmarinus officinalis* Linn toothpaste.

*Allium cepa* L red onion skin chaff is a medicinal vegetable consumed worldwide and is a rich source of dietary flavonoids, anthocyanins, phenolics, fructans, and organosulfur compounds [9,10] These phytochemicals have been reported to be responsible for the antimicrobial anticancer, antibacterial, and antiviral potential of onions [9] *A Indica* (neem ) belongs to the family *Meliaceae*. It is a very good chewing stick, and many bioactive compounds from its parts have been employed as antibacterial, antiulcer, anti-inflammatory, and antimalarial [11] in dentistry, neem is used as an antiplaque, anticaries, and anti-inflammatory [12] It contains phytochemicals like tannins, saponins, phenols, flavonoids, and alkaloids [13] *Tetrapleura tetraptera* ( *T tetraptera* ) (Schun & Thonn) Taub. Is a typical Western African plant. The pharmacological properties and the volatile chemical compounds. Of *A. Cepa* skin chaff. *A indica* seed, and *T. Tetraptera* pod ethanol extracts. The research study also produces and characterises various plant extracts of toothpaste formulated compared to some commercial toothpaste.

The plant extracts yield ranged from 9.7 % to 16.55 %

Phytochemical constituents and antioxidants of plant extracts

The presence of phenolic, flavonoid, alkaloid saponin, oxalate, and tannin were confirmed from the phytochemical analysis of the plant extract. *A cepa* has the highest total phenolic content ( 1.20 mg GAE /g) tannin ( 0.11 mg/g), saponin ( 14.8 % ) and antioxidant (82.8%) in contrast *T. Tetraptera* has the highest alkaloid ( 20.4 mg /mL), and flavonoid value ( 0.33 mg/g) highest oxalate ( 77.5 mg/g ) was found in *A indica* All these phytochemicals contributed to the biological activities of the extracts.

### Gas chromatography / mass spectrometer (GC-MS) analysis

Different bioactive chemical compounds were detected in the various plant extracts. The GC-MS chromatogram of *A Indica* revealed the presence of twenty-six bioactive compounds (Table S2) twenty in *A cepa* (Table S1) and forty seven in *T tetraptera* ( Table S3) some detected bioactive compounds are ester, essential oil, and acid Those compounds have been reported to have bioactive activities beneficial to human health.

### Physicochemical and organoleptic properties

pH 7.30 to 8.10 formability 16.70% - 44.44% and there were no hard edge particles in all the toothpaste, The organoleptic properties of the toothpaste had a brown colour, pleasant smell and bitter taste. Antimicrobial activity of the formulated and commercial toothpaste

### Zone in inhibition of the formulated and commercial toothpaste

The toothpaste bioactiveness was tested against bacterial (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*) and fungi (*Candida albicans* and *Candida parapsilosis*) The zone of inhibition of the bacteria ranged from 0 mm to 19 mm while fungi had 0 mm to 18 mm.

### Minimum inhibitory concentration

The microbes bacteria and fungi recorded MIC *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella oxytoca* (bacteria) and *Candida parapsilosis* (fungi) recorded 1.25 mg/mL – 5.00 mg /mL. *Klebsiella pneumoniae* (bacteria) and *Candida albicans* (fungi) had 1.25 mg / mL – 10.00 mg / mL

### MATERIAL AND METHODS

The extracts from the plant present different yields, *Allium cepa*, has the lowest value (9.7%) *T. tetraptera* 15.10 % and the highest 16.55 % of *A. Indica*. Seed materials are sometimes tagged as an oil producers, which is probably the reason for the highest percentage yield recorded by *A. indica*.

### Phytochemical constituents and antioxidant of plant extracts.

Quantitative phytochemicals in the plant materials reveal their secondary metabolites, flavonoid, alkaloid, saponin, oxalate and tannin were present in all the plant materials to a different extent. *A. cepa* has the highest total phenolic content of 1.20 mg GAE/g followed by *T. tetraptera* 0.80 mg GAE /g and *A. Indica* at 0.30 mg GAE/g. The phenolic obtained in this study for *T. tetraptera* was lower than 3.37 mg/g [14] and 3.4 mg/g [15] reported in the literature. *A. indica*'s phenolic value of (0.30 mg /g) reported in this study was lower than, 3.94 mg/g, 1.74 mg/g, and 0.97 mg/g recorded using water, ethanol, acetone, ethyl acetate, and petroleum ether as extractants [16] However, the value was higher than 0.0032 mg/g reported in the literature [17]. *A. cepa* also recorded a lower phenolic value (1.20 mg/g) than 6.4 mg/g [18] However, the values of this study are higher than 0.43 mg/g (aqueous extract) and 0.70 mg /g (ethanol extract) [19] The differences in the reported literature value and those in this study could be linked to the sample preparation method, soil type, sample species, and analysis method, The high phenolic content of *A. cepa* suggests that it would have better antioxidant properties and thus can be a good material for anticancer, antitumor, and antibacterial.

Alkaloid was highest in *T. tetraptera* (20.4 mg/mL) followed by *A. Indica* (12.3 mg/mL for *Allium cepa*. Ogbugu and Chinagoram [20] reported 44 mg/g for *T. tetraptera* fruit, while 0.16 mg/g was ascribed to *A. Cepa* [17] Alkaloids are known to form salts with acids [21] thus the presence of alkaloids in the analysed plant materials could trigger a neutralisation reaction between the acid produced through the microbial breakdown in the mouth, causing a stoppage of mineralisation of the tooth and enamel. Also, the produced salt could act as a preservative in the mouth.

Among this study's analysed phytochemicals, flavonoid has lowest value. However, amongst the plant materials, T tetraptera has the highest value, 0.33 mg/g ( petroleum extract) but lower than 0.23 mg/g ( methanol extract ), 16.5 mg/g ( ethanol extract) , 278.4 mg/ g (70 % methanol extract ) and 212.3 mg / g ( 70 % ethanol extract ) [22] The difference in the results obtained by this study and Singh et al. [22] might be linked to the different standards used. While Singh et al. [22] used quercetin, this study used Rutin. The differences in the values reported by this study and other could be connected to factors such as the plant condition before usage, the plant species, and the experimental procedure. The presence of flavonoids in the analysed plant extract could be an added advantage to the proposed toothpaste's medicinal value since flavonoids have anti-inflammatory, anticancer, antibacterial, antiparasitic, and antiviral properties [23].

Plant extract with compounds that could boost oral hygiene is essential to formulating medicinal toothpaste. Tannin medicinal properties have been found to include anti-tumour, antimicrobial, anti-inflammatory, wound and bleeding treatment [15] Thus, there is a need to determine tannin in the plant extracts. A cepa ( 0.11 mg /g ) has the highest values in this ) study, followed by T.tetraptera ( 0.07 mg/g ) and A Indica. (0.03 mg/g ) Ogbugu and Chinagororom [20] reported tannin of 11.09 mg/g for Tetrapleura tetraptera, while Ajayi et al. [17] reported 0.0041mg/g for A indica, plant species, harvest period, and analysis methods could have caused the differences in the reported values in this study and those of others.

Saponin has been credited with antioxidant, anticancer, anti-inflammatory, and antifungal properties [24] Thus, the availability of saponin in the examined plants of this study will positively contribute to the medicinal value of the anticipated toothpaste. A. Cepa has the highest saponin, 148%, followed by T. Tetraptera (9.61%) and 6.13% for A. Indica In a separate study 0.60% was reported for T.tetraptera [34] and 2.50% was reported for A.indica [25]

The determination of oxalate is essential because an oxalate intake could cause oxalate stone formation [26] Therefore, the anticipated toothpaste should have low amount of oxalate. Oxalate daily intake should not exceed 50-200 mg/day [27] to prevent the occurrence of kidney stones. Oxalate recorded in this study is 34.2 mg/g, 75.00mg/g, and 77.5 gm/g, respectively, for Allium cepa, Tetrapleura tetraptera, and A indica The obtained values are within the daily recommended intake. Thus, including the extracts from allium cepa, Tetrapleura tetraptera, and A, indica would probably not cause any adverse health effects. In the literature, 5.6 gm/g and

The DPPH result revealed that the extract with the highest antioxidant activities also has the highest phenolic content, indicating a direct proportionality between phenolic and antioxidant activities (Fig.3) The antioxidant results show that A. cepa has the highest values ( 82.8%), followed by T. Tetraptera (73.6%) and A. Indica (73.00%) The values obtained in this study are higher than some of the literature values. Different DPPH values have been reported like 3.74% to 50.57% ( aqueous extract ) and 6.09%-89.71% ( ethanol extract ) for T. Tetraptera, using a concentration range from 1000ug/mL to 31.25ug/mL [33]. While, 65.76% and 53.43% were reported for yellow- and red-A. cepa chaff [42], 74.3% (aqueous ethanol) and 54.66% ( ethyl acetate ) [28]. The high antioxidant properties of the extracts will probably assist the antimicrobial and cleaning action of the proposed toothpaste.

## RESULT

Appraisal of the produced toothpaste showed that have suitable pH(7.30-8.10) and potent antimicrobial power against bacteria and fungi with zone of inhibition ranges from 4 to 18. These properties could be linked to various phytochemicals (such as phenolic, flavonoid, alkaloid, saponin, oxalate, and tannin) and chemical compounds (1H-3a, 7-methanoazulene, octahydro-1,4,9,9-tetramethyl, 1-heptatriacotanol, 2-dodecen-1-yl(-) succinic anhydride from *A. Ceba* while *A. indica* contained 9-octadecenoic acid (E), methyl ester, 9-octadecenoic acid, methyl stearate and *T. tetraptera* has cyclooctane 1,2-cyclopentanedione and benzofuran, 2,3-dihydro). Thus, the produced toothpaste can be employed in oral hygiene as a replacement for synthetic kinds of toothpaste, that have been credited with various negative effects like fluorosis.

## Author contribution statement

Olugbenga O. Oluwasina: Conceived and designed the experiments; performed the experiments; Analysed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. Clement Olusola ogidi, Festus O. Igbe: performed the experiments; Analysed and interpreted the data; contributed reagents, materials, analysis tools or data.

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## Date availability statement

No data was used for the research described in the article

## DECLARATION OF INTEREST'S STATEMENT

The authors declare no competing interest.

## DISCUSSION

Herbal Medicines are a few emerging tried in recent times, as they provide safer, effective and economical treatment to patients. *Azadirachta Indica* (neem) has been an integral part of health and oral care since times immemorial. It has shown to be an effective antimicrobial, anti-inflammatory and anticancer agent. In India it has been an indispensable part of oral care and is still a popular dental care agent in use. India. Incorporation of neem extract in oral health care products provides a natural solution to oro-dental problems and in maintaining oral health.

*Azadirachta indica* (Neem) belongs to the mahogany family *Meliaceae* and is a species of genus *Azadirachta*. *Meliaceae* and is a species of genus *Azadirachta*. Neem has two species: *A. Indica*, *A. Juss* and *M. Azedarac* which are known as Indian neem (margosa tree) or Indian lilac and Persian lila respectively [29]. Neem is a perennial plant found in tropical and semi-tropical regions of India, Pakistan, and Bangladesh [30]. *Azadirachta indica* is derived from Persian *Azad* meaning free and *dirakat* meaning tree, *indica* means of Indian origin hence it signifies free tree of India. Neem has been an indispensable herb in Ayurveda, Unani and other traditional medicine therapies since prehistoric times, Neem continues to be a cheap and effective drug for various health ailments in Indian folk medicine and thereby popularly

termend as village dispensary [31] siddiqui was first to identify medicinal properties of neem in 1942 He isolated Nimbin and nimbinin along with a bitter component nimbidin from neem. He stated that nimbidin has antiarthritic, antiulcer and inflammatory properties whereas former two had antipyretic and anti inflammatory properties [32] Various parts of neem plant have shown medicinal properties such as anti-tumourogenic, antioxidant, antiulcer and immunostimulant activity [33]

Part of tree	Pharmacological activity
Leaf	Antifungal activity, Antibacterial activity, Antiviral Activity, Anticarcinogenic activity, antiulcer effect, Hypoglycaemic activity, Hepatoprotective activity, central nervous system depressant, anxiolytic.
Bark	Anti-inflammatory, antibacterial, analgesic, antiseptic, Antiulcer and immunomodulatory
Seed	Antimalarial Activity, Antifungal activity, Antibacterial Activity, Antioxidant activity, Antifertility effect.

### Therapeutic Effect of Neem in oral Diseases

**Anti bacterial action** – Azadirachtin and nimbinin are principal constituents of neem extract responsible for its antibacterial properties [34] Neem leaf extract has shown significant reduction in plaque index and bacterial count especially *S. Mutans* and *Lactobacilli* species [35] Elavarasu et al also demonstrated in their study definite anti plaque activity of neem oil [36] Hedge & Kesaria compared antimicrobial efficacy of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against *E. Faecalis* and *C. Albicans* in their study. They showed excellent efficacy of neem extract in inhibition of most resistant species *E. Fecalis* and *Candida* in root canal disinfection [37] Adyanthaya et al studied antimicrobial effect of methanol extract of neem twig. They found efficacy of neem extract in reducing cariogenic as well as periodontal disease causing bacteria and suggested incorporation of methanol extract of neem twig into oral care products [38]

Neem is an omnipotent tree and nature’s gift to mankind for prevention and treatment of various health ailments. In past years extensive research on therapeutic benefits of neem in oral and dental problems had proved of neem in oral and dental problems had proved its efficacy as an excellent and cheap antimicrobial, antiinflammatory and anticancer agent. It’s time that neem extracts are incorporated in present day oral and dental care products as well as in treatment of various oral premalignant and malignant lesions.

### Plant material and preparation of extract

Leaves of *Azadirachta indica* juss. Were collected Lucknow district, Uttar Pradesh ( India ) during the month of januray 2012 The specimen was identified, authenticated and submitted at CSIR National Botanical Research Institute Lucknow ( Voucher specimen No LWG 98571 ) Leaves of the collected plants were washed thoroughly with distilled water and shade dried for ten days. A 1000g dried leaves were ground to a fine powder using mixer grinder and subjected to extraction thrice in 50% ethanol, using cold maceration technique. The extract was concentrated in rotary vacuum evaporator and stored at 40c until further use ( yield 21.24 % )



### QUALITATIVE ANALYSIS OF PHYTOCHEMICAL

The Azadirachta indica extract (AILE) was subject to preliminary phytochemical screening. Presence of alkaloids (Mayer's test), flavonoids (alkaline reagent test), tannins (Braymer's test), carbohydrates (Molisch's test), triterpenoids (Liebermann Burchard test), proteins and amino acids (Ninhydrin test) were tested.

### QUANTITATIVE ESTIMATION OF TOTAL FLAVONOID AND TANNINS

The total phenol content was determined using Folin-ciocalteu reagent and the total flavonoid content was estimated using aluminium chloride method [39]. The tannin estimation in crude drug followed the method mentioned by Schander [40] with slight variations.

### IDENTIFICATION OF PHYTOCONSTITUENTS IN AILE using HPTLC

The HPTLC plates are coated with high performance silica gel which is of very small and uniform in size (about 5  $\mu$ m). These high performance silica gels give more efficient and reproducible separation than conventional grades of silica gel. Known quantity of test and standard solutions were applied on a pre-coated silica gel GF254 plate of uniform thickness with help of LINOMAT 5 applicator attached of CAMAG HPTLC system. HPTLC profile of 50% ethanolic extract for various marker compounds were developed in solvent systems, toluene; ethyl acetate; formic acid for  $\beta$ -sitosterol and lupeol; toluene; ethyl acetate; formic acid for ellagic acid, ferulic acid, ferulic acid and quercetin; ethyl acetate: acetic acid: formic acid: water for rutin. The plates were scanned densitometrically by CAMAG scanner 3 by using software WinCATS and fingerprint profile was recorded. Standard peak of the reference marker compounds were scanned for their spectral analysis at the range of 200-700 nm wavelength and  $\lambda_{max}$  was recorded. Identification of all the marker compounds in extract were confirmed by overlaying absorption spectra at three different levels i.e. peak start, peak apex and peak end position of the spot of the respective marker compounds.

### DPPH FREE RADICAL SCAVENGING ASSAY

The free radical scavenging activity (antioxidant capacity) of AILE on stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by method mentioned by Brand Williams et al. [41]. Briefly, 2 ml of AILE at varying concentrations (50  $\mu$ g/ml) was 2.0 ml of DPPH solution in methanol (0.004% w/v). The mixture was allowed to stand at room temperature in dark for 20 min. Then the mixture was vortexed and absorbance was recorded at 517 nm using spectrophotometer. Ascorbic acid was used as a reference standard and control consisted of DPPH solution without extract. The test was performed in triplicate and percentage scavenging of DPPH free radical by extract was calculated using the equation:  $(A_{control} - A_{test}) / A_{control} \times 100$ , where  $A_{control}$  is the absorbance of control and  $A_{test}$  is the absorbance in presence of extract or standard.

### DETERMINATION OF IN-VITRO ANTIBACTERIAL ACTIVITY

The anti-bacterial activity was tested using agar well diffusion method according to Lino A, et al [42] and Arshad H et al [43]. The MTCC cultures were obtained from the department of Pharmacology, CSIR – NBRI. The AILE was tested for its antibacterial property against *Escherichia coli* (ATCC 10536) and *Staphylococcus aureus* (ATCC 33591). A 1 ml of test culture (10<sup>7</sup> CFU/ml) was inoculated into a sterile plate with 20 ml Muller Hinton agar which was then made to solidify. Three wells of

approximately 6 mm diameter were made on the surface of agar plate using a sterile cork – borer. Stock solution of AILE was dissolved in DMSO at varying concentrations (0.50 to 1.50 mg /ml ) A 50 ul extract of each concentration was pipetted in the well. A 50 ul DMSO served as negative. The plates were then incubated at 37°C for 24 hr and the zone of inhibition was recorded.

## SUMMARY

The Azhadiraction in Indian society. Has been considered higher than kalpavriksha, the mythological and ethanobotanical beneficial properties. The efficacy of neem as a medicine has been documented in several different ancient treatments like the Atharvana Veda, the Ghryasutra and Sutragrantha and in puranas. The acidic principles in neem as margosic acid with three acetive constituents. Viz nimbin, nimbidin and nimbinene. Chemically, for example, pesticides, antifeedants and cytotoxic properties, also show a whole range of biological activity.

The Sanskrit word nimbi means ( Nimbat sincati svasthyamiti, svastha vrtikaramiti yavat ) that which gives health [44] The neem tree is an extraordinary plant that has been proclaimed by the United Nations as the Tree of the 21<sup>st</sup> century The neem twigs are used as tooth brushes as it possess prophylactic effect widely used by 500 million people of india alone [45,46] Neem is used is Ayurveda, Siddha, Unani and Homeopathic medicine. In India, Pharmaceutical chemists isolated margosic acid during 1919, an acidic principle in neem oil containing three active constituents, such as nimbin nimbidin and nimbinene, since they were chemically identical and biogenetically derived from tetracycliterpenes [47] The components are also referred ro as liminoids as it contains azhdirachtin, meliantrol, salanin etc, ( chen et al., 2018 ) These compounds also exhibits a wide range of biological activity.

## MEDICINAL USES OF NEEM

Neem has more than 140 compounds isolated from different parts of the tree [48] The parts of neem such as leaves, flowers, seeds fruts, roots and bark have been used traditionally in treating inflammation, infections, fever, skin diseases, dental disorders and removing the evil effects of spirits. Quercetin ( flavonoid ) and nimboesterol ( B-sitosterol ) are present in the leaves, as is of the liminoids ( nimbin and its derivatives ) Quercetin is a polyphenolic flavonoind that tends to have antibacterial and antifungal properties and possess curative effects for sores and scabies [49] At a dosage ranging between 100 and 500 ppm, limonoids such as nimocinolide and isonimocinolide affect fertility in house flies and mutagenic properties are also founds against mosquitoes yields intermediates. New matured leaves develop on adorous viscous essential oil that exhibits fungal antifungal activity against Trichophyton mentagrophytes at invitro conditions [50] White crystalline flakes obtained from petroleum ether extract of leaves has C 14, C 24, C 31 alkanes were found to exceed or equal the larvicidal activity of pyrethrum extract [51] Proteins carbohydrate minerals calcium, phosphorus, vitamin c, carotene, etc. Are the main constituents of neem leaves Amino acids such as glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine, cystine and other fatty acids such as dodecanoic acid, tetradecanoic acid, elcosanic anci, etc. Are present.

## VALUABLE PRODUCTS EXTRACTED FROM THE NEEM

1. Antimalarial	2. Antiuberculosis	3. Antiviral
4. Anti allergic	5. Anti Enzemic	6. Antiscabic

7. Anti Dermatic	8. Anti Gingivits	9. Antinflammatory
10. Anti Perodontici	11. Ameobicial	12. Diuretic
13. Spermicidal	14. Antipyrhoic	15. Anti furancular
16. Anti freedant	17. Anti fungal	18. Anti furancular
19. Bactericide	20. Insecticidal	21. Larvicidal
22. Piscidal	23. Anti cardiac arrest	24. Nematicidal

Nimbosterol and flavonoids as kaempferol, melicitrin are present in flowers which are one of the essential oil consisting of sequiterpene derivatives while flowers yields a waxy material which contains fatty acids such as behenic ( 0.7 percent ), arachidic ( 0.7 percent ), stearic ( 8.2 percent ), palmitic ( 13.6 percent ), oleic (6.5 percent ) and linolec (8.5 percent ) [52] There are several amino acids in the neem pollen, including glumatic acid, tyrosine, arginine, methione, phenylanine, histidine, arminocaprylic, acid, and isolecuine [53]

Nimbin ( 0.04 percent ) nimbinin ( 0.001 percent ) nimbidin ( 0.4 percent ) nimbosterol ( 0.03 percent ) , essential oil ( 0.02 percent ), tannin ( 6.0 percent ) bitter margosin and 6 desacetyl nimbinene are found in the trunk bark [49] The stem bark is rich in tannins ( 12-16 percent ) and non tanni ( 8 – 11 percent ) The bark contains anti inflammatory polysaccharide consisting of glucose, arabinose and fructose at a molar ratio 1:1:1 with molecular weight of 8400 [54] The bark also yields an antitumor polysaccharide [55] [56] stem bark and root bark yields diterpenoids such though heartwood contains iron salts, potassium and calcium, heartwood provides charcoal (30 percent ) and pyroligeneous acid (38.4 percent) by the process of destructive distillation. [57] Neem wood contains ellulose,hemicelluloses (14.00%) and lignin (14.63%) while wood oil contains B-sitosterol,cycloeucaenol and 24-ethylenecyceloartenol [58] The gum exuded by the tree on hydrolysis yields, D-glucoronic acid L-arabinose, D-galactose and L-fucose as the older tree exudes a sap which has free sugars such as glucose, fructose, mannose and xylose and amino acids includes asparagines, praline, alanine, aminobutyric, acid, arginice, aspartic acid, glycine, norvaline, and amino acids viz., citric, malonic, succinic and umaric acids. [57] The sap is reported to be useful in the trreatment of general weakness and skin diseases. Seed is very important both because of its high lipid content and vecause of the presence of a large number of bitter principles that include, in substantial amounts, azadirachtin, azadiradione, fraxinellone, nimbin, salannin, salannol, vepinin, and vilasinin.

**Table 2: Chemical Composition of Neem Leaves**

S.No.	Chemical composition	Unit as prescribed in parenthesis
1.	Moisture	59.4 (%)
2.	Fat	1.0 (%)
3.	Carbohydrates	22.9(%)
4.	Calcium	510(mg/100g)
5.	Iron	17(mg/100g)
6.	Niacin	1.40(mg/100g)
7.	Carotene	1998(ug/100g)
8.	Glutamic acid	73.30(mg/100g)

9.	Asparic acid	15.50(gm/100g)
10.	Proline	4.00(gm/100g)
11.	Proteins	7.1(%)
12.	Fiber	6.2(%)
13.	Minerals	3.4(%)
14.	Phosphorus	80(gm/100g)
15.	Thiamine	0.04(gm/100g)
16.	Vitamin C	218(gm/100g)
17.	Calorific value	1290(kcal/kg)
18.	Tyrosine	31.50(gm/100g)
19.	Alanine	6.40(gm/100g)
20.	Glutamine	1.00(gm/100g)

[49]

### Conclusion

Neem (*Azadirachta indica* Linn) is a spiritual divine tree mainly grown in Indian subcontinent, all parts of the neem is used as medicine for curing various diseases in human as well as animal beings. The active compound present in neem play a major role to relieve different pains, fevers, infections and other problems. The multipurpose benefit behind in neem tree as a uncountable and more valuable gift to living forms is the ecosystems.

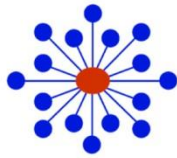
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