**Journal of Multidisciplinary Innovations and Perspectives (JMIP)**
**Maiden Edition**
www.fedpodjournal.com
**Volume 1; Issue 1; November 2024; Page No. 93-103.**

# PHYTOCHEMICAL SCREENING, PROXIMATE AND GAS CHROMATOGRAPHY FLAME IONIZATION DETECTOR (GC-FID) IDENTIFICATION OF BIOACTIVE COMPOUNDS OF ETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* LEAVES.

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**Abstract**
*Azadirachta indica*, commonly known as neem is one of the most promising medicinal plants having a wide spectrum of biological activities**.** This study was aimed at screening the phytochemical and identification of the bioactive constituents of the ethanolic extract of neem leaves. About 1kg of fresh matured leaves was collected, washed and dried for one week and pulverized into a fine powder using an electric blender. Using ethanol as the solvent, Soxhlet extraction method was employed to extract the bioactive ingredients and concentrated using rotary evaporator. The vitamins, proximate parameters and phytochemical screenings were determined following standard methods. The minerals were done using Atomic absorption spectrophotometer (AAS). The bioactive compounds were analyzed using GC-FID techniques. The macro mineral contents were; Na (18.87 ± 0.01 ppm), Ca (6.03 ± 0.00 ppm) and P (10.45 ± 0.02 mg/kg), while vitamins (mg/100g) were 6.78 ± 0.01, 10.08 ± 0.07, 4.67 ± 0.04 for B, C and carotenoid, respectively. The proximate compositions (%) were; fat (12.50 ± 0.02), protein (29.00 ± 0.10) etc. The phytoextract revealed moderate level of all the bioactive compounds including alkaloid, saponin, etc., except glycoside which was high. The GC-FID analysis revealed the presence of twelve (12) constituents: resveratrol, catechin, ribalinidine, flavanones, flavan-3-ol, flavones, aglycone, lunamarin, gallocatechin, isoflavonoids and kaempferol. The significant presence of these compounds suggest that neem leaf may hold promise in traditional and contemporary medicinal practices.

**Keywords: *Azadirachta indica*, ethanolic, extraction, phytochemicals.**

## Introduction

Medicinal plants are endowed with rich sources of bioactive metabolites with potential for drug discovery and development (Yusuf *et al*., 2020). These plants and their parts e.g., stems, leaves, bark, fruits, nuts, oils, and whole grains contain bioactive compounds which contribute to their positive metabolic and immunological actions in the body required for good health (Shrinet *et al*., 2021). The bioactive compounds prevent the oxidative damage of cells by detoxifying the free radicals, thus minimizing the incidence of diseases such as: neurodegenerative diseases, cardiovascular disease (CVD), type 2 diabetes, cancer etc. (Xiao and Bai, 2019).

## Research problems

There has been a lot of attention focused on producing medicines and products that are natural in recent times. Herbal medicines are of beneficial use in the developing countries but of recent, there has been rise in the use of herbal medicines in the developed world (Atanasov *et al*., 2021). It is regarded as one of the foremost medical



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practices believed to have been central to ushering in the present modern pharmaceuticals. Plant-based therapies as an excellent alternative for antibiotics began to be offered in Nigeria because of their least toxic impact on humans and environment (Ugboko *et al*., 2020). The ever-increasing emergence of drug resistance threats health worldwide and great efforts are being made to reverse this trend through phytochemical screening and utilization of their phytoextracts with the hope to getting some newer, safer, and more effective agents that can be used to fight infectious diseases (Khan *et al*., 2021) thus, resulting in growing interests and acceptability of medicinal plants worldwide.

**Significance of the study**

The phytoextracts from these plants have wide spectra of biological activities and enormous nutritional benefits, yet untapped for human development. Existing research has predominantly focused on the basic classes in neem leaves, however, there is a notable gap in comprehensive phytochemical analysis. Therefore, a holistic examination of these components is necessary to fully elucidate their potential health benefits and provide a more nuanced view of the synergistic effects between different compounds and their collective impact. This quest will help find their potential health benefits and provide an intelligent view of their combined effects (Abhishek *et al*., 2024). Analyzing neem leaves through phytochemical screening will qualitatively assesses the presence of various secondary metabolites and their compounds that might be responsible for the plant's medicinal properties. The proximate composition is important for understanding the nutritional value and potential uses of neem-based products. The mineral contents are crucial for their various physiological processes and understanding their presence and levels in neem leaves can help in understanding its nutritional and medicinal value. GC-FID identifies and quantifies specific volatile and semi-volatile compounds in the neem extract. This allows for a more precise characterization of the bioactive compounds, such as azadirachtin, nimbin, lunamarin, flavon-3-ol and other terpenoids, and their concentrations.

On this note, this study was undertaken to screen and identify bioactive compounds of ethanolic extract of neem (*Azadirachta indica*) leaves using the gas chromatography flame ionization detector (GC-FID) techniques.

**Objectives of the study were to;**

- Extract the bioactive compounds of neem leaves using ethanolic Soxhlet extraction method.
- Determine the vitamins, proximate parameters and qualitative screening of the phytochemicals following standard methods.
- Evaluate some macro minerals using Atomic Absorption Spectrophotometer (AAS).
- Identify and analyze the bioactive compounds using (GC-FID) techniques.

**Literature Review**

Indigenous medicinal plants including; neem (*Azadirachta indica*) (Amadi *et al*., 2017; Khan *et al*., 2021), guava (*Psidium guajava*) (Sandeep *et al*., 2023), lemon grass (*Cymbopogon citatus*) (Wifek *et al*., 2016) etc. are reputed to hold protective and therapeutic properties owing to the presence of pharmacologically active components such as alkaloids, flavonoids, phenols, tannin, saponin, glycoside etc., which have been isolated from fresh and dried parts (Bassey, 2016; Maria and Romilly, 2017), besides each plant possess unique nutrient contents that satisfy the energy requirements for various metabolic processes (Shrinet *et al*., 2021).

*Azadirachta indica,* a medicinal plant commonly known as neem or Indian neem (Margosa tree) (Family-Meliaceae) (Figure 1), is an evergreen, attractive perennial tree that is native to the Indian region. In addition, it is grown in numerous Latin American nations, Southeast Asia, Australia, East and Sub-Saharan Africa, Fiji, Mauritius and Nigeria (Puvan *et al*., 2015).


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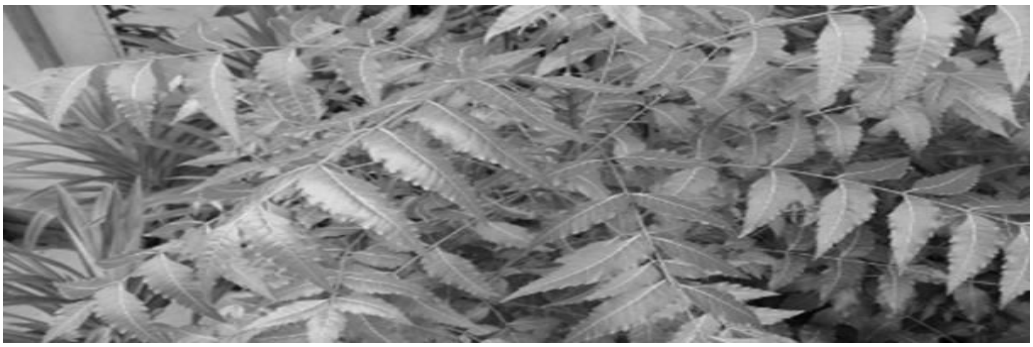

**Figure 1. Fresh neem leaves**

In Nigerian, neem plant popularly known as "Dogonyaro“ have been used to treat illnesses such as cardiovascular disorders, diabetics, eczema, malaria etc., production of toothbrushes, lubricants, insect repellants, pesticides etc. (Virshette *et al*., 2020).

Available research had shown that many bioactive chemicals with antibacterial, antifungal and antiviral properties had been found in plant extracts from stem, leaves, bark, and root (Khanal, 2021). These biological capabilities of this medicinal plant are attributed to numerous active components. as well as wide range of pharmacological activities, such as anti-inflammatory, anti-mutagenic, anti-carcinogenic, antioxidant, antihyperglycemic, antiulcer, and anti-diabetic features (Khanal, 2021).

Global, agricultural, environmental, and health issues have long been addressed using it in health as innovational tools (Santhosh and Navartnam, 2013). Many illnesses, including diabetes and TB, are treated with neem oil, which is also used as a lubricant, pesticide, and medication (Virshette *et al*., 2020).

It had been reported that numerous physiologically active substances, such as alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones, have been isolated from neem as chemical contents. Azadirachtin, an active compound, is truly a combination of seven isomeric molecules identified as azadirachtin A-G, and azadirachtin E is more effective (Zillich *et al*., 2015). Other molecules that have biological activity are salannin, volatile oils, meliantriol, and nimbin (Khanal, 2021).

## Materials and methods

### *Collection of sample*

Fresh matured leaves of *A. indica* were collected from Carmelite Garden, Amorji- Nike, Enugu, Nigeria.

### *Preparation of plant material*

The fresh leaves of *A. indica* were washed with water to remove debris, dried for one week and pulverized into a fine powder using an electric blender. Powdered leaves were subjected to extraction by Soxhlet method using absolute ethanol. Five hundred (500) gram of the sample was weighed out, wrapped in filter paper and then put in the thimble of the Soxhlet apparatus compartment. Thereafter, the condenser was carefully and efficiently connected. An initial 500 ml volume of the solvent (ethanol) were added with the aid of a funnel by passing it through the thimble containing the sample to the round bottom flask system of the Soxhlet. The inlet and outlet of the condenser were connected to a hose respectively, for the recycling of the cold water during the extraction. Thereafter, the heat source was switched on about 5cm from the flask. Finally, the crude extract was concentrated using rotary evaporator and the dried phytoextract was stored in a sterile screw-capped bottle and kept in cool place for further use.



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***Determination of proximate parameters***

The method described by A.O.A.C (2006) was adopted for all the parameters such as; ash, moisture, fat, fiber and protein. Carbohydrate was estimated by difference; % carbohydrate = 100 - % (protein + moisture + fat + fiber + ash).

***Determination of vitamin composition***

The vitamin content was analyzed by a modification of A.O.A.C, (2006). The sample was subjected to the laboratory atmospheric condition on the bench after removing the samples from the storage chamber at 40C. The sample was pressed and completely homogenized in the mortar carefully with pestle to avoid forming balls. The homogenized sample (0.10g) was weighed into a beaker after extraction; the extract was concentrated to 1. 0 ml and for analysis, using a HP 6890 Gas chromatographic apparatus fitted with pulse flame photometric detector (PFPD) using Nitrogen as carrier gas. Split ratio 20:1 with flow rate of 1.0ml/min, 0ml/minimum, inlet temperature 2500C, and column type HP-5 with 30m x .25mm x .25 µm column dimensions. Oven temperature: initial temperature @ 500C for 2 min, detector temperature maintained 3200C; pressure 20psi and compressed air pressure 30psi.

***Determination of mineral contents***

Wet digestion of samples (5ml) using a mixture of concentrated HNO3 and 60% (v/v) $HClO_4$ was carried out according to the method of A.O.A.C (1990) where the organic matter in the sample was digested and afterwards diluted to a final volume of 25 ml with deionized distilled water. The levels of Na, Ca and P, in the sample were thus evaluated using an atomic absorption spectrophotometer (AAS) (Buck Scientific model 210 VGP) and flame photometer (Jenway model).

***Qualitative Phytochemicals Analysis***

Preliminary qualitative phytochemicals screening was carried out following standard protocols. A modified method of Ankita and Sapan, (2018) was used in these analyses. Essentially, phytochemical screenings were done to identify the presence of secondary plant metabolites. The saponin, alkaloids, tannin, flavonoids, glycoside, phenol and steroid were determined following these protocols.

***Analysis of bioactive compounds by gas chromatography flame ionization detector (GC-FID)***

The analysis of bioactive compounds were performed using a gas chromatography flame ionization detector (GC-FID) system (Burk scientific M910) as described by Alcalde-eon et al. (2006). The GC-FID analysis was carried out on ethanol fraction of *A. indica* leaves to identify and quantify bioactive compounds present in the sample. A syringe was used to draw 0.1 ml of the fraction and injected into the gas chromatography (GC) machine equipped with FID. In principle, FID uses a flame to ionize organic compounds containing carbon. Following separation of the sample in the GC column, each analyte passes through a flame, fueled by nitrogen and zero air, which ionizes the carbon atoms.

*Instrumentation*

Analysis was performed on a GC-FID system (Buck scientific M910). The GC was equipped with a HP-5MS of 30 m length and 0.25 mm internal diameter capillary column (RESTEK 15 METER MIX-1), with 0.25 µM film thickness. The carrier gas was Nitrogen (At 5 pounds per square inch (P.S.I)) set to flow at 1.5 ml/min. The injector was operated in spitless mode at the 280 °C temperature. The chromatographic working conditions were optimized for complete separation of the target compounds. The oven was programmed from 50 °C (3.0 min.) to 310 °C at the rate of 5 °C/min. and maintained at this temperature for 5.0 min. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The



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concentrations of the different phytochemicals were expressed in microgram per gram (µg/g) and parts per million (ppm).

***Statistical analysis***

Data collected were subjected to statistical analysis using IBM Statistical Package for the Social Sciences (SPSS) version 21.0. Descriptive statistics were used and the differences in mean were considered significant at $p < 0.05$. Experiments were performed in triplicate ($n = 3$) and results were expressed as mean ± standard deviation (SD).

**Results/Findings**

The results of proximate composition, vitamin, and mineral analysis of the phytoextract of A. *indica* is shown in Table 1.

**Table 1: Results of the proximate composition (%), vitamins (mg/100g) and mineral contents (ppm) and (mg/kg) of the phytoextract.**

| Proximate compositions (%) | Results |
|---|---|
| Ash | 6.38 ± 0.02 |
| Moisture | 5.15 ± 0.13 |
| Fat | 12.50 ± 0.02 |
| Fiber | 9.60 ± 0.20 |
| Protein | 29.00 ± 0.10 |
| Carbohydrate | 36.31 ± 0.03 |
| **Vitamins (mg/100g)** | |
| B | 6.78 ± 0.10 |
| B1 | 2.67 ± 0.05 |
| B6 | 4.58 ± 0.03 |
| C | 10.08 ± 0.07 |
| Carotenoid | 4.67 ± 0.04 |
| **Minerals** | |
| Sodium (Na) (ppm) | 18.87 ± 0.01 |
| Calcium (Ca) (ppm) | 6.032 ± 0.00 |
| Phosphorus (P) (mg/kg) | 10.45 ± 0.02 |

Key: % = percentage, mg/100g = milligram per 100 grams; ppm = parts per million; mg/kg = milligram per kilogram. Values are means of triplicate results ± SD.

**Table 3: Results of the qualitative phytochemical analysis**

| Parameters | Qualitative |
|---|---|
| Alkaloids | + + |
| Saponin | + + |
| Flavonoid | + + |
| Glycoside | + + + |
| Tannin | + + |
| Phenol | + + |
| Steroid | + + |

Key: + + = indicates moderate; + + + = indicates high


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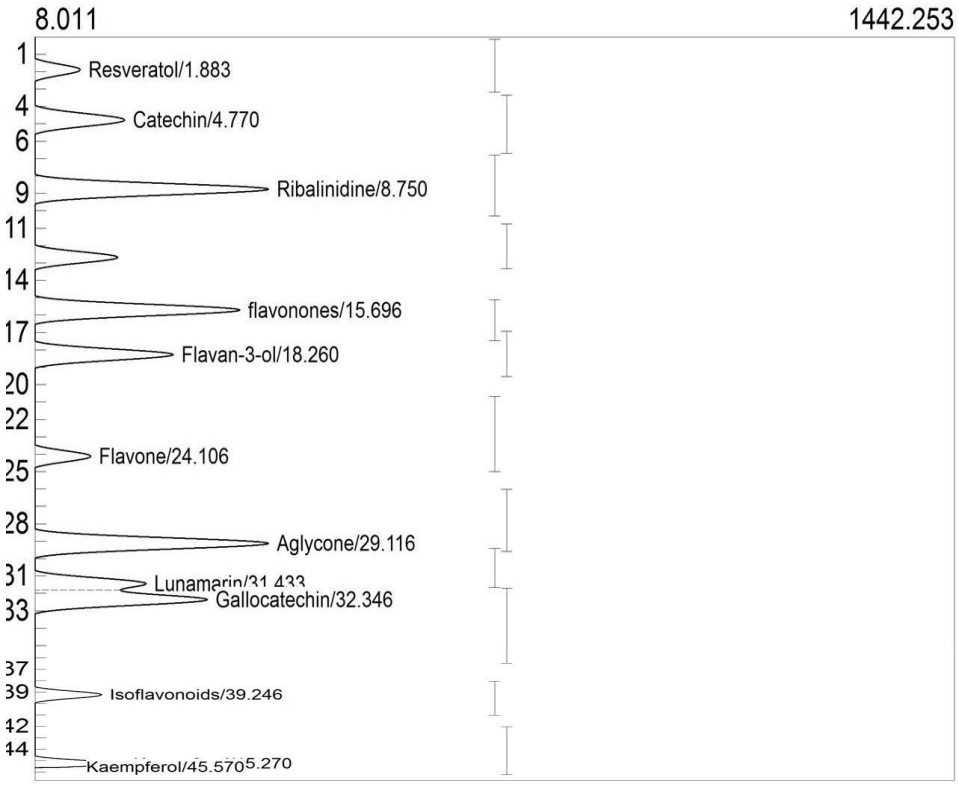
Temperature program:

| Init temp | Hold | Ramp | Final temp |
|---|---|---|---|
| 50.00 | 5.000 | 10.000 | 180.00 |
| 180.00 | 2.000 | 5.000 | 220.00 |
| 220.00 | 0.000 | 5.000 | 310.00 |

Events:

| Time | Event |
|---|---|




| Component | Retention | Area | Height | External | Units |
|---|---|---|---|---|---|
| Resveratol | 1.883 | 10310.4636 | 79.009 | 11.3824 | ug/ml |
| Catechin | 4.770 | 7981.3190 | 148.708 | 22.5308 | ug/ml |
| Ribalinidine | 8.750 | 17777.7308 | 374.284 | 64.0834 | ug/ml |
| flavonones | 15.696 | 15628.3172 | 330.432 | 33.4475 | ug/ml |
| Flavan-3-ol | 18.260 | 10767.5819 | 226.431 | 30.4883 | ug/ml |
| Flavone | 24.106 | 4624.6554 | 97.990 | 12.6566 | ug/ml |
| Aglycone | 29.116 | 17704.3660 | 374.940 | 41.6918 | ppm |
| Lunamarin | 31.433 | 8046.0438 | 183.516 | 76.3193 | ppm |
| Gallocatechin | 32.346 | 13533.5810 | 279.188 | 48.5752 | ppm |
| Isoflavonoids | 39.246 | 5364.3732 | 113.520 | 7.2342 | ppm |
| Kaempferol | 45.270 | 5218.4190 | 149.341 | 27.9606 | ug/ml |
| Kaempferol | 45.570 | 137.3300 | 75.440 | 0.7358 | ug/ml |
| | | 117094.1809 | | 377.1061 | |

Fig. 2. GC-FID graph (chromatogram) showing different constituents of bioactive compounds identified in ethanolic fraction.

*Key:* Retention = Retention Time (min.); Area = Peak Area ($cm^2$); Height = Peak height of each compound; External = Concentration measured in their various units



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**Discussion**

The proximate composition of extract of *Azadirachta indica* is shown in Table 1 below. The moisture content of the extract was significantly ($p \leq 0.05$) low. This value (5.20) was observed to be lower than the reports for neem leaf extract as reported by Madakil *et al*., (2016). This could be due to the fact that the sample was sun-dried. However, such value is a good attribute for storage (Sadowsky and Ishi, 2008) because low moisture could reduce the activities of microorganisms. The ash and fiber contents of this extract were high when compared with the report of Madakil *et al*., (2016), but lower than that reported by Enin *et al*., (2014) on Sida acuta leaf as well as leaf extracts from *Vernonia amygdalina, and Gongronema latifolium* (Atangwho *et al*., 2009). The implication with such high fibre content (9.60 mg/100g) is that the leaves of neem plant might be suitable to relieve constipation. This also indicates that *A. indica* leaves contained fibre which can aid in peristalsis. The ash content may imply poor mineral composition of this plant under study. The ash content indicates the degree of the inorganic matter constituents of the samples. The ash content may imply relatively adequate mineral composition of this plant under study. The carbohydrate content of the extract was low. Madakil *et al*. 2016, in their study on proximate and mineral composition of neem leaves reported 78.12 % ±0.35 carbohydrate. The fat and protein contents of this extract was in agreement with the reports of Atangwho *et al*., (2009) for neem plant, but significantly differed with the work of Madakil *et al*., (2016). The protein content of *A. indica* in this study is higher than the percentage recommended by the Food and Agriculture Organization (FAO, 2003) which is in the range of 12-15%. It shows that this plant is highly nutritious and needs not be supplemented. High protein (29 mg/100g) and fat (12.51 mg/100g) as well as other phytonutrients recorded in this investigation, point to the plant's medicinal value. It's anti-oxidant, anti-inflammatory, anti-diabetic, anticancer, and anti-malaria properties of this plant had been linked to presence phytonutrients and bioactive compounds. Available literatures have demonstrated that various neem parts: leaf, bark, and seed oils, have a wide range of pharmacological activities, such as anti-inflammatory, anti-mutagenic, anti-carcinogenic, antioxidant, antihyperglycemic, antiulcer, and anti-diabetic features (Khanal, 2021).

The result of vitamin analysis revealed low vitamin contents as shown in Table 2. Madakil *et al*. (2016) reported high concentration of vitamin A and C from a fresh leaves of A. indica. Vitamin C is known for its protective function and it is water soluble. The recommended dietary allowance for vitamin C are based on its known physiological and antioxidant functions in white blood cells and are much higher than the amount required for protection from deficiency. The dietary allowance for vitamin C for female is 75 mg while for male is 90 mg. Vitamin C (L- ascorbic acid) is naturally present in some food which is required for the biosynthesis of collagen and also involved in protein metabolism (Li and Schellhorn, 2007). The abysmally lower vitamin contents could be due to its extraction method, resulting in loss of these vitamins, including the carotenoid.

The result of mineral analysis depicted low concentrations. These findings are in agreement with the reports of Madaki *et al*. (2016) on their extract from fresh neem leaves which showed low concentration of minerals such as Na, P, K, and Ca. The mineral contents in this study is lower than the percentage recommended by the FAO, (2003): sodium is 2400 mg, phosphorus (700 mg), potassium (4700 mg), calcium (1000 mg). This suggests the need to supplement diets based on *A. indica* leaves with a complementary mineral element source to make it more nutritious. Sodium, calcium, and phosphorus are all essential minerals that help the body function normally. Sodium Helps maintain fluid balance, while calcium helps in muscles contract and transmission of nerve signals. Calcium and phosphorus are involved in blood clotting and blood pressure, maintenance of healthy bones and teeth. Phosphorus equally contribute to muscle contractions, normal heartbeat and with nerve signaling. The rate of extraction of phytocompounds from medicinal plants depends on the solvent dielectric constant. According to Nawaz *et al*. (2020), extraction and purification are generally affected by such factors including time, temperature, solvent concentration and solvent polarity. The qualitative phytochemical compositions of



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ethanol fractions of *A. indica* leaves showed the distributions of bioactive metabolites. The qualitative phytochemical screenings carried out following the methods reported in literature revealed the presence of alkaloid, saponin, flavonoid, glycoside, tannin, phenol and steroid (Table 2). All the bioactive compounds detected showed moderate concentration, except glycoside which was highest. In addition to the above bioactive substances detected in the present study, Yusuf *et al*. (2020) reported the presence of terpenoids, reducing sugars, and phenols in substantial quantities, thus giving credence on the anti-oxidants, antimicrobial, anti-inflammatory, analgesic, anti-arthritic and wound healing effect of methanol and ethyl-acetate extracts of *A. garckeana.*
The bitter taste of this plant under study may be attributed to its tannin content. Tannins are naturally occurring plant polyphenols, used as the astringent substance in the treatment of burns that precipitate the proteins of exposed tissues to form a protective covering. They are used as mild antiseptics in the treatment of diarrhea, and to check small hemorrhages, and as healing agents in leucorrhoea, gonorrhea, and piles. Tannins are reported to possess anti-inflammatory, antibacterial, antiviral, anti-parasitic, anti-ulcer, and antioxidant properties (Achikanu et al., 2022). The presence of flavonoids and phenols indicate a possible antioxidant property of *A. indica,* protection against allergies, free radicals, platelet aggregation, ulcers, hepatoxins, viruses and tumors, scavenging, anti-aging, anti-inflammatory, anti-microbial, anti-leukemic, vasodilator, anticancer, and antibacterial properties, and are reported to be useful for improving blood circulation in the brain of alzheimeric patients (Achikanu *et al*., 2022). Ejoba, (2012) reported the absence of saponins from aqueous extract of neem plant, contrary the findings of this present study. The presence of saponins indicates possible usage as a cleaning agent. On the other hands, alkaloids play some important metabolic role in living organisms as anticancer, antimalarial, analgesic, antispasmodic and bactericidal, antioxidant and stimulating activities (Achikanu *et al*., 2022).

Figure 2 shows the chromatogram of phytochemical analysis of ethanolic extract of neem plant using GC-FID techniques. A total of 12 peaks were detected and 12 bioactive compounds were identified and quantified using their peaks and retention times (Fig. 2). These results are comparable with that of Duru (2020), who reported similar finding in the concentration of phytochemical of ethanolic extract of *Z. mays* husk using GC-FID. Ethanol has proved its effectiveness in the extraction of the various compounds of flavanonoid including; resveratrol, flavonones, flavan-3-ol, and flavone; the quinoline alkaloid such as lunamarine, and ribalinidine. Other compounds detected are aglycone, gallocatechin, isoflavonoid and kaempferol, a compound of flavonol exhibiting different concentrations. The forkloric claims of this plant usage in medicine for the stimulation of the cardiac and uterine muscles in childbirth might be related to these alkaloid activities.

The compound lunamarin was found to be the most abundant with a concentration of 76.3193 ppm and a retention time (RT) of 31. 433 min, followed by ribalinidine with a concentration of 64.0834 ug/ml and retention time of 8.750 min. However, the compound: kaempferol was the least with a concentration of 0.7358 ug/ml and a retention time of 45.570 min. Kaempferol is a natural flavonol, a class of flavonoid known to increase intracellular ATP content under hypoxic conditions. It scavenges different types of radicals, inhibits reactive oxygen species (ROS) – generating enzymes, and increases the expression of antioxidant enzymes (Achikanu et al., 2022). Resveratrol is also a flavonol that has been reported to have shown anticancer activity as well as in preventing heart damage after a cardiac arrest. It also assists in the reduction of oxidative damage of the liver during ethanol intoxication. Ribalinidine, a quinoline alkaloid, is known to have radical scavenging function, and pharmacological activities (Duru, 2020). Flavan-3-ol oligomers and monomers have been reported to be potent antioxidant compounds. Flavanone is a flavonoid that has been linked to cardiovascular disease and cancer prevention. Intake of catechin – rich foods for instance, have been associated with the prevention and treatment of chronic diseases in humans, such as inflammatory bowel disease (IBO) (Achikanu *et al*., 2022). Spartein, lunamarine and ribalinidine are quinoline alkaloids known to be pharmacologically active compounds with biological activities such as antimalarial, anti-inflammatory, antimicrobial, anti-protozoal, antioxidant as well


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as metal chelating activities. Lunamarine and ribalinidine have been reported to have radical scavenging function. Lunamarin also possess anti-amoebic activity (Duru, 2020; Achikanu et al., 2022). In essence, the presence of these various bioactive compounds in *A. indica* leaves may be responsible for their numerous physiological potentials and biological activities.

**Conclusion**

This study has shown that the extract of *A. indica* possesses considerable number of bioactive compounds: Lunamarin, ribalinidine, gallocatechin and aglycone are the most abundant. This implies that *A. indica* leaves are good source of biologically active compounds suitable to be used for nutraceutical and pharmacological development. The findings of this study were indicative of suitability of ethanol for extraction and the results of the proximate compositions, vitamins and minerals not only fulfill nutritional requirement, but will also have a great role in therapeutic purposes.

**Recommendations and policy implications**

- We recommend that further research is needed to fully explore the potential of *Azadirachta indica* and its bioactive compounds.
- Local communities should be encouraged to participate in the cultivation and processing of neem, thus, contributing to economic development and environmental sustainability.
- Standardization of neem extracts is important for ensuring quality and safety in pharmaceutical and other applications.
- Government's policies for sustainability of agriculture through the use of neem-based products would help to reduce reliance on synthetic pesticides and promote sustainable farming practices.
- Government should promote research and development through investment in research to further explore the potential of neem's bioactive compounds, thus leading to new discoveries and drug applications

**References**

Abhishek, K., Kuljeet K. and Hina C. (2024). Formulation and evaluation of polyherbal floor disinfectant. *Journal of Emerging Technologies and Innovative Research (JETIR)*. Vol.11, issue 12 www.jetir.org d819 (ISSN-2349-5162).

Achikanu, C.E., Ujah, I.I. and Ezenwali, M.O. (2022). Proximate and phytochemical composition of Phyllantus amarus *World Journal of Advanced Research and Reviews*, 15 (01): 041-047, 10.30574/wjarr.2022.15.1.0517.

Alcalde-eon, C., Escribano-Bailon, M.T., Santos-Buelga,C. and Rivas-Gonzalo, J.C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study. *Analyitical Chimica Acta*, 563: 238-254.

Amadi, B., Emelieze, M., Agomuo, E., Ogunka-Nnoka, C. and Amadi, P. (2017). Proximate, GC-FID, and micronutrient analysis of extracts of azadirachta indica. *International Journal of Advanced Chemistry*, 5 (2): 73-79. doi: 10.14419/ijac. v5i2.8124.

Ankita, S., and Sapan P. (2018). Preliminary phytochemical screening and quantitative analysis of secondary metabolites of Mentha arvensis and *Azadirachta indica. International Journal of Advanced Research and Development*. 3(1): 114-118.

AOAC (2006). Official Methods of Analysis (Horwitz,W editor) 18th edition. Association of Official Analytical Chemists (AOAC) Washington, DC, USA.

AOAC. (1990). Official methods of analysis (15th edn.,). Washington DC, USA. Association of official analytical chemists' inch 400-2200 Wilson Boalevard, Arlinton Virginia USA, 2: 910-92.



*Cite this article as*:
Agbo A.O. et al. (2025). Phytochemical screening, proximate and gas chromatography flame ionization detector (GC-FID) identification of bioactive compounds of ethanolic extract of Azadirachta indica leaves.. *Journal of Multidisciplinary Innovations and Perspectives (JMIP), 1*(1) 93-103.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M and Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. Nature review: Drug Discovery, **20**:200-216.

Atangwho, I. J., Ebong, P. E., Eyong, E. U. Williams, I. O. Eteng, M. U. and Egbung, G. E. (2009) Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium*. *African Journal of Biotechnology* 8 (18): 4685-4689.

Bassey, E. E., Mohammed, G. A., Bala, H. M., Ogonna, U. S., Yawuri, B. B, and Maduchi, O. C. (2016). 'Phythochemical analysis and antimicrobial activity of methanolic, ethanolic and acetonic extracts of stem bark and leaf of Neem plant (*Azadirachta indica*). *Journal of Diseases and Medical Plants*, 2 (3): 14-25.

Duru, C. E. (2020). Mineral and phytochemical evaluation of Zea mays husk. *Scientific African*, 7 (2020), p. e00224.

Ejoba, R. (2012) Phytochemical constituents of some leaves extract of Aloe vera and Azadirachta indica plant species. *Global Advanced Research Journal of Environmental Science and Toxicology*, 1(2): 014-017.

Enin, G. N. Antia, B. S. and Enin, F. G. (2014). Chemical assessement of the proximate, minerals, and anti-nutrients composition of Sida acuta leaves. *Elixir Org. Chem*. 71: 24654- 24660.

FAO (2003). Law and sustainable development in Rio: Legal trends in agriculture and natural resource management. FAO Legislative Study. 2003;73.

Khan M. A., Yaqoob S. and Ahmad, S. (2021). Antimicrobial activity of Azadirachta indica, against target pathogens and its utility as a disinfectant and floor cleaner. *Evolution Med Dent Sci*. 10 (25):1899-1903. DOI: 10.14260/jemds/2021/392.

Khanal, S. (2021). Qualitative and Quantitative Phytochemical Screening of Azadirachta indica Juss. plant parts. *International Journal of Applied Science and Biotechnology.* 9(2): 122-127.

Li, Y. and Schellhorn, H.E. (2007). New developments and novel therapeutic perspectives for vitamin C. *J. Nutr*., 137: 2171-2184.

Madakil, F. M., Kabiru1, A.Y., Bakare-Odunola1, M.T., Mailafiya1, S.C., Hamzah1, R.U and Edward, J. (2016). Phytochemical and Proximate Analyses of Methanol Leaf Extract of Neem *Azadirachta indica. European Journal of Medicinal Plants* 15(2): 1-6, 2016, Article no. EJMP.25191 ISSN: 2231-0894, NLM ID: 101583475.

Maria, C and Romilly, M. M. (2017). 'Phytochemical screening and the antimicrobial activity of the leaves of *Azadirachta indica. International Journal of Scientific and Engineering Research*, 8 (5): 721-724.

Nawaz, H., Shad, M., Rehman, N., Andaleeb, H .and N. Ullah (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds.

Puvan., A. A., Irfan, M., Rosdan, S and Zeehaida, M. (2015). 'Antifungal effect of Malaysian Neem leaf extract on selected Fungal specie causing Otomycosis in-vitro culture medium', *Malaysian Journal of Medicine and Health Sciences*, 11 (2): 69-84.

Sadowsky M J and Ishii S. (2008). Escherichia coli in the environment: Implications for water quality and human health. Journal Microbes and Environments, 23(1): 101-108.

Sandeep, H. H., Amritha., G. K and Vedamurthy A. B. (2023). Phytochemical screening and antibacterial activities in leaf extract of *Psidium guajava* (Guava). *International Journal of Recent Scientific Research*, 14 (01) (A): 2836-2840, DOI: http://dx.doi.org/10.24327/ijrsr.2023.1401.0581

Santhosh, V. K, and Navartnam, V. (2013). Neem prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine*, 3(7):505-519.

Shrinet, K., Singh, K., Chaurasia, R.K., Tripathi, A.K and Kumar, A. (2021). Bioactive compounds and their future therapeutic applications. *Natural Bioactive Compounds: Technological Advancements*, 17: 337-355, 10.1016/B978-0-12-820655-3.00017-3

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H and Omonhinmin, C. A. (2020). Antimicrobial importance of medicinal plants in Nigeria. *Scientific World Journal*. 2020:7059323. doi:10.1155/2020/7059323.

Virshette, S. J., Patil, M. K., Deshmukh, A. A., Shaikh, J. R., and Dhas, M. S. (2020). Phytochemical constituents of different extracts of *Azadirachta indica* leaves in urine solvent of a non-pregnant cow. *Journal of Pharmacognosy and Phytochemistry*, 9 (2): 1324- 1328.

Wifek M, Saeed A, Rehman R and Nisar S. (2016). Lemongrass: a review on its botany, properties, applications and active components. *International Journal of Chemical and Biochemical Sciences*, 9 :79-84.

Xiao, J and Bai, W. (2019). Bioactive phytochemicals Critical Reviews in Food Science and Nutrition, 59 (6): 827-829, 10.1080/10408398.2019.1601848.

Yusuf, A.A, Lawal, B, Sani, S, Garba, R, Mohammed, B.A and Oshevire D.B, (2020). Pharmacological activities of *Azanza garckeana* (Goron Tula) grown in Nigeria, *Clinical Phytoscience*, 6 (27): 1-8.

Zillich, O.V., Schweiggert-Weisz, U., Eisner, P. and Kerscher, M., (2015). Polyphenols as active ingredients for cosmetic products. *International Journal of Cosmetic Science*, 37(5): 455-464.