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Research Article

# FTIR Studies of Bioactive Molecules in Methanolic Extract of *Azadirachta Indica* (Neem) Leaves

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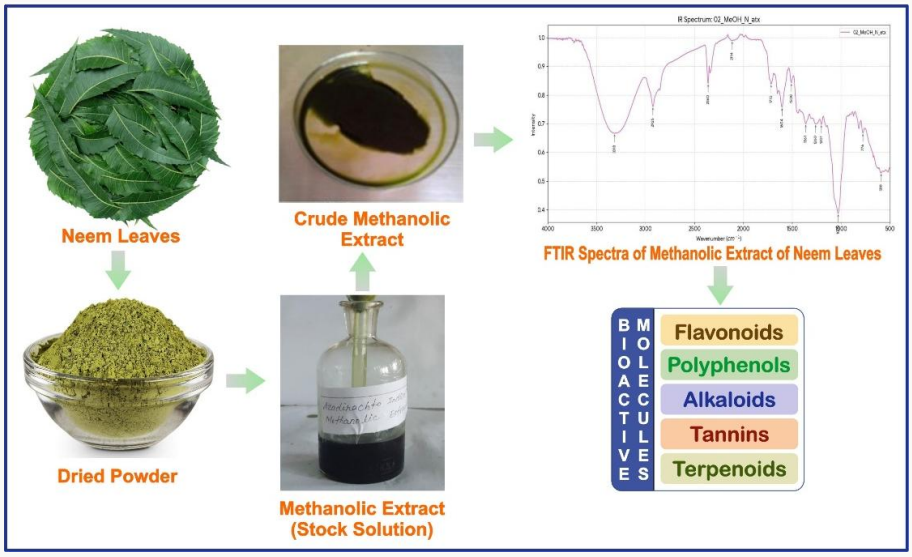
## Abstract

*Azadirachta Indica (Neem) is known as medicinal plant for its antimicrobial, anti-inflammatory and antioxidant properties. Now a days, India found many therapeutic uses from neem leaves extract. The neem tree generally found in Indian subcontinent and South Africa. The present study investigates the functional groups present in the methanolic extract of neem leaves using Fourier Transform Infrared (FTIR) Spectroscopy. The spectrum was recorded in the region of 4000–500 $cm^{-1}$. Several absorption peaks displayed to hydroxyl, alkane, carbonyl, aromatic and alkoxy functional groups. Detailed spectral interpretation suggests the presence of bioactive phytochemicals such as polyphenols, flavonoids, terpenoids, alkaloids and tannins. These compounds are responsible for the pharmacological activities of neem leaves. The results demonstrate that FTIR spectroscopy is a rapid and reliable analytical technique for phytochemical characterization of plant extracts. Finally, the study focuses to isolate and characterize lead molecules from methanolic neem leaves extract for further uses of biological activities.*




**Keywords:** *Azadirachta Indica (Neem) leaves, FTIR spectroscopy, Bioactive molecules, Methanolic extract.*

## 1. INTRODUCTION:

Medicinal plants play a vital role in traditional and modern medicine. Among these plants, *Azadirachta Indica* (commonly known as neem) is widely recognized for its therapeutic properties[1], [2]. Neem belongs to the family Meliaceae and has been used for centuries in Ayurvedic medicine[3]. Various parts of the plant including leaves, bark, seeds and oil possess medicinal value[3], [4], [28].

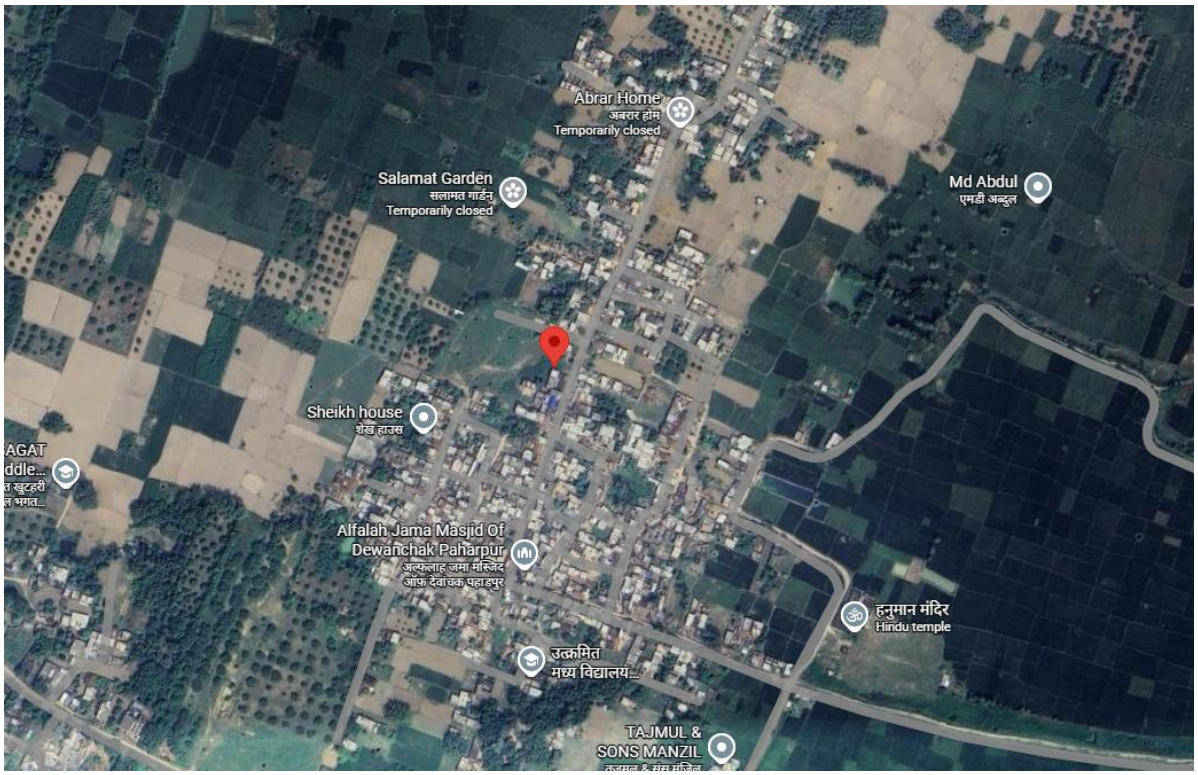



**Fig. 1: Google Image of Study Area.**

Neem leaves contain several bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids and phenolic compounds[5], [6], [7], [8], [9], [10], [11], [12], [27], [28]. These phytochemicals exhibit antibacterial, antiviral, antifungal, antioxidant and anti-inflammatory activities[13], [14], [15], [16], [17], [18], [19], [28]. Because of these properties, neem has attracted significant interest in pharmaceutical and biomedical research.

**Fig. 2: Tree of Neem (*Azadirachta Indica*).**

**Fig. 3: Leaf of Neem (*Azadirachta Indica*).**

Fourier Transform Infrared Spectroscopy (FTIR) is an important analytical tool used for identifying functional groups present in organic compounds. FTIR works by measuring the absorption of infrared radiation by molecules[11], [20], [21], [22], [23]. Different functional groups absorb infrared radiation at characteristic frequencies, producing a unique spectral fingerprint.

The present study aims to identify the functional groups present in the methanolic extract of neem leaves using FTIR spectroscopy and to correlate these groups with possible bioactive phytochemicals[24], [27], [28].

## 2. MATERIALS & METHODS:

### Equipment

- Grinder (Morphy Richards)
- Electronic balance (DJ602A)
- Refrigerator (SAMSUNG)
- Hot air oven (OPTICS TECHNOLOGY)
- FTIR Spectrophotometer (INVENIO S, BRUKER OPTIK, GmBH)

### Reagents and Chemicals

- Sample Material – Dried Neem Leaves
- Extraction Solvent – Methanol (ZENITH)

### Consumables

- Glassware – Reagent bottles, Measuring cylinder, Funnels, Watch glass, Beakers (BOROSIL)
- Filter papers
- Aluminium foil
- Protective Equipment – Apron, Gloves, Glass

### 2.1 Plant Material:

Fresh and mature leaves of neem (*Azadirachta Indica*) were collected from the local area of Dewanchak, Godda, Jharkhand, India in January 2026 (Fig. 2 & Fig. 3).

**Fig. 4: Neem Leaves.**

**Fig. 5: Dried Neem Leaves.**

**Fig. 6: Neem Leaves Powder.**

### 2.2 Preparation of *Azadirachta Indica* (Neem) Leaves Powder:

The collected healthy neem leaves were cut into small pieces and washed thoroughly with distilled water to remove dust, soil particles and other impurities[3].

The cleaned leaves were then spread on clean trays for dry under shade at room temperature (24–27°C) for approximately 12 days to remove moisture while preserving the bioactive molecules[3] (Fig. 4 & Fig. 5).

After drying, the leaves were ground into a fine powder using a clean mixer grinder (Fig. 6). The powdered sample was then stored in an airtight container to prevent moisture absorption and contamination until further use[3].

### 2.3 Preparation of Methanolic Extract of *Azadirachta Indica* (Neem) Leaves:

The general method was used to prepare the methanolic extract of neem leaves.

Approximately 50 g of neem leaves powder was accurately weighed using an electronic balance and transferred into a clean reagent bottle[3].

A measured volume of methanol solvent was added to the bottle to ensure complete immersion of the plant material[3], [25] (Fig. 7).

The mixture was shaken occasionally to enhance the extraction process and was kept at room temperature for 48 hours to allow efficient extraction of phytochemicals[1], [3], [10].

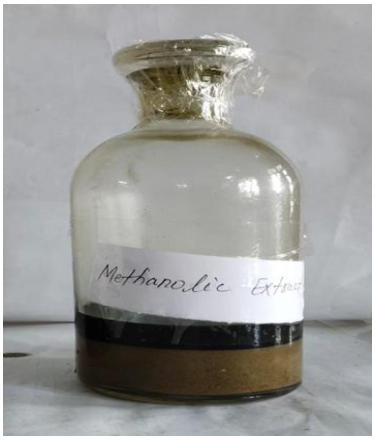



**Fig. 7: Extraction Process.**

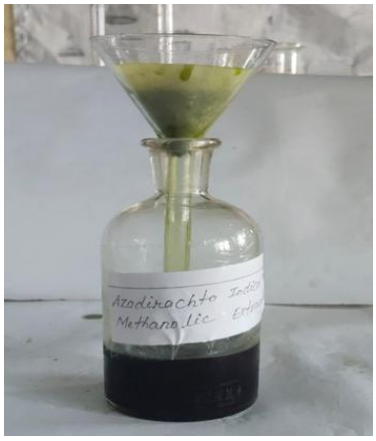



**Fig. 8: Filtration for Stock Solution.**

After the extraction period, the mixture was filtered using Whatman filter paper to separate the liquid extract from the solid residue.

The methanol extract was filtered and collected in a clean reagent bottle (Fig. 8). Excess methanol solvent was dried under in a hot air oven at 50°C to obtain the crude methanolic extract[10], [21], [24], [25].

To improve the extraction efficiency, the same extraction procedure was repeated other two additional times using fresh solvent. The stock solution and different spectral analysis samples solution were sent to IIT (ISM) Dhanbad.

| S. No. | Methanol (mL) | Neem Leaf Powder (g) | Date |
|---|---|---|---|
| **1** | 120 | 50 | 03.02.2026 |
| **2** | 120 | 50 | 05.02.2026 |
| **3** | 120 | 50 | 07.02.2026 |

**Table 1: Extraction Procedure of Methanolic Neem Leaves Extract.**

## 2.4 FTIR Spectrophotometer Analysis:

FTIR spectra were recorded as KBr pellets using Bruker Optik (GmBH) FTIR spectrometer in the spectral range 4000-500 $cm^{-1}$.

## 2.5 Analytical Procedure (KBr Pellet):

The FTIR optical bench was flushed with nitrogen gas for 2 hours at a rate of approximately 200 liters/hr before analysis to minimize the effect of moisture and other gasses. Hydraulic press (~6 ton for 5 minutes) was used to make the KBr pellets using a mix of KBr powder (IR spectroscopy grade) and powdered samples (-200 mesh). The circular thin KBr pellets were inserted in the standard sample holder with a quick lock base plate. The door of the sample analysis chamber was closed for analysis. The FTIR spectra was collected in absorbance/transmittance mode and saved as a separate file. The spectra were clipped for the desired frequency range (wave number: 4000-500 $cm^{-1}$) and baseline correction (rubber band correction) was performed. The resultant FTIR data was plotted as absorbance/transmittance vs frequency plot (Y-X Plot).

## 3. RESULTS AND DISCUSSION:

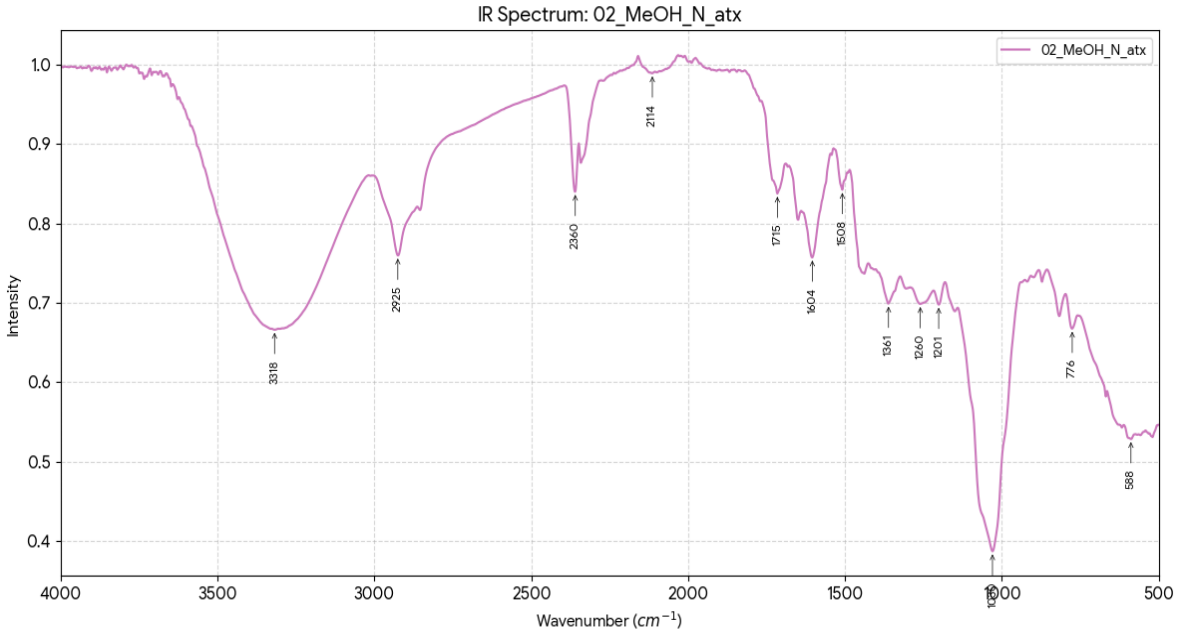



**Fig. 9: FTIR Spectra of Methanolic Extract of Neem Leaves.**

The FTIR spectrum of *Azadirachta Indica* (Neem) leaves extract of from methanol shows several characteristic absorption bands corresponding to different functional groups present in phytochemical constituents (Table 2).

| Sr. No. | Functional Group | Observed Frequencies ($cm^{-1}$) | Reported Frequencies ($cm^{-1}$) |
|---|---|---|---|
| **1** | O-H | 3318 | 3700-3070 |
| **2** | C-H | 2925 | 3000-2840 |
| **3** | C=C | 2360 | 2500-2300 |
| **4** | C≡C | 2114 | 2060-2100 |
| **5** | C=O | 1715 | 1760-1650 |
| **6** | N-H Bending | 1604 | 1650-1515 |
| **7** | N-H | 1508 | 1550-1504 |
| **8** | C-H | 1361 | 1450-1360 |
| **9** | C-O | 1260 | 1260-1000 |
| **10** | C-C-O | 1201 | 1260-1000 |
| **11** | C-O-C | 1036 | 1260-1000 |
| **12** | C-H | 736 | 900-650 |
| **13** | C-H | 588 | 900-650 |

**Table 2: FTIR Absorbed Peaks of Methanolic Extract of *Azadirachta Indica* (Neem) Leaves [26].**

### 3.1 FTIR SPECTRAL PEAKS ANALYSIS OF *AZADIRACHTA INDICA* (NEEM) LEAVES (Fig. 9 and Table 2):

The broad peak observed 3318 $cm^{-1}$ corresponds to O–H stretching vibrations. This peak is characteristic of hydroxyl groups present in polyphenolic compounds and flavonoids. These compounds are known for their antioxidant activity and antiulcer activity [20], [21].

The absorption peak observed 2925 $cm^{-1}$ corresponds to C–H stretching vibrations of aliphatic hydrocarbons. This indicates the presence of alkane chains that may be present in various phytochemical constituents [20], [21].

The absorption peak observed 2360 $cm^{-1}$ corresponds to C=C (alkene) conjugated a C≡C (alkyne) stretching vibrations. This indicates the presence of alkene/alkyne group that may be present in various phytochemical constituents [20], [21].

The absorption peak observed 2114 $cm^{-1}$ corresponds to C≡C (alkyne) group that may be present in various phytochemical constituents [20], [21].

A strong absorption peak observed 1715 $cm^{-1}$ indicates the presence of carbonyl (C=O) stretching vibrations. Carbonyl groups are commonly found in aldehydes, ketones and esters, which are important components of plant secondary metabolites [3], [5], [20], [21].

The band observed 1604 $cm^{-1}$ corresponds to N-H bending vibrations associated with aromatic rings or aliphatic hydrocarbons. This suggests the presence of aromatic compounds such as flavonoids and polyphenols [20].

The peak observed 1508 $cm^{-1}$ corresponds to the $NH_3^+$ group of the salt absorbed may be present in bioactive molecules [20].

The absorption peaks observed 1361 $cm^{-1}$ display to C–H aliphatic bending group may be present in different phytochemical constituent [21].

The C-O, C-C-O and C-O-C absorption peaks observed 1260 $cm^{-1}$, 1201 $cm^{-1}$ and 1036 $cm^{-1}$ respectively due to C-O stretching vibration of alcohol, ethers and esters associated with plant derived phytochemical [20], [21].

The peak observed 736 $cm^{-1}$ and 588 $cm^{-1}$ corresponds to out of Plane C-H bending of aromatic and hetro-aromatic compound may be present [21].

## 4. CONCLUSION:

The FTIR analysis confirmed the presence of several functional groups including hydroxyl, carbonyl, alkane, aromatic and alkoxy groups. These groups indicate the presence of important phytochemicals such as polyphenols, flavonoids and terpenoids in neem leaves. The present FTIR study confirmed the presence of various bioactive molecules in the methanolic extract of *Azadirachta Indica* (neem) leaves. These groups are associated with medicinally important phytochemicals responsible for the biological activities of neem. FTIR spectroscopy help to be a simple and efficient method for phytochemical characterization.

## 5. SCOPE OF FUTURE WORK:

Further research may be helpful for the identification of lead bioactive molecules using advanced analytical techniques such as GC–MS, HPLC and NMR spectroscopy. The biological assays may also be performed to evaluate antimicrobial, antioxidant, anticancer, antiulcer and antidiabetes activities of the methanolic neem leaves extract.

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## REFERENCES

1. Yadav, D. K., et al. (2017). Tamarixetin 3-O-β-D-glucopyranoside from *Azadirachta indica* leaves: Gastroprotective role through inhibition of matrix metalloproteinase-9 activity in mice. *Journal of Natural Products, 80*. https://doi.org/10.1021/acs.jnatprod.6b00957
2. Rajamanickam, S., & Siddavaram, N. (2005). Medicinal properties of neem leaves: A review. *Current Medicinal Chemistry – Anti-Cancer Agents, 5*, 146–149. https://doi.org/10.2174/1568011053174828
3. Yadav, D. K., Bharitkar, Y., Chatterjee, K., Ghosh, M., Mondal, N. B., & Swarnakar, S. (2016). Importance of neem leaf: An insight into its role in combating diseases. *Indian Journal of Experimental Biology, 54*, 708–718.

4. Pankaj, S., Lokeshwar, T., Mukesh, B., & Vishnu, B. (2011). Review on neem (*Azadirachta indica*): Thousand problems one solution. *International Research Journal of Pharmacy, 2*, 97–102.
5. Sarah, R., Tabassum, N., Idrees, N., & Hussain, M. (2019). Bioactive compounds isolated from neem tree and their applications. In *Natural bio-active compounds: Production and applications* (Vol. 1). https://doi.org/10.1007/978-981-13-7154-7_17
6. Pandey, G., Verma, K., & Singh, M. (2014). Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* leaves. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*, 444–447.
7. Hossain, M., Al-Toubi, W., Weli, A., Al-Riyami, Q., & Al-Sabahi, J. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science, 7*. https://doi.org/10.1016/j.jtusci.2013.05.003
8. Islas, J. F., et al. (2020). An overview of neem (*Azadirachta indica*) and its potential impact on health. *Journal of Functional Foods, 74*, 104171. https://doi.org/10.1016/j.jff.2020.104171
9. Gopala Krishna, P., & Krishnaiah, G. M. (2014). Chemical composition of the leaves of *Azadirachta indica* Linn (neem). *International Journal of Advanced Engineering Technology Management and Applied Science, 1*, 21–31.
10. Hashim, N., Abdullah, S., Hassan, L., Ghazali, S., & Jalil, R. (2021). A study of neem leaves: Identification of method and solvent in extraction. *Materials Today: Proceedings, 42*. https://doi.org/10.1016/j.matpr.2020.11.726
11. Sani, G. D., Saidu, A., Rilwanu, A., & Sahabi, S. (2025). FTIR and UV–Vis characterization of ZnO produced using neem (*Azadirachta indica*) leaves extracts. *IJIREEICE, 13*. https://doi.org/10.17148/IJIREEICE.2025.131212
12. Biswas, K., Chattopadhyay, I., Banerjee, R., & Bandyopadhyay, U. (2001). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science, 82*.
13. Seriana, I., Akmal, M., Darusman, D., Wahyuni, S., & Khairan, K. (2021). Phytochemical characterizations of neem (*Azadirachta indica*) leaves ethanolic extract: An important medicinal plant as male contraceptive candidate. *Rasayan Journal of Chemistry, 14*, 343–350. https://doi.org/10.31788/RJC.2021.1415899
14. Dang, J., et al. (2024). Neem leaf extract exhibits anti-aging and antioxidant effects from yeast to human cells. *Nutrients, 16*, 1506. https://doi.org/10.3390/nu16101506
15. Paul, R., Prasad, M., & Sah, N. (2011). Anticancer biology of *Azadirachta indica* L (neem): A mini review. *Cancer Biology & Therapy, 12*, 467–476. https://doi.org/10.4161/cbt.12.6.16850
16. Puri, H. S. (1999). *Neem: The divine tree Azadirachta indica*. CRC Press. https://doi.org/10.4324/9780203304310
17. Kumawat, K., & Kumar, R. (2018). *Pharmacological and therapeutical overview of neem (Azadirachta indica): A nature's drugstore*.
18. Abd El-Moez, S., Tawfeeq, S., Amer, H., & Zaki, F. (2014). Antimicrobial activities of neem extract (*Azadirachta indica*) against microbial pathogens of animal origin. *Global Veterinaria, 12*, 250–256. https://doi.org/10.5829/idosi.gv.2014.12.02.82123
19. Schumacher, M., Cerella, C., Reuter, S., Dicato, M., & Diederich, M. (2011). Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (*Azadirachta indica*) leaf extract are mediated via modulation of the nuclear factor-κB pathway. *Genes & Nutrition, 6*, 149–160. https://doi.org/10.1007/s12263-010-0194-6
20. Kamble, V., & Gaikwad, N. (2016). Fourier transform infrared spectroscopy studies in *Embelia ribes*. *Asian Journal of Pharmaceutical and Clinical Research, 9*, 41. https://doi.org/10.22159/ajpcr.2016.v9s3.13804
21. Rani, K. S., Mumtaj, M., Priyanka, D., & Chandran, M. (2021). Phytochemical screening by FTIR spectroscopic analysis of methanol leaf extract of *Andrographis echioides*. *Journal of Ayurvedic and Herbal Medicine, 7*, 257–261. https://doi.org/10.31254/jahm.2021.7408
22. Othman, F., Motalleb, G., Peng, S., Rahmat, A., Basri, R., & Chong, P. (2012). Effect of neem leaf extract (*Azadirachta indica*) on c-Myc oncogene expression in 4T1 breast cancer cells of BALB/c mice. *Cell Journal, 14*, 53–60.
23. Khanal, S. (2021). Qualitative and quantitative phytochemical screening of *Azadirachta indica* plant parts. *International Journal of Applied Sciences and Biotechnology, 9*, 122–127. https://doi.org/10.3126/ijasbt.v9i2.38050
24. Dikwa, K., Alkali, K., Ajibade, G., Magaji, Y., & Ige, O. (2025). Effects of methanol leaf extracts of *Azadirachta indica*, *Senna occidentalis*, and *Senna siamea* on the in vitro growth of *Plasmodium falciparum*. *Sahel Journal of Life Sciences, 3*, 287–298. https://doi.org/10.33003/sajols-2025-0301-35
25. Yusuf, A., et al. (2025). Methanol leaf extract of *Azadirachta indica* mitigates isoproterenol-induced myocardial infarction through modulation of oxidative stress and signaling pathways in rats. *Avicenna Journal of Phytomedicine, 15*, 1328–1340. https://doi.org/10.22038/ajp.2024.25277
26. Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2005). *Spectrometric identification of organic compounds*. Wiley.
27. Kumar, A., Jose, S., Hemalatha, G., & Tyagi, D. (2024). *Introduction to medicinal chemistry*. AG Publishing House.
28. Kumar, A., et al. (2024). In-depth review on taxonomy, phytochemistry, traditional uses and pharmacological significance of *Azadirachta indica*. *European Journal of Biomedical and Pharmaceutical Sciences, 11*, 277–284.

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