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Potential health and environmental benefits of the identified phytochemicals screening of (*Azadirachta indica*) neem leaves in Bauchi Metropolis, Bauchi State, Nigeria

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Abstract

The phytochemical screening of (*Azadirachta indica*) Neem leaf extract was conducted to identify and quantify the presence of bioactive compounds. The study conducted various tests to assess the phytochemical components. About 1kg of matured fresh leaves of (*Azadirachta indica*) neem was collected from five (5) different location in Bauchi metropolis, these locations are; Kobi Street, Gida Dubu, Yelwan Tudu, Wuntin dada and Federal Low-cost areas in Bauchi metropolis, Bauchi State, Nigeria. The leaves of the plant, which were ground into a fine powder using a mortar and pestle. An ethylacetate solvent was used to derive the A. indica leaf extract. The results of the research shows that the presence of alkaloids, saponins, phenols, and cardiac glycosides in the neem leaf extract is significant, and flavonoids, terpenoids, and steroids were not detected in the neem leaf extract. The presence of alkaloids, saponins, phenols, and cardiac glycosides suggests that neem leaves may hold promise in traditional and contemporary medicinal practices. Alkaloids, known for their pharmacological significance, may contribute to the plant's therapeutic properties. Saponins, with their frothing and foaming characteristics, have been associated with immunomodulatory effects and their potential in agrochemical applications. Phenolic compounds, as evidenced by the positive response to the Ferric chloride test, indicate the antioxidant potential of neem leaves, which can combat oxidative stress. Conversely, the absence of flavonoids, terpenoids, and steroids suggests that these specific phytochemical classes may be limited in neem leaves. These findings open avenues for further research and utilization of neem as a valuable natural resource.

Keywords: *Azadirachta indica*; Mortar and Pestle; Ethylacetate solvent; Cardiac glycosides.

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1. Introduction

Medicinal plants are generated from a variety of plantgrouos and families and are used as essential oils, plant extract, or both (Srivastava and Kumar 2010). Many bioactive chemicals with antibacterial and antifungal properties may be found in plant extracts from stem plants, rhizomes, bark, stem, and root (Khanal, 2021). Currently, a number of scientific disciplines are quite interested in studying urban environments. Urban environments are changing as a result of human activities such urbanization, carbon emissions, and biodiversity changes. This emphasizes the significance of continuous environmental assessment to protect the health and livability of urban residents (Mudele et al., 2020; Onivefu et al., 2024; Onivefu, 2023).

According to Khanal (2021) the biological capabilities of medicinal plants are attributed to numerous active components. The primary aim of this research is to employ an ethyl acetate extract to detect the phytochemical features of the (*Azadirachta indica*) Neem leaves in the study area. The specific objective of the study was; To identify and quantify the phytochemicals present in neem leaves, to assess the potential health and environmental benefits of the detected phytochemicals

Neem (Azadirachta indica) is a tree in the *Meliaceae* family. Neem is known by the Sanskrit name "arista," which means "perfect, complete, and perishable" (Girish and Shankara, 2008). It is an 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, and Mauritius (Puvan et al., 2015). In the mid-1900s, a large amount of chemical research was done on the byproducts of the neem tree. The United Nations decided to name the amazing neem tree as the Tree of the 21st Century (Puri, 1999). The most often prescribed remedies in historical medical books for digestive disorders, diarrhea and intestinal infections, skin ulcers, and malaria are extracts from the Neem tree (*A. indica*), also known as "Dogonyaro" in Nigeria (Virshette et al., 2020). The most often prescribed remedies in historical medical books for illnesses such as diarrhea and intestinal infections, skin ulcers, and malaria are extracts from the Neem tree (A. indica), also known as "Dogonyaro" in Nigeria (Virshette et al., 2020).

Global, agricultural, environmental, and health issues have long been addressed using in health innovational tools (Santhosh and Navartnam, 2013; Bassey et al., 2016; Mudele et al., 2021). Its leaves can be taken as medication to treat eczema, diabetes, and lower fever. Neem leaves are used to produce toothbrushes, and the plant's roots are insect- and disease-repelling Neem tree seeds are extremely rich in oil. Many illnesses, including diabetes and TB, are treated with neem oil, which is also used as a lubricant, pesticide, and medication (Kumar et al., 2009; Virshette et al., 2020). Neem leaf extracts have been used in Africa to create a variety of therapeutic medicines. Additionally, it has been demonstrated that various neem parts, including the 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 (Reddy et al., 2013).

Figure 1 *Azadirachta indica leaves*

The robust, evergreen neem (*Azadirachta indica*) tree can be found mainly in tropical and subtropical regions of the world, primarily in Africa and Asia. possess thick, round, pinnate leaves and bark that ranges from brown to dark gray. They range in size from medium to big (Susmitha et al., 2013). The reason for their survival in dry and semi-arid regions of the planet is their vast deep root system. Plants generate molecules known as phytochemicals that help protect against bacteria, viruses, fungus, and weariness in insects and other animals. The name originates from Greek (phyton) 'plant', which is thought to be responsible for protective health benefits (Webb, 2013). Numerous physiologically active substances, such as alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones, may be isolated from neem and added to the chemical contents. Azadirachtin 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).

As antibiotic resistance is a serious problem, the creation of novel compounds derived from plants may help address the need for new antimicrobial agents with enhanced safety and efficacy (Srivastava et al., 2010). Alkaloids, flavonoids, tannins, saponins, polyphenols, and reducing sugars are among the bioactive substances found in neem extracts' leaf and stem bark, according to Bassey (2016). These chemicals also have antibacterial properties. Further research by Maria and Romilly (2017) revealed the presence of volatile oils, triterpenes, alkaloids, flavonoids, tannins, saponins, steroids, resins, bitter cardiac glycosides, reducing sugar, and flavonoids in both fresh and sun-dried neem leaves. These compounds have a significant potential for antibacterial effects.

This research is justified by the need to elucidate the phytochemical constituents of *Azadirachta indica* (neem leaves) when extracted using ethyl acetate. The results of this research may contribute to the development of new medicines and the ethical use of this valuable plant in a variety of environments by expanding our understanding of the health, nutritional, and safety benefits of neem leaves.

A total of five (5) sample points were selected based on the geographical representative in each direction (see Fig. 2). During the sorting of the sample, out of the 5 samples selected and collected, only one important point (Kobi Street) was selected and proceeded with for the research because of its geographical location in the study boundary line and the highest density of the population in the research area.

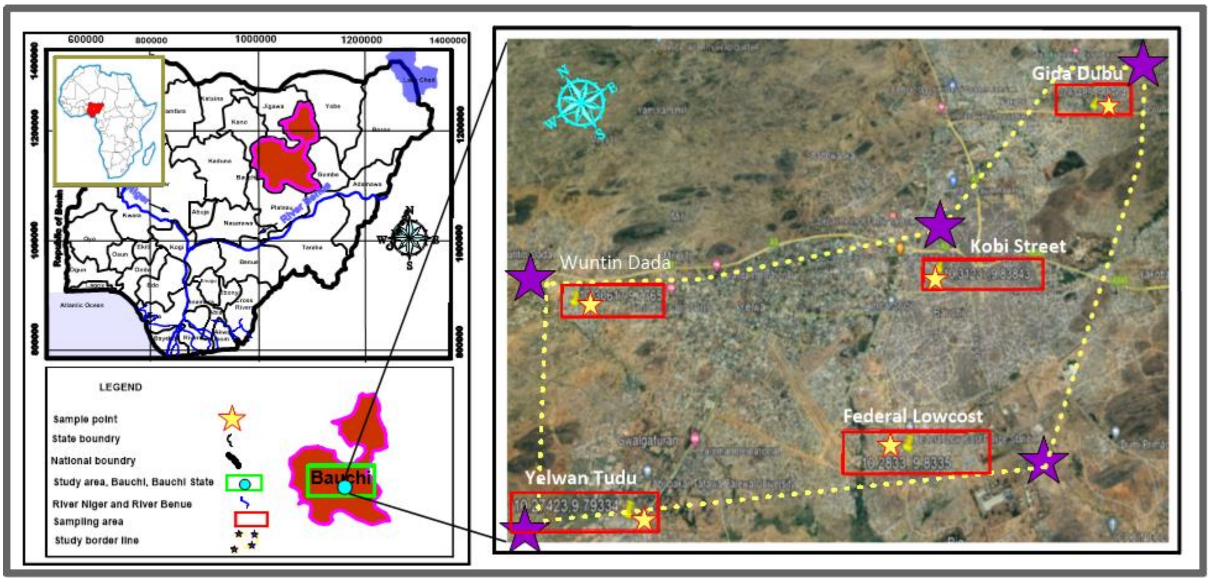

Figure 2 Map of the Study area and the sample points (Modified after Akagbue 2023a; Abdulbariu et al. 2023; Akagbue 2023b.)

Neem tree belongs to the family *Meliaceae* which is widely distributed in tropical and semitropical areas such as Bangladesh, Pakistan, Nepal and India. Additionally, neem has been introduced to various other tropical and subtropical regions worldwide, including Africa, the Caribbean, and parts of the Americas (Girish and Neem, 2008). The tree grows quickly, reaching a height of 20–23 m tall, its trunk is straight, with a diameter of around 4-5 feet. although it can grow taller under favorable conditions (Girish and Neem, 2008).

According to Alzohairy (2016), the complex, imparipinnate leaves are made up of five to fifteen lance-shaped, serrated-edged leaflets per leaflet. They are arranged alternately on the branch. Its fruits are green drupes which turn golden yellow on ripening in the months of June– August. The fruits are olive-like drupes that are typically oval or round in shape. These fruits are green when young and turn yellow as they ripen. Each fruit contains a single elongated seed with a kernel inside (Biswas *et al*., 2012). The bark of the neem tree is brown to grayish-brown and is characterized by its rough texture. The root has a shallow but widespread root system, making it well-adapted to arid and semi-arid regions (Biswas *et al*., 2012). Table 1 below shows the taxonomic classification of (*Azadirachta indica*) neem.

Table 1 Taxonomic position of (*Azadirachta indica*) neem

Kingdom	Plantae
Order	Rutales
Suborder	Rutinae
Family	Meliceae
Sub-family	Meliodae
Tribe	Melieae
Genus	Azadirachta
Species	Indica

Azadirachta indica L. shows a therapeutic effect in health management because of its abundance of various types of ingredients. *Azadirachtin* is the most significant active ingredient; the others include *quercetin*, sodium *nimbinate*, *gedunin, salannin, nimbin, nimbidin*, and *nimbidol*. Ascorbic acid, n-hexacosanol, *amino acids*, *nimbin*, *nimbanene*, 6-*desacetylnimbinene*, *nimbandiol*, *nimbolide*, 7-*desacetyl*-7-*benzoylazadiradione*, 7-*desacetyl-7-benzoylgedunin*, 17-h*ydroxyazadiradione*, and *nimbiol* are among the ingredients found in leaves Hossain et al., 2011). ß-sitosterol, Polyphenolic and Quercetin flavonoids, were extracted from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents such as *azadirachtin* and *gedunin*. Neem is renowned for its diverse array of bioactive compounds, which contribute to its therapeutic potential (Hossain *et al*., 2011). Below are the key phytochemical constituents found in neem leaves, seeds, and other parts of the tree.

1.1. Alkaloid

Neem leaves and seeds contain various alkaloids, including nimbin, nimbidin, and nimbinene. These alkaloids have been associated with various pharmacological activities, such as anti-inflammatory, analgesic, and antipyretic effects (Ramesh and Suganya, 2018).

1.2. Flavonoids

Neem leaves are a good source of flavonoids, including quercetin, kaempferol, and rutin. Flavonoids are well-known for their antioxidant properties, which can help combat oxidative stress and reduce the risk of chronic diseases (Kikani *et al*., 2020).

1.3. Saponin

Neem seeds are particularly rich in saponins. Saponins have demonstrated various biological activities, including anti-inflammatory, immunomodulatory, and antitumor effects (Verma *et al*., 2017).

1.4. Phenolic Compounds

Neem leaves contain phenolic compounds, such as gallic acid and catechin. These compounds contribute to the antioxidant properties of neem, which can safeguard the cells from harm caused by free primitives (Gupta *et al*., 2014).

1.5. Terpenoids

Neem is a source of various terpenoids, including limonoids, triterpenes, and essential oils. Limonoids found in neem, such as azadirachtin, have insecticidal properties and are used in organic pest control (Isman, 2021).

1.6. Steroids

Neem seeds contain steroids, including beta-sitosterol. Beta-sitosterol has been studied for its potential cholesterol-lowering effects and anti-inflammatory properties (Dwivedi and Dubey, 2007).

1.7. Cardiac Glycosides

Neem leaves have been found to contain cardiac glycosides. Cardiac glycosides have a well-documented role in cardiac health and have been studied for their potential in managing heart conditions (Cui *et al*., 2018).

1.8. Tannins

Tannins are also present in neem leaves and seeds. They have astringent properties and can form complexes with proteins, which may contribute to their medicinal use (Dasgupta and Rao, 2018).

Potential Health and Environmental Benefits of Neem Phytochemicals

Neem (*Azadirachta indica*) has been utilized traditionally for its various phytochemicals in both medicine and agriculture. Additionally, contemporary research has shed light on numerous applications of neem-derived compounds. Below are the traditional and contemporary uses of neem phytochemicals.

1.8.1. Potential Health Application of Neem Phytochemicals

Neem's bioactive compounds, such as nimbidin and nimbin, have been traditionally applied in Ayurvedic medicine for their antibacterial and antifungal properties. Neem-based formulations have been used to alleviate inflammation and pain in various conditions, including arthritis (Subapriya and Nagini, 2015).

Antioxidant and Anti-Inflammatory Effects

Neem's phytochemicals, particularly phenolic compounds, exhibit strong antioxidant properties. Antioxidants neutralize free radicals in the body, protecting cells from oxidative stress and inflammation (Gupta *et al*., 2014). These properties suggest a potential role in preventing and managing inflammatory conditions. Neem extracts, owing to their antimicrobial and anti-inflammatory properties, are used for treating skin conditions like acne, eczema, and psoriasis (Pazyar *et al*., 2012).

Cardioprotective Properties

The presence of cardiac glycosides in neem suggests potential cardioprotective effects. Cardiac glycosides have been studied for their positive impact on heart health, including their use in managing cardiac conditions (Cui *et al*., 2018). Further research can explore neem's role in cardiovascular health.

Antimicrobial and Antibacterial Activity

Alkaloids, identified in moderate concentrations in neem, contribute to its antimicrobial properties. Neem has been traditionally used to treat various infections due to its antibacterial and antifungal effects (Biswas *et al*., 2012). This suggests applications in the development of natural antimicrobial agents. Neem twigs have been used traditionally as a natural toothbrush due to their antibacterial properties, helping to maintain oral hygiene (Chatterjee and Pakrashi, 1991; Kikani *et al*., 2020).

Immune System Modulation

Saponins, present at a low concentration, are known for their immunomodulatory properties. These compounds may play a role in enhancing the immune system's response to infections and diseases (Verma *et al*., 2017). Neem's immunomodulatory potential can be explored for therapeutic applications.

1.8.2. Potential Environmental Applications of Neem Phytochemicals

Organic Pest Control

Azadirachtin, a prominent neem-derived compound, is widely used in organic pest control due to its insecticidal properties (Isman, 2021). Neem extracts can serve as eco-friendly alternatives to synthetic pesticides, minimizing environmental impact.

Phytoremediation

Neem trees are planted in contaminated areas to help purify soil and groundwater due to their ability to absorb and neutralize pollutants. Immunomodulatory Properties: Neem-based products may have applications in immunomodulation and supporting the immune system (Verma *et al*., 2017). Ineffective protein utilization in food that has disastrous effects on the ecosystem due to nitrogen pollution (Adeniyi *et al*., 2023: Akagbue *et al*., 2023b)

Sustainable Agriculture

Neem-based fertilizers, such as neem cake, provide a sustainable alternative to chemical fertilizers. The organic matter in neem cake enhances soil fertility and promotes sustainable agriculture practices (Isman, 2021).

Biopesticides for Crop Protection

Neem extracts, containing a combination of bioactive compounds, can be formulated into biopesticides. These biopesticides offer environmentally friendly alternatives for crop protection, reducing reliance on synthetic chemicals (Isman, 2021).

A study by Biswas *et al*. (2012) provides a comprehensive overview of the phytochemical composition of neem leaves, highlighting the presence of alkaloids, flavonoids, and other bioactive compounds. It discusses the traditional uses and medicinal properties of these phytochemicals. Subapriya and Nagini (2015) conducted a study that explores the traditional use of neem leaves for their antibacterial and antifungal properties. The paper discusses the potential of neem phytochemicals in combating microbial infections.

Isman (2021) provides an overview of the history and production of azadirachtin, a prominent neem-derived compound used in organic pest control. The study discusses the applications of neem-based pesticides in agriculture. Kumar and Dubey (2014) explore the use of neem trees in phytoremediation for environmental cleanup. The paper discusses how neem's phytochemicals can help purify soil and groundwater in contaminated areas. According to Patil et al. (2013), a reseach was conducted to assess the 70% alcoholic neem root bark extract (NRE) in diabetes. The findings indicated that the 800 mg/kg dosage of neem root bark extract produced statistically significant outcomes.

Further research was conducted to investigate the pharmacological hypoglycemic action of *Azadirachta indica* in diabetic rats. The research revealed that the rats' glucose levels were significantly lower in the glucose tolerance test using 250 mg/kg of neem extract than in the control group, and that the administration of *Azadirachta indica* significantly decreased the glucose levels in the diabetic rats by the fifteenth day (Dholi et al., 2011).

Existing research has predominantly focused on specific classes of phytochemicals present in neem leaves, such as alkaloids, flavonoids, and saponins. However, there is a notable gap in our understanding when it comes to a more comprehensive phytochemical analysis. Neem leaves contain a vast array of bioactive compounds, and a holistic examination of these components is necessary to fully elucidate their potential health benefits. A comprehensive analysis would provide a more nuanced view of the synergistic effects between different compounds and their collective impact. This knowledge gap calls for research that explores the full spectrum of neem leaf phytochemicals to better grasp their therapeutic potential (Jeyasankar *et al*., 2014).

While individual phytochemicals in neem leaves have been studied, the bioavailability of these compounds and their potential synergistic effects when present together is an area that requires more attention. Understanding how neem phytochemicals are absorbed and utilized by the body is critical for designing effective interventions. Additionally, the potential synergies between these compounds can enhance or alter their therapeutic properties. Research addressing these bioavailability and synergy aspects will shed light on the practical implications of using neem in healthcare (Isman, 2021).

To establish the therapeutic potential of neem leaves and their phytochemical components in humans, particularly for specific health conditions, well-designed clinical trials are required. While preliminary research shows promise, robust clinical studies are necessary to provide evidence-based insights into the efficacy of neem-based interventions. Clinical trials would bridge the gap between laboratory findings and practical healthcare applications, allowing for more informed and evidence-based recommendations for neem usage (Isman, 2021).

The establishment of quality control measures and standards for neem-based products, such as dietary supplements and cosmetics, is an important yet underdeveloped area. Quality control ensures the consistency and safety of products available to consumers. It is imperative to create guidelines and regulations that guarantee the quality and efficacy of neem-based products, addressing this crucial aspect of neem utilization. For the long-term availability and preservation of *Azadirachta indica*, research on sustainable cultivation and harvesting practices is essential. Sustainable practices not only support the ecological balance but also ensure a continuous supply of neem leaves. Research in this domain aims to develop environmentally responsible approaches for neem cultivation (Kumar and Dubey, 2014). Addressing these knowledge gaps is vital for advancing our understanding of neem's phytochemical composition, its potential health applications, and its sustainable utilization. These research areas will contribute to a more comprehensive and evidence-based approach to the use of neem in various fields, from medicine to agriculture.

2. Materials and methods

2.1. Data Collection and Preparation of Plant Sample

Fresh leaves of *Azadirchta indica* (neem) was collected from Yelwan Tudu, Federal Low cost, Gida Dubu, Wuntin Dada and Kobi Street of Bauchi, Bauchi State. An approximate 1 kg of fresh, maturated leaves of the plant was thoroughly cleaned with tap water, after which they were rinsed with distilled water. The leaves were then allowed to air dry for a week at ambient temperature (27 ^{o}C). The sample was then manually pounded into a fine powder using a sterile, clean mortar and pestle to enhance surface area, and the powder was weighed, assembled, and packaged into clean cellophane bags and labeled to avoid mix-ups. The sample was stored in a cool, dry location until needed, while fresh leaves were used promptly after being collected from the tree branches.

2.2. Extraction of Plant Material

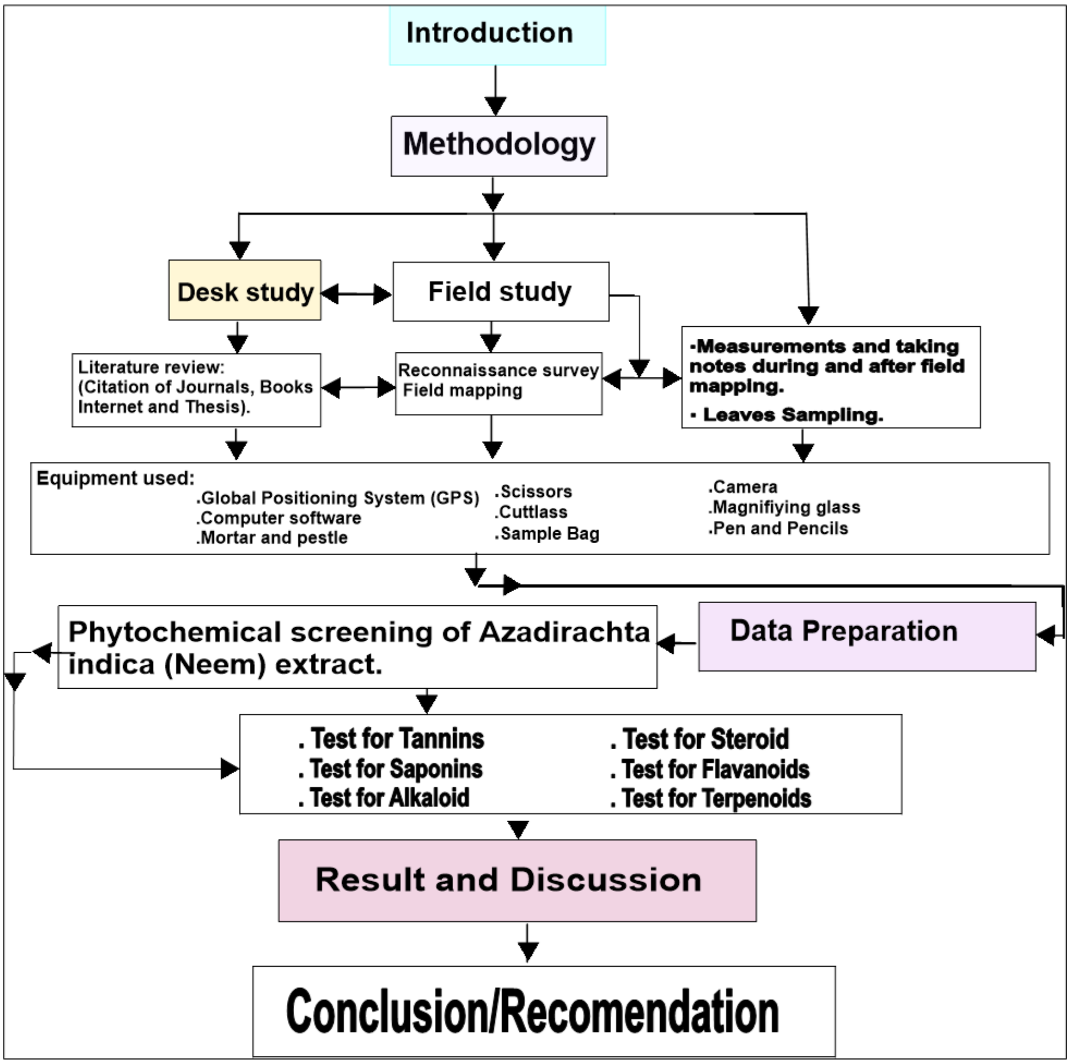

Figure 3 Research Methodology Flow chat (Modified after Baba Aminu et al., 2023a; Akagbue et al., 2023c; Abdulbariu et al., 2023.

The extraction was prepared using maceration method. Thus, 100g of the powdered plant material was weighted and extracted with 250ml of ethyl acetate for 72 hours. Proper filtration was carried out using filter paper after which the filtrate was boiled for proper extraction. The extract was tested with several reagents to determine the presence and quantity of Tannin, saponins, alkaloids, flavonoids, terpenoids, steroids, phenolic compound and *cardiac glycoside*.

2.3. Phytochemical screening of *Azadirachta indica* Extract

To identify the chemical components of the plant's ethyl acetate extracts, routine phytochemical tests were carried out.

2.3.1. Test for Tannins

A small amount of 0.5g of the crude extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds (Bassey *et al*., 2016).

2.3.2. Test for Saponins

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins (Edeoga *et al*., 2005).

2.3.3. Test for Alkaloid

The presence of alkaloids in the sample was shown by the appearance of a dark orange or purple hue when 1 ml of each solvent's extract and 1 ml of Wagner's reagent were added and well mixed (Bassey et al., 2016).

2.3.4. Test for Steroid

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, steroid is present (Bassey *et al*., 2016).

2.3.5. Test for Flavanoids

After extracting around 4 g of neem from fresh and dried leaves that had been shaded, 6 ml of a 50% methanol, ethanol, and acetone solution was added. After warming the solutions, 1.5 g of metal magnesium was added to the solution containing neem leaf extract. Then 4-5 drops of concentrated hydrochloric acid were added (Bassey *et al*., 2016).

2.3.6. Test for Terpenoids

A reddish-brown color at the interface indicates a good result for the presence of terpenoids. The crude extract was mixed with 2 ml of chloroform, and 3 ml of concentrated sulfuric acid was carefully added to produce a layer (Bassey et al., 2016).

2.3.7 Test for Phenol

Distilled water was used to dissolve a small quantity of extract. A 5% ferric chloride solution (two milliliters) was added to this solution. Phenolic chemicals are present when a blue, green, or violet color forms

2.3.7. Test for Cardiac Glycosides

About 2ml of the crude extract was dissolved in 1ml of glacial acetic acid. A few drops of 5% ferric chloride solution and few drops of concentrated sulphuric acid were added. Formation of green blue precipitate indicate the presence of glycosides (Bassey *et al*., 2016).

3. Result

3.1. Result of Phytochemical screening of the Ethyl acetate extracts

Alkaloids, saponins, phenols, and cardiac glycosides were found in the phytochemical screening of the *Azadirachta indica* leaf extract, as shown in Table 2 below. Other components analyzed, including flavonoids, terpenoids, and steroids, were not found in the extract.

Table 2 Phytochemical screening of *Azadirachta indica* leave extract

Phytochemical components	Test	Results
Alkaloid	Wagner's test	++
Flavonoid	Ammonia test	-
Saponin	Frothing/foam test	+
Phenol	Ferric chloride (FeCl3) test	+
Terpenoids	Terpenoid test	-
Steroid	Salkowski's test	-
Cardiac glycoside	Keller-Killiani test	++
Tannins	Ferric chloride	+

Key: - = not detected; + = low concentration ;++ = moderate concentration; +++ = high concentration

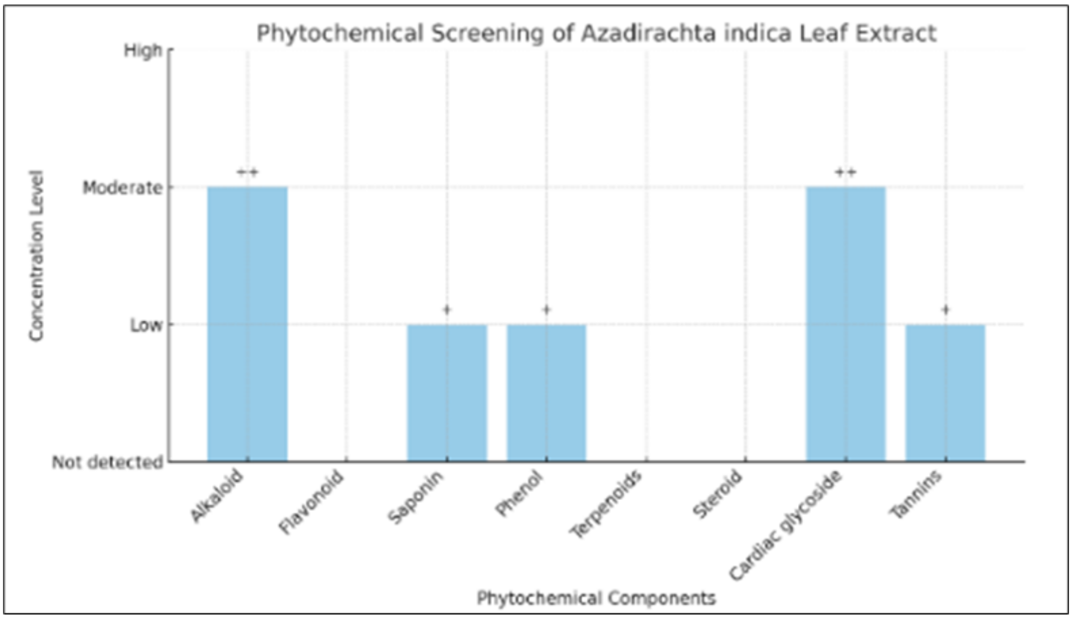

Figure 4 A bar chart pictorial representation of the Phytochemical screening of *Azadirachta indica* leave extract

The bar graph represents the phytochemical screening of *Azadirachta indica* leaf extract. Each bar corresponds to a phytochemical component, and the height of the bar indicates the concentration level detected in the extract:

"Not detected" is represented by the absence of a bar (see fig. 4)

"Low concentration" is indicated by a bar with a single plus sign above it (see fig. 4).

"Moderate concentration" is indicated by a bar with two plus signs above it (see fig. 4).

4. Discussion

4.1. Phytochemical screening of *Azadirachta indica* leave extract

The phytochemical analysis of *Azadirachta indica* (neem) leaves revealed the presence and concentration of various bioactive compounds, including alkaloids, saponins, phenols, and cardiac glycosides, which are important for understanding the potential health and environmental applications of neem. As a result, no flavonoids, terpenoids, or steroids were detected. The results indicate the following findings:

The presence of alkaloids in *ethylaceatate* neem leaves, as determined by the Wagner's test, is significant at a moderate concentration (++). Alkaloids have been shown to have a variety of biological functions, including analgesic and antibacterial characteristics. In their study on Phytochemical analysis of different extract of *Azadirachta indica* Leaves, Virshette *et al*. (2020) detected alkaloids, tannins, phytosterols, and saponins in all extracts of *A. indica*. This finding is also matching with the research of Madaki *et al*. (2016), who discovered alkaloid and other components in their study of Phytochemical and proximate analyses of methanol leaf extract of Neem *Azadirachta indica*. Alkaloids are primarily found in plant species, where they often serve as secondary metabolites.

The presence of alkaloids also suggests that A. indica extracts may have biological actions, including anti-cholinergic, anti-tumor, anti-hypertensive, cough expectorant, anesthetic, analgesic, muscle relaxant, anti-pyretic, and anti-malarial (Emran et al., 2015). Susmith *et al*. (2013). Recent research has looked into the therapeutic qualities of neem alkaloids, such as their antibacterial, anti-inflammatory, and immunomodulatory effects (Ahmad and Amin, 2014). Some alkaloids, for example nicotine and caffeine, are well-known for their effects on the human body and are frequently used recreationally or in foods and beverages (Ahmad and Amin, 2014). Alkaloids like caffeine can increase heart rate and blood pressure. Prolonged excessive consumption of caffeine may contribute to cardiovascular issues, including arrhythmias, high blood pressure, and an increased risk of heart disease. High doses of certain alkaloids can lead to nervous system disturbances, including anxiety, restlessness, tremors, and in severe cases, seizures or hallucinations (Sajid *et al*., 2021).

The presence of saponins in neem leaves is considerable, as evidenced by a low concentration (+ frothing/foam test). Saponins can be utilized as natural detergents and emulsifiers, among other things. Saponins are also being investigated as immunomodulatory and anticancer medicines (Oleszek *et al*., 2011). Saponins are polycyclic aglycones connected to one or more sugar side chains. Saponins have a variety of health benefits, including decreased blood cholesterol, cancer prevention, bone health, and immune system activation. Saponins contain antifungal, antibacterial, and antiprotozoal effects, according to Kumar *et al*. (2018). Aqueous leaf extract of *A. indica* contained alkaloids and saponins with nematocidal activity (Muhammad *et al*., 2018). Excessive saponin consumption might cause gastrointestinal problems. Saponins have a foaming property and can form compounds in the digestive tract with lipids and proteins. This can cause nausea, vomiting, diarrhea, and abdominal cramps (López, 2015). It can also irritate the mucous membranes of the mouth, throat, and gastrointestinal system in some situations. This inflammation can be painful and contribute to gastritis or other inflammatory diseases (Schönthal, 2018). It also contains antioxidant properties that can help the body neutralize harmful free radicals. This characteristic could contribute to its potential health benefits, such as resistance to oxidative stress-related illnesses (Liu *et al*., 2016). According to study, some saponins present in ginseng and soy may have anticancer properties. They have the ability to inhibit the growth of cancer cells and induce apoptosis (programmed cell death) in these cells. The presence of saponins in neem leaves is considerable, as evidenced by a low concentration (+ frothing/foam test). Saponins can be utilized as natural detergents and emulsifiers, among other things. Saponins are also being investigated as immunomodulatory and anticancer medicines (Oleszek *et al*., 2011).

The presence of phenols, as demonstrated by a positive ferric chloride (F_eCl3) test result, shows that antioxidant effects are possible. Anti-oxidative action has been attributed to phenolic compounds contained in A. indica leaf extracts, which protects cells and tissues from free radicals (Kumar *et al*., 2018).

Among the most potent natural antioxidants found in plants are phenolic chemicals. By scavenging free radicals and minimizing oxidative damage, they help protect cells from oxidative stress. This feature has been linked to a variety of health advantages (Salehi *et al*., 2019). Phenols have anti-inflammatory characteristics that can aid in the reduction of chronic inflammation. Chronic inflammation has been linked to a variety of disorders, including cardiovascular disease and cancer (D'Archivio *et al*., 2010). Some phenolic chemicals, such as resveratrol (found in red grapes and wine), have been studied for their ability to limit cancer cell development and trigger apoptosis, or programmed cell death (Salehi *et al*., 2019). Phenolic chemicals have been linked to cardiovascular benefits, such as lowering the risk of heart disease. They can help lower blood pressure, improve blood vessel function, and lower LDL cholesterol oxidation (Visioli *et al*., 2011). Excessive consumption of phenolic compound-rich foods, such as certain fruits and vegetables, can cause digestive discomfort, including abdominal pain and diarrhea. This is especially important for people with sensitivities or allergies (Gu *et al*., 2017). While phenolic chemicals are generally benign, certain highly concentrated phenols, such as hydroquinone, can be poisonous and hazardous if taken in large quantities (Gu *et al*., 2017).

The Keller-Killiani test indicated the presence of cardiac glycosides at a moderate concentration (++). Cardiac glycosides have been studied for their potential utility in heart disease. They have the potential to make an impact on cardiovascular medicine (Ahmad and Amin, 2014). Traditional medicine has used cardiac glycosides' cardiotonic effects for millennia. They increase the force and efficiency of cardiac contractions, which makes them effective in illnesses such congestive heart failure (Chen *et al*., 2015). Cardiac glycosides alter cardiac muscle contractility, which can help enhance heart pumping efficiency (Wu *et al*., 2020). Recent research has explored the potential anticancer properties of certain cardiac glycosides. These compounds have shown promise in inhibiting the growth of cancer cells and inducing apoptosis (Zhang *et al*., 2020). Some cardiac glycosides have been investigated for their anti-inflammatory activities, which can have implications for various inflammatory conditions (Grossman *et al*., 2017). One of the primary concerns with cardiac glycosides is their narrow therapeutic window, meaning there is a small margin between the therapeutic dose and a toxic dose. Slight overdosage can lead to serious toxicity (Chan *et al*., 2020). Excessive use of cardiac glycosides can cause severe cardiac toxicity, potentially resulting in arrhythmias, heart block, and even death (Yeh *et al*., 2019). Nausea, vomiting, stomach pain, and diarrhea are early signs of cardiac glycoside overdose (Chan *et al*., 2020). Cardiac glycosides can also disrupt other physiological systems, including electrolyte imbalances, renal dysfunction, and hyperkalemia (high potassium in the blood) (Yeh *et al*., 2019).

The presence of tannins, as revealed by the positive ferric chloride test result, implies that neem leaves contain these astringent chemicals. Tannins have been researched for their antiviral, antibacterial, and antioxidant activities, making them appealing for both medicinal and industrial applications (Scalbert, 1991). It is well-known for its powerful antioxidant effects. They can help protect cells and tissues from oxidative damage by scavenging free radicals and avoiding lipid peroxidation (Liu *et al*., 2016). It has also been shown to have anti-inflammatory characteristics, making it potentially effective in decreasing chronic inflammation, which plays a critical role in many chronic diseases (Salehi *et al*., 2019). Some tannins have antibacterial properties that help to keep bacteria, fungi, and viruses at bay. Cushnie and Lamb (2015) state that this can be effective for food preservation and infection prevention. Tannins, notably those contained in red wine and some fruits, have been linked to cardiovascular benefits. They can improve blood vessel function, lower LDL cholesterol oxidation, and assist regulate blood pressure (Visioli *et al*., 2011). Excessive consumption of tannin-rich foods or beverages can cause digestive discomfort, including abdominal pain, nausea, vomiting, and constipation (Kasote *et al*., 2015). Tannins are astringent and can cause tooth discoloration and erosion. Tannin-containing beverages such as tea and red wine may have a deleterious impact on tooth health (He *et al*., 2018).

The absence of flavonoids, as shown by the negative ammonia test result, is quite unexpected. Flavonoids are renowned for their antioxidant and anti-inflammatory qualities, and their presence is frequently associated with plant leaves. However, it is important to remember that the absence of flavonoids in the specific sample studied does not rule out their presence in other portions of the neem plant or under different conditions (Rice-Evans *et al*., 2007). The absence of flavonoids in the studied neem ethyl acetate extract could be attributed to changes in textural, chemical, and nutritional soil variables that vary between geographic areas (Dash *et al*., 2017; Saiyam, 2018). Flavonoids are renowned for their strong antioxidant properties, which help protect cells and tissues from oxidative damage by neutralizing harmful free radicals (Salehi *et al*., 2019). Flavonoids have been shown to exhibit anti-inflammatory properties, making them valuable in reducing chronic inflammation, a key factor in many chronic diseases (Salehi *et al*., 2019). Some flavonoids have natural antimicrobial properties and can help inhibit the growth of bacteria, fungi, and viruses, which can be beneficial for food preservation and combatting infections (Cushnie and Lamb, 2015). Excessive consumption of flavonoid-rich foods or supplements may cause digestive discomfort such as abdominal pain, nausea, vomiting, and diarrhea (Boyer and Liu, 2014). While rare, some people may be allergic to particular flavonoids and have skin rashes and itching after consuming them (Mertens-Talcott *et al*., 2018). Flavonoids, particularly those found in tea and certain fruits, have been demonstrated to restrict the absorption of non-heme iron (the iron found in plant-based meals) in the stomach. This could result in iron insufficiency in persons who don't obtain enough iron from their diet (Mertens-Talcott *et al*., 2018).

The absence of terpenoids and steroids, as evidenced by the negative findings in the terpenoid and Salkowski's tests, shows that neem leaves may be deficient in these phytochemical groups. Terpenoids, which are renowned for their varied biological actions, are frequently present in plant essential oils, whereas steroids are key components of many bioactive chemicals (Nishino *et al*., 2019). Terpenoids, which include carotenoids and tocopherols, have substantial antioxidant action. They help protect cells and tissues from oxidative stress by neutralizing damaging free radicals (Nishino *et al*., 2019). Certain terpenoids, particularly essential oils containing terpenes, have inherent antibacterial effects. They have the ability to prevent the growth of bacteria, fungus, and other microorganisms (Raut and Lakkakula, 2019). In some cases, the consumption of terpene-rich essential oils or concentrated terpenoid supplements can lead to gastrointestinal discomfort, including nausea and diarrhea (Kim *et al*., 2016). Direct skin contact with certain terpenes, particularly in essential oils, may cause skin irritation or allergic reactions in sensitive individuals (Foti *et al*., 2015).

Plant steroid phytosterols have cholesterol-lowering effects and are frequently added to functional foods. They can lower dietary cholesterol absorption in the intestines (Musani *et al*., 2018). Some plant steroids, such as beta-sitosterol, have been demonstrated to have anti-inflammatory properties. They may help reduce inflammation and are being studied for their possible function in the treatment of illnesses such as arthritis (Bouic, 2015). High dosages of phytosterols or other plant steroids can impair fat-soluble vitamin absorption, particularly vitamins A, D, E, and K (Ostlund, 2014). Although it is uncommon, some people are allergic to plant sterols and may develop allergic reactions when eating foods supplemented with high quantities of phytosterols (Pascual *et al*., 2016)

5. Conclusion

The phytochemical analysis of *Azadirachta indica* (neem leaves) revealed the presence of alkaloids, cardiac glycosides, saponins, phenols, and tannins. However, flavonoids, terpenoids, and steroids were either absent or identified in low amounts. These findings reveal that neem leaves contain a variety of bioactive chemicals, including those with potential antibacterial, anti-inflammatory, antiarrhythmic, antiviral, antifungal, cardioprotective, and antioxidant effects, which are consistent with the universal usance of neem in herbal medicine. Further study and clinical trials are needed to better understand the precise health consequences and therapeutic applications of the phytochemicals found in neem leaves.

Recommendations

Base on the findings of the present study, the following recommendation were made:

- Research efforts should continue to examine and identify the whole spectrum of phytochemicals found in *Azadirachta indica* (neem leaves) in order to get a comprehensive grasp of the plant's phytochemistry.
- Future research should concentrate on the possible medicinal applications of neem leaves and their phytochemical constituents.
- In light of the moderate concentrations of cardiac glycosides and alkaloids, it is imperative to conduct in-depth toxicological studies.
- Clinical trials should be initiated to evaluate the therapeutic potential of neem leaves and their phytochemical components in treating specific health conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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