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Study of Pharmacological and Therapeutic Potential of Neem Leaves (*Azadirachta Indica*)

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ABSTRACT:

The research investigates the pharmacological and therapeutic potential of *Azadirachta indica* (neem) leaves, with a focus on their phytochemical composition and antimicrobial efficacy. Fresh neem leaves were collected, processed, and extracted using ethyl acetate for subsequent biochemical and antimicrobial analysis. Phytochemical screening revealed the presence of key bioactive constituents such as alkaloids, tannins, terpenoids, flavonoids, and fatty acids. Antibacterial assays using the agar well diffusion method demonstrated pronounced inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* sp, while antifungal testing indicated effectiveness against *Penicillium*, *Aspergillus niger*, and *Aspergillus flavus*. Fourier-transform infrared (FTIR) spectroscopy further identified functional groups corresponding to amino acids, alkenes, nitrates, ethers, and carbohydrates, confirming the presence of pharmacologically relevant compounds and the absence of toxic cyanide residues. These findings underscore the significant antimicrobial and phytochemical properties of neem leaves, supporting their potential role in developing natural therapeutic agents. Nonetheless, clinical application should be guided by scientific validation and medical supervision due to the complexity of bioactive interactions.

Key words: Azadirachta indica, FTIR spectroscopy, Natural therapeutics, Plant-based bioactives, Non-toxic botanical extract

1. Introduction:

Neem (*Azadirachta indica A. Juss*.), an evergreen tropical forest tree, is a renewable source of numerous beneficial products. There are several applications for this tree's seeds, leaves, roots, bark, trunk, and branches, to mention some of them. Despite the fact that many plants are very effective, environmentally safe, and non-toxic ways to eradicate or control insect infestations that lower agricultural productivity, neem has the ability to produce insecticides and insect repellents (Govindacharya *et al*., 1992). Azadirachtin, which can be extracted from the seed kernels, is the most important of the several bioactive compounds found in different parts of the tree (Thengane *et al*., 1995). Neem is a great and beautiful gift from the natural world. The tropical evergreen tree is indigenous to India and can also be found in other Southeast Asian nations. Neem has been designated as the "Tree of the 21st century" by the United Nations. It is an incomprehensible plant, hence the term "century" a widely disseminated study entitled "Neem: A tree for the US National Academy of Science gave a lecture on global problem resolution in 1992. *Azadirachta* Indicated as "Azad" for "free," indica is a Latin word that has Persian roots. "dirakht" for "tree" and "I" for "Hind" signify that anything is rooted in India. Nimem is "arista" is a term used in Sanskrit that translates as "complete and imperishable".

The neem tree is one of the few trees that are known to exist on the Indian subcontinent. This tree, which belongs to the Meliaceae family, is growing rapidly in tropical and subtropical climates. It is also mentioned that the tree could survive in arid and desert conditions. Despite being an evergreen, neem trees can have temporary leafless spells under certain conditions. Numerous parts of Indonesia, such as Bali, Lombok, East Java (Situbondo Ngawi), West Java, Central Java, and Nusa Tenggara Barat, are home to neem trees. All parts of the neem plant, including the advantages of the bark, leaves, blossoms, fruits, seeds, and roots for use in industrial and therapeutic applications. It has been demonstrated that neem plant extracts are toxic to certain fungus, such as *Aspergillus flavus* from soybean seeds and Poria monticolad, which infects wood (Dholi *et al*., 2011). *Pyricularia oryzae* infects rice crops and field-grown plants (Arumugam *et al*., 2014). Therefore, it may be expected that the required substances will likewise be efficient in preventing fungi from damaging harvested fruits. There is currently very little evidence available regarding neem extract's ability to prevent plant-caused fruit deterioration pathogens during storage (Killedar *et al*., 2025).

2. Materials and Methods:

2.1. Sample collection, preparation and extraction:

Fresh neem leaves was collected from the campus of Davanagere University in polythene covers and brought to the laboratory and then washed thoroughly 2-3 times with tap water and once with sterile water and plot dried. After collecting the healthy neem leaves and washed with fresh water, then, leaves were crushed by using the mortar and pestle, later the sample was filtered using Whatman's filter paper and the filtrate was collected in clean bottles and stored at refrigerator temperature for further process (Paul *et al*,.2002).

2.2. Ethyl acetate extraction:

In this method, 20 ml of neem extract and 40 ml of ethyl acetate were taken in a separating funnel in 1:2 ratio and mixed well (Ramalingappa *et al.,* 2025). Later the mixture was placed in a separating funnel stand with its stopcock closed. Stopper was fixed, firmly a stand was allowed to leave the mixture in the funnel undisturbed for a while. Extract was remained below. Now without shaking the funnel, extract was collected in clean beaker and stored at refrigerator temperature for further work (Sharma *et al*., 2014).

2.3. Phytochemical test of neem leaves:

2.3.1. Test for Tannins:

About 200 mg of the neem leaves extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture, which was then observed for blue-black coloration indicating the presence of tannins.

2.3.2. Test for Alkaloids:

The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of yellow or white precipitate indicated the presence of alkaloids.

2.3.3. Test for Saponins:

About 0.5 millilitres of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

2.3.4. Test for Steroids:

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

2.3.5. Test for Terpenoids:

The presence of terpenoids was determined by the formation of a reddish-brown colour in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

2.3.6. Test for killer kilane:

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides (Killedar *et al.,* 2024).

2.4. Determination of antibacterial activity:

Agar well diffusion method was used to evaluate the antibacterial activity of plants or microbial extract. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface, then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer, and a volume (20µl) of extract solution at dilution is introduced into the well. Then agar plates are incubated under suitable conditions for 24 hours at 37^0C (Sithisarn *et al*., 2005).

2.5. Determination of antifungal activity:

Potato dextrose agar medium plate's surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer, and a volume (20µl) of the antifungal agent or extract solution at dilution is introduced into the well. Then agar plates are incubated under suitable conditions for 48-72 hours at room temperature (Sithisarn *et al*., 2005).

2.6. FI-IR spectrometer method:

Fresh *Azadirachta indica* leaf extract samples were finely grinded using a mortar and pestle. A 2 to 3 mg portion of the extract was mixed with 5ml of Ethyl and Chloroform respectively, (FT-IR) and stalled to form layer oil. This oil was placed in the sample holder of a Bruker FT-IR spectrometer, and FT-IR spectra were recorded across the 400 to 4000 cm^{-1} absorption range and results were reported.

3. Results and Discussion:

Samples were collected from different locations around the college campus, near canteen using polythene covers, then, the samples was washed with the tap water and then rinsed with distilled water then allowed to plot dried. After washing the sample the leaves were crushed by using the mortar and pestle and sample was filtered through whatman filter paper, later filtrate was collected in a clean conical flask for further analysis. The steps involved in the process as shown in the Figure.1.

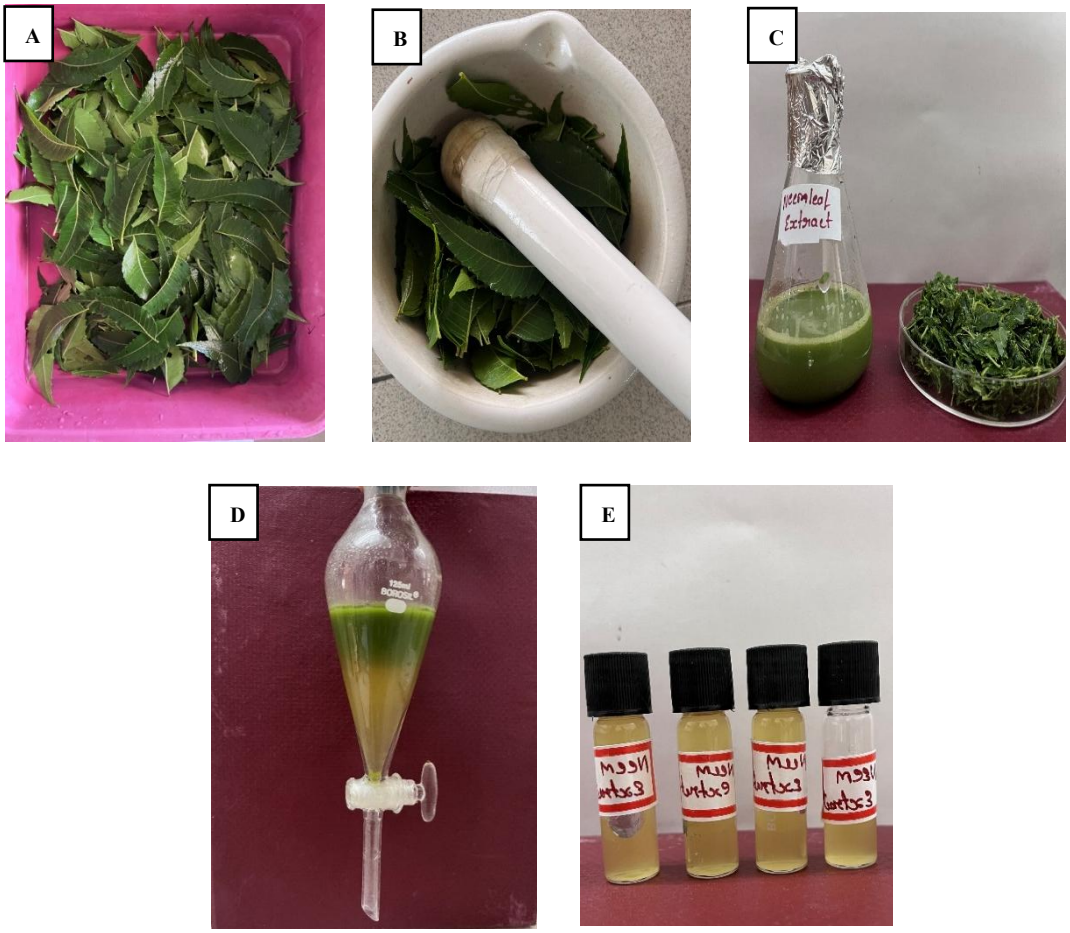

Figure.1. Steps involved in the extraction of neem. A. Collection of neem. B. Crushing of neem leaves.

C. Filtrate of Neem leaves. D. Ethyl acetate extraction. E. Ethyl extract.

20 ml of neem filtrate and 40 ml of ethyl acetate was taken in separation funnel. After separation ethyl extract was collected in sterilized collection tubes for further analysis. Phytochemical analysis of neem extract was done. It showed the presence of phytoconstituents such as terpenoids, saponins, steroids and glycosides. The presence of deoxysugar, a feature of cardenolides, is shown by the reddish-brown ring not forms at the interface of two liquids, it indicates the negative results for glycosides. The extracts of neem turn blue or green color after addition of two drops of ferric chloride, so it indicates the positive results for tannins. The test for saponins showed positive result by formation of stable foam. Terpenoids test showed positive result by formation of reddish-brown color ring which indicates the presence of phytosterols. The extracts of neem does not showed greenish black

color in the lower chloroform layer indicates absence of flavonoids. Lead acetate test, where the appearance of yellow color precipitate is formed indicates the presence of flavonoids. There is no appearance of red or violet color is indicates the presence of proteins so the test showed negative result for proteins. In fatty acids or fixed oil testing, oil stains on filter paper shows the presence of fixed oils and fats and it indicates the positive result as shown in the Table.1.

Table.1. Phytochemical analysis of Azadirachta indica

Bioactive compound	Tests	Ethyl acetate Extract
Alkaloids	Mayer's test	**Positive (+)**
Glycosides	Benedict's test	**Negative (-)**
Tannins	Lead test	**Positive (+)**
Saponins	Foam test	**Negative (-)**
Terpenoids	Salkowski test	**Positive (+)**
Flavonoids	Lead acetate test	**Positive (+)**
	Ferric chloride test	**Negative (-)**
Proteins	Biuret test	**Negative (-)**
Fatty acids	**Spot test**	**Positive (+)**

Seriana *et al*., (2021) investigates the phytochemical characteristics of neem (*Azadirachta indica*) leaves, focusing on their potential as a male contraceptive. Jose *et al*., (2023) focused on the phytochemical analysis of aqueous extracts from *Azadirachta indica* (neem) leaves, employing qualitative biochemical tests and Fourier Transform Infrared (FTIR) spectroscopy. The analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, terpenoids, glycosides, phenols, steroids, tannins, and saponins. FTIR spectroscopy further identified functional groups such as alcohols, carboxylic acids, and amine salts, indicating that *A. indica* is a rich source of bioactive compounds with significant potential for pharmaceutical applications. Khanal *et al*., (2021), focuses on the qualitative and quantitative phytochemical screening of various parts of the *Azadirachta indica* (neem) plant. The research reveals the presence of alkaloids, flavonoids, saponins, and terpenoids across all examined parts, with the highest concentration of alkaloids found in the stem-bark (12.8%) and the highest flavonoids in the leaves (13.8%). The findings suggest that neem contains significant bioactive compounds that may have potential medicinal applications.

Antibacterial activity of neem extract was carried out against three bacterial species, *Staphylococcus aureus*, *E coli*, and *Pseudomonas* sp. It indicates neem leaf extract should more activity against bacterial species. After incubation it have been found to show zone of inhibition around the well, *Staphylococcus aureus* showed more zone of inhibition compare to other two bacterial species as shown in the Figure.2 and Figure.3.

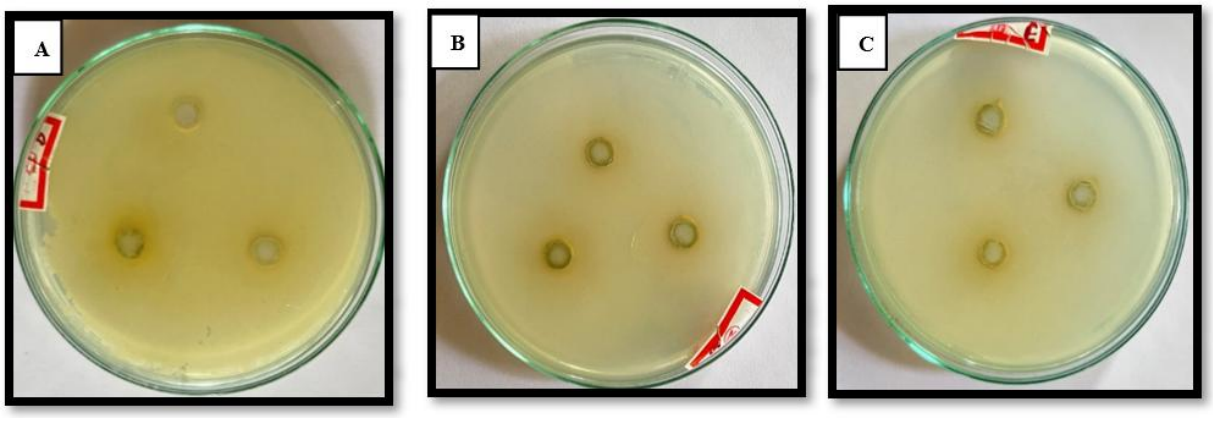

Figure.2. Antibacterial activity of neem extract. A. *S.aureus*; B. *E.coli*; C. *Pseudomonas* Sp

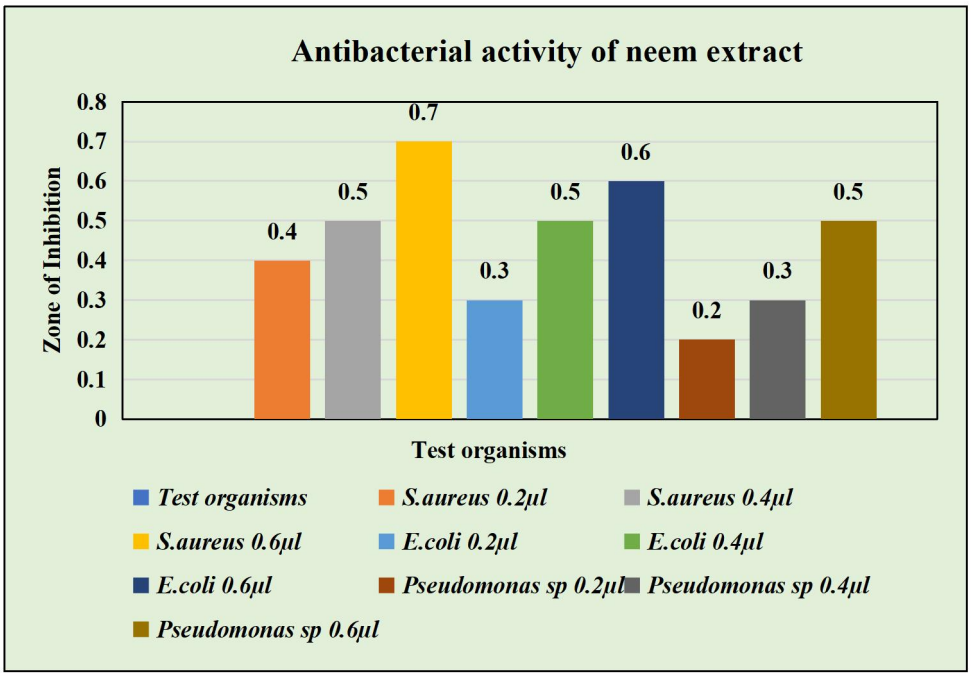

Figure.3. Antibacterial activity of neem extract against bacterial species.

Antifungal activity was carried out against two fungal species, *Penicillium*, *Aspergillus Niger*. *Penicillium* showed maximum zone of inhibition followed by *Aspergillus niger* and *Aspergillus flavus*. The results as shown in the Figure.4.

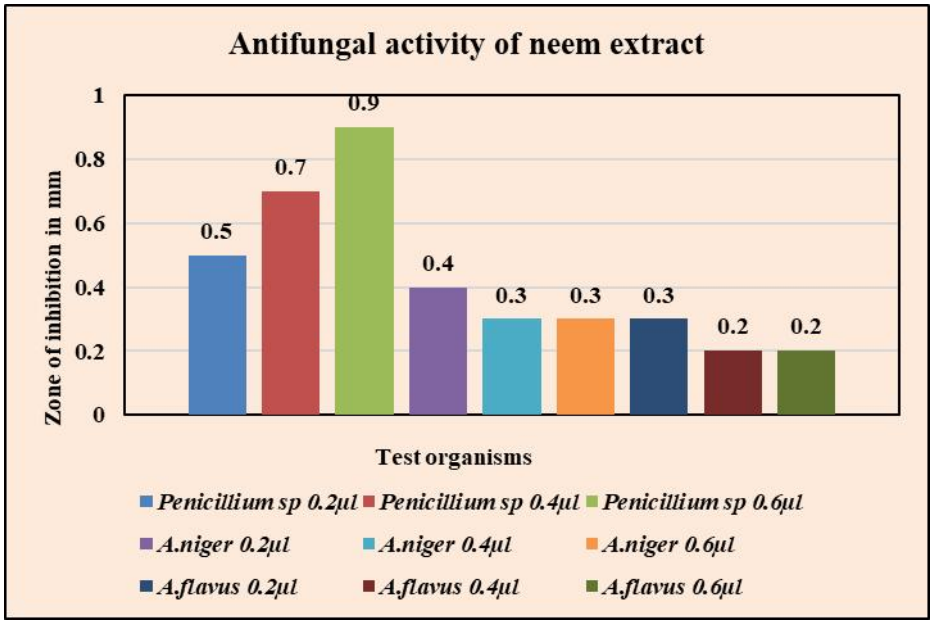

Figure.4. Antifungal activity of neem extract against fungal species.

Fatima *et al*., (2020) investigates the antibacterial effects of *Azadirachta indica* (neem) extracts on uropathogens, employing methods such as agar well diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibiotic sensitivity tests. The findings reveal significant antibacterial activity against *S. aureus, E. coli,* and *Pseudomonas,* with the highest inhibition observed against *S. aureus*. The presence of active phytochemicals, including alkaloids, glycosides, tannins, and saponins, suggests that neem could serve as a potential alternative to synthetic antibiotics for treating urinary tract infections. In our study, we explored the pharmacological and therapeutic potential of neem leaves. The neem leaves were collected, cleaned and crushed to obtain the crude neem extract. The crude extract was extracted with ethyl acetate and filtrate was used for the phytochemical analysis, antimicrobial activity. The neem extract showed that tannins, alkaloids and saponin were present in phytochemical analysis. For antimicrobial activity, the extract showed antibacterial activity against *E. coli* and *S. aureus* and *Pseudomonas* sp. The neem extract showed antifungal activity against, *Penicillium* sp *Aspergillus niger, and Aspergillus flavus*. It is important to note that while neem leaves offer numerous potential health benefits and the use of neem leaves should be done with caution and under the guidance of a healthcare professional, especially when used in medicinal or therapeutic applications, to avoid potential side effects or interactions with medications.

Fourier transform infrared spectroscopic analysis:

Using FTIR at wavelengths between 4000 and 400 cm^{-1}, the chemical species and chemical bonds between these species are examined in order to determine the chemistry of the functional group affixed to the Neem sample. Multiple scans were performed with a 0.4 cm^{-1} resolution. Extract of *Azadirachta indica* leaves FTIR spectra in Standard exhibit distinctive peaks at 2986.53 cm^{-1} (C-H stretching), 1733.86 cm^{-1} (C-O stretching), 1235.98 cm^{-1} (C-O stretching), and 776 cm^{-1} (C-H bending), in that order. Additional high bands are visible at 3363.31 cm^{-1}, 2986.53, 1733.86, 1641.87, 1449.53, 1373.36, 1097.60, and 775.08 cm^{-1}, which correspond to O-H/N-H, C-H, C-O, and C-CS. The (Fig.1, A. Standard) states that stretching and bending vibrations, respectively, indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds, and carbohydrates in plants. Ethyl acetate exhibits distinctive peaks at 3331.86 cm^{-1} (C-H stretching), 1637.00 cm^{-1} (C-O stretching), 1373.54 cm^{-1} (C-O stretching), and 1043.48 cm^{-1} (C-H bending), in that order. The presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds, and carbohydrates in neem plants is indicated by the more intense bands in ethyl acetate at 3331.86 cm−1, 1637.00, 1373.54, 1238.45, 1080.50, and 1043.48 cm^{-1}, which correspond to O-H/N-H, C-H, C-O, and C-Cl/C-CS stretching/bending vibrations, respectively (Fig. 1,B: Ethyl acetate extract). *Azadirachta indica* extract in chloroform exhibits distinctive peaks in the FTIR spectra at 3020.03 cm^{-1} (C-H stretching), 1735.19 cm−1 (C-O stretching), 1239.76 cm^{-1} (C-O stretching), and 717.64 cm^{-1} (C-H bending), in that order. One possible explanation for the extremely strong absorption band seen between 3363.31 and 3331.86 cm^{-1} is the presence of bonded N-H/C-H/O-H stretching of amines and amides (Fig.1, C. Chloroform Extract). Amino acid presence is indicated by the very strong absorption band seen in the 1500–1000 cm^{-1} region. There are polymeric hydroxyl derivatives present, as indicated by the strong absorption band seen between 3500 and 3000 cm^{-1}. The presence of a primary amine is indicated by the N-H vibration. The C-H symmetric stretching of the ethyl and chloroform groups in aliphatic compounds is represented by the band seen at about 2986.53 cm^{-1}. The range of the C-C stretching region is 1641.87–1235.98 cm^{-1}. As a result, the chelated C-O stretching vibrations fall between 1097.60 and 1042 cm^{-1}, which is the lower wave number side. The absence of absorbance in the 2220–2260 cm^{-1} range suggests that none of the extracted *Azadirachta indica* plant contain cyanide groups. This demonstrates that there are no harmful substances present in the study samples. The absence of cyanide was confirmed. It is nontoxic because there was no cyanide present (Joselin *et al.,* 2024).

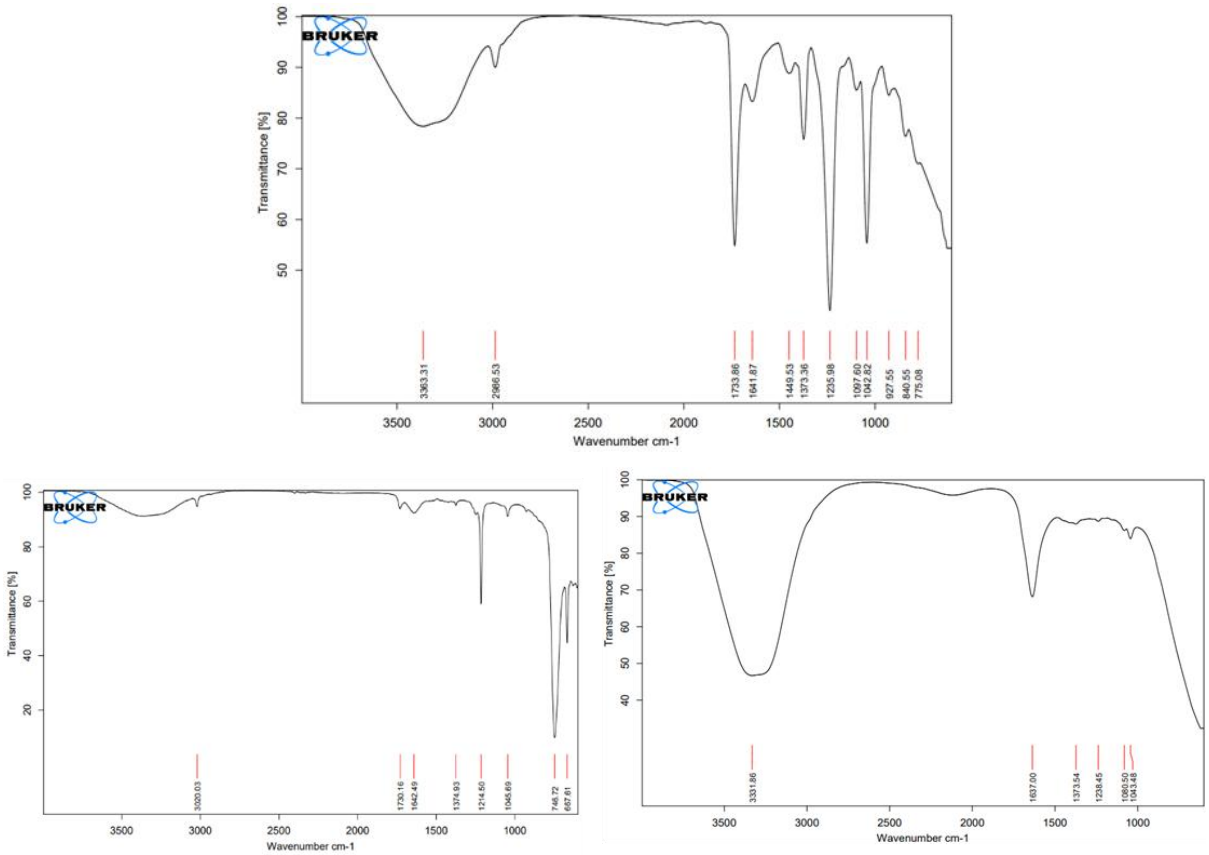

Fig.1. FT-IR spectra of Azadirachta indica. A: Standard extract for the comparison, B: Ethyl acetate extract of Azadirachta indica, C: Chloroform extract of Azadirachta indica.

Conclusion:

The study of the pharmacological and therapeutic potential of neem leaves is an important area of research due to the wide range of medicinal properties associated with neem (*Azadirachta indica*). Neem is a tropical evergreen tree native to the Indian subcontinent and has been used for centuries in traditional medicine for its various health benefits. Neem leaves contain compounds like quercetin, catechin, and various other flavonoids

and polyphenols, which have strong antioxidant properties. Antioxidants help in neutralizing harmful free radicals in the body, reducing oxidative stress, and preventing cellular damage. This is particularly important in preventing chronic diseases such as cancer and cardiovascular diseases. Neem leaves have been traditionally used to treat inflammatory conditions (Ghoshal *et al*., 2024). These anti-inflammatory effects can be attributed to compounds like nimbin, nimbidin, and quercetin present in neem leaves. Neem leaves may help in managing conditions like arthritis, skin inflammations, and gastrointestinal inflammation. Neem leaves exhibit potent antimicrobial activity. They contain compounds like azadirachtin, which have strong antibacterial, antifungal, and antiviral properties. Neem leaves can be used topically to treat skin infections and wounds, and they are also effective against oral infections. While neem leaves offer promising therapeutic potential, it's crucial to acknowledge that further research is needed to better understand their mechanisms of action, dosage recommendations, and potential side effects. The use of neem leaves for medicinal purposes should be approached with care and under the guidance of healthcare professionals. Nonetheless, neem leaves remain a valuable resource in traditional medicine and continue to be studied for their various health benefits.

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