Azadirachta Indiaca (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, Rutales (order), Rutina (Suborder), Meliaceae (Family), Melioidea (Subfamily), Melieae (Ttibe), 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, Rutales (order), Rutina (Suborder), Meliaceae (Family), Melioidea (Subfamily), Melieae (Ttibe), Azadirachta (Genus), and indica (Species).

TAXONOMY OF AZADIRACHTA INDICA :
Kingdom - Plantae
Division - Magnoliophyta
Class - Dipsacales
Order - Rutales
Sub-order - Rutinae
Genus - Azadirachta
Species - Indica

HISTORY:
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 Disease” 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, ophthalmia 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 twings 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 tertrapleura 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 tetraperta 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 hydrxy – 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 tetraperta 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, chlorchexidine 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 buring. 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, antiinflammation, and antimalarial [11] in dentistry, neem is used as an antiplaque, anticaries, and anti-inflammatory [12]. It contains phytochemicals like tannins, sapoins, phenols, flavonoids, and alkalodis [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 sapoain, 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. Antimicrobrial 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 pneumonia, 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.

Mimimum inhibitory concentration
The microbes bacteria and fungi recorded MIC Pseudomonas aeruginosa, Escherichia coli and Klebsiella oxytoca (bacteria) and candidia parapsilosis (fungi) recorded 1.25 mg/mL – 5.00 mg /mL. Klebsiella pneumonia (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 tetraperta 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.tetraperta 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 repoted literature value and those in this study could be linked to the sample prepration 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 alkalodis 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, flavonoids 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 querctin, 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 Chinagorom [20] reported tannin of 11.09 mg/g for Tetrapleura tetraptera, while Ajayi et al. [17] reported 0.0041 mg/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, 75.00 mg/g, and 77.5 mg/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 proportion 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. Cepa while A.indica contained 9-octadeconic acid (E), methyl ester, 9-octadecenoic acid, methyl stearate and T.tetrapera 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, antiflammatory and anticancer agent. In India it has been an indespensible part of oral care and is still a popular dental care agent in rule. 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 perinenial 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 indespensible herb in ayurveda, unani and other traditional medicine therapies since prehistoric times, Neem continue 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]

<table>
<thead>
<tr>
<th>Part of tree</th>
<th>Pharmacological activity</th>
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<tbody>
<tr>
<td>Leaf</td>
<td>Antifungal activity, Antibacterial activity, Antiviral Activity, Anticarcinogenic activity, antiulcer effect, Hypoglycaemic activity, Hepatoprotective activity, central nervous system depressant, anxiolytic.</td>
</tr>
<tr>
<td>Bark</td>
<td>Anti-inflammatory, antibacterial, analgesic, antiseptic, Antiulcer and immunomodulatory</td>
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<tr>
<td>Seed</td>
<td>Antimalarial Activity, Antifungal activity, Antibacterial Activity, Antioxidant activity, Antifertility effect.</td>
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**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 or 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, antinflammatory 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 Azaidrachta indica juss. Were collected Lucknow district, Uttar Pradesh ( India ) during the mouth of januray 2012 The specimen was identi ied, 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 ine 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 futher us ( 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, FLAVONOIDS AND TANNINS
The total phenol content was determined using Folin-ciocalteau 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 modifications.

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 μm). These high performance silica gels give more efficient and reproducible separation than conventional grades of silica gel. Known quantities of test and standard solutions were applied on a pre-coated silica gel GF254 plate of uniform thickness with the help of LINOMAT 5 applicator attached to CAMAG HPTLC system. HPTLC profile of 50% ethanolic extract for various marker compounds were developed in solvent systems, toluene; ethyl acetate; formic acid for β-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 the 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 y mass 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 μg/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: \[ \frac{A_{control} - A_{test}}{A_{control}} \times 100 \]
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 antibacterial 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 Pharmacognosy, 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 (107 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 370c 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 acetic constituents. Viz nimbin, nimbidin and nimbinene. Chemically, for example, pesticides, antifeedants and cytoxic 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 21st 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 tetracyclicterpenes [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 compunds 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 nimbosterol ( 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**

<table>
<thead>
<tr>
<th>1. Antimalarial</th>
<th>2. Antiuberculosis</th>
<th>3. Antiviral</th>
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11. Anti Dermatic
12. Anti Gingivits
13. Anti Perodontici
14. Anti Gingivits
15. Antinflammatory
16. Anti Perodontici
17. Anti Perodontici
18. Anti Perodontici
19. Ameobicidal
20. Anti furancular
21. Diuretic
22. Spermicidal
23. Antipyrhoeic
24. Anti furancular
25. Bactericide
26. Insecticidal
27. Larvicidal
28. Piscidal
29. Anti cardiac arrest
30. Nematicidal

Nimbosteral and flavonoids as kaempferol, melicitrin are present in flowers which are one of the essential oil consisting of sequipterene 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 glutamic acid, tyrosine, arginine, methione, phenylalanine, histidine, arminocaprylic acid, and isolate con[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 nimbisterol 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 pyrogenelous 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, cycloeucalenol and 24-ethylenecycloartenol. [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 treatment of general weakness and skin diseases. Seed is very important both because of its high lipid content and because of the presence of a large number of bitter principles that include, in substantial amounts, azadirachtin, azadiradiradione, fraxinellone, nimbin, salannin, salannol, vepinin, and vilasinin.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chemical composition</th>
<th>Unit as prescribed in parenthesis</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Moisture</td>
<td>59.4 (%)</td>
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<tr>
<td>2.</td>
<td>Fat</td>
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<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>22.9(%)</td>
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<tr>
<td>4.</td>
<td>Calcium</td>
<td>510(mg/100g)</td>
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<tr>
<td>5.</td>
<td>Iron</td>
<td>17(mg/100g)</td>
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<tr>
<td>6.</td>
<td>Niacin</td>
<td>1.4(mg/100g)</td>
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<tr>
<td>7.</td>
<td>Carotene</td>
<td>1998(ug/100g)</td>
</tr>
<tr>
<td>8.</td>
<td>Glutamic acid</td>
<td>73.3(mg/100g)</td>
</tr>
<tr>
<td></td>
<td>Nutrient</td>
<td>Value</td>
</tr>
<tr>
<td>---</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>9</td>
<td>Asparic acid</td>
<td>15.50 (gm/100g)</td>
</tr>
<tr>
<td>10</td>
<td>Proline</td>
<td>4.00 (gm/100g)</td>
</tr>
<tr>
<td>11</td>
<td>Proteins</td>
<td>7.1 (%)</td>
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<tr>
<td>12</td>
<td>Fiber</td>
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<tr>
<td>13</td>
<td>Minerals</td>
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<tr>
<td>14</td>
<td>Phosphorus</td>
<td>80 (gm/100g)</td>
</tr>
<tr>
<td>15</td>
<td>Thiamine</td>
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<tr>
<td>16</td>
<td>Vitamin C</td>
<td>218 (gm/100g)</td>
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<tr>
<td>17</td>
<td>Calorific value</td>
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<tr>
<td>18</td>
<td>Tyrosine</td>
<td>31.50 (gm/100g)</td>
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<tr>
<td>19</td>
<td>Alanine</td>
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</tr>
<tr>
<td>20</td>
<td>Glutamine</td>
<td>1.00 (gm/100g)</td>
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**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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<td>Investigational New Drugs</td>
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Course Completion Date: 3 January 2023
CTN Expiration Date: 3 January 2026

Eve Jelstrom

Eve Jelstrom, Principal Investigator
NDAT CTN Clinical Coordinating Center

Good Clinical Practice, Version 5, effective 03-Mar-2017

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