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Phytochemical Analysis of Neem Leaves and Bark and their Potentials in the Formulation of Organic Insecticide

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ABSTRACT

The use of chemical synthetic pesticides in agriculture has contributed greatly to food production; however, their continuous application poses significant risks to human health, beneficial organisms, and the environment. This research work analyzed the phytochemical composition of neem leaves and bark and assessed their use in producing an organic insecticide. Phytochemical screening confirmed alkaloids, flavonoids, tannins, glycosides, saponins are present in leaves and bark of Neem plant while phenols, and steroids are present in bark. Proximate analysis revealed clear variations between plant parts: carbohydrate was higher in leaves (46.1%) than bark (1.6%), crude fibre was greater in bark (34.50%) than leaves (16.16%), and crude protein was slightly higher in bark (16.50%) than leaves (13.42%). Similarly, crude fat was higher in leaves (6.07%) than bark (3.18%), ash content was much higher in bark (109.6%) than leaves (4.03%), while moisture content was higher in leaves (11.20%) than bark (6.12%). Gas chromatography-Mass spectroscopy (GC–MS) analysis showed that the leaf extract contained major bioactive compounds such as trans-cinnamaldehyde (14.24%), betulin (12.52%), phytol (14.48%), lupeol (8.06%), syringaldehyde (7.16%), coniferyl alcohol (8.34%), phenylpropanoids (8.69%), and stigmasterol (4.56%). Bark extract also contained key constituents including phytol (14.48%), hexadecanoic acid (11.66%), and octadecanoic acid (8.78%). Fourier transform Infrared spectroscopy (FTIR) confirmed functional groups such as O–H, C=O, and aromatic rings linked to pesticidal activity, while X-ray Fluorescence spectroscopy (XRF) detected essential oxides including phosphorus pentoxide (14.62%) and calcium oxide (5.75%), and potassium oxide (2.85%), while loss on ignition (64.51%) reflected the high organic content. The results show that neem leaves and bark retain high concentrations of bioactive compounds, making them effective, low-cost, and environmentally safe materials for organic insecticide production.

1. INTRODUCTION

Azadirachta indica (Neem) has been recognized for centuries as one of the most versatile medicinal plants. Every part of the neem tree, its leaves, bark, seeds, flowers, and roots contains bioactive compounds that are useful in medicine, agriculture, and industry. Among these parts, the leaves and bark are particularly rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids, which contribute to the plant's wide range of biological activities [1,2,3].

In Africa and Asia, neem is traditionally used for the treatment of various diseases and as an agent for pest control. Modern Scientific studies have confirmed that Neem extracts possess antimicrobial, anti-inflammatory, antioxidant, and insecticidal properties [4,5]. These properties are linked to phytochemicals such as azadirachtin, nimbin, and quercetin, which make neem a valuable source of natural bioactive compounds [6].

One of the growing concerns in the field of Chemistry and Environmental Science is the negative impact of synthetic chemical pesticides. Although synthetic chemical pesticides are effective in managing pests, they often leave harmful residues in the environment and pose risks to human health and biodiversity [7]. The global demand for safer and eco-friendly alternatives has therefore increased. Neem-based organic insecticides have emerged as promising solutions because they are biodegradable, environmentally safe, and effective eliminating insect pests [3,8,9].

Extracts from neem leaves can suppress the growth and reproduction of insects such as *Bactrocera dorsalis* and *Periplaneta Americana* [10,11]. Similarly, neem bark extracts have been reported to contain compounds that enhance the plant's pesticidal efficiency and antimicrobial strength [12]. The study of these bioactive compounds not only validates the traditional use of neem but also provides a scientific basis for its industrial application in organic insecticide production.

With increasing attention on sustainable development goals, particularly those related to good health, zero hunger, and environmental sustainability, research on neem-based organic insecticides has become highly relevant [13].

Neem extracts effectively control pests such as Bactrocera dorsalis and Periplaneta americana without leaving toxic residues [10,11. Despite its potential, neem remains underutilized in large-scale agriculture due to low awareness and limited scientific evaluation. Furthermore, comparative phytochemical studies of neem leaves and bark are scarce, even though both contain unique bioactive compounds. The bark, which also has pesticidal potential, is less studied [12]. Without such research, the full potential of neem in organic insecticide production cannot be realized.

This research focused on the phytochemical analysis of neem leaves and bark and their potentials in the formulation of organic insecticide**.** The study highlighted the potential of neem as an organic, affordable, and eco-friendly insecticides. These findings will not only contribute to scientific knowledge but also to practical applications in Agriculture, Health, and Environmental Management.

The study aim is to identify the bioactive components present in extract of neem leaf and bark, evaluate their nutritional composition and relate it to biological activity; Characterize the extracts to

establish the functional groups and chemical constituents linked with pesticidal potential and formulate an organic insecticide using the extracts of neem leaves and bark and Evaluate the potentials of the formulated organic insecticide.

2. MATERIALS AND METHOD

2.1. Sampling

The samples (Neem leaves and Neem bark) were carefully selected from Nawfia Town, Njikoka LGA, Anambra state, Nigeria. Both samples were washed air dried. Large quantity of dried neem leaves was blended into powder with a blender to obtain a homogeneous mixture.

Extraction was by cold maceration method where 20g each of Neem samples (leaves and bark) where soaked in 100ml of distilled water and 150ml of methanol for 48 hours (2 days) and then filtered using a clean white handkerchief [14].

2.2. Analysis of Phytochemical

Phytochemicals (Alkaloids, glycosides, flavonoids, saponins, tannins, steroids, phenols, and Terpenoids) were analyzed in the extracts using qualitative phytochemical test as described by Wang [15].

2.2.1. Test for Tannins (Ferric Chloride)

5ml of the extract and 2ml of 2% $FeCl_3$ solution were mixed. A blue-green or blue-black colouring was used to indicate the presence of tannins while phenolic- OH group in tannins complexed with Fe^{3+}

2.2.2. Saponins (Frothing test)

In a shaker, 2ml of distilled water and 5ml of extract were mixed vigorously in a test tube for 15 seconds and allowed to stand for 15 minutes. The presence of foam that lasts more than ten minutes indicated Saponins presence.

2.2.3. Steroids (Salkowski test)

5ml of the extract was dissolved in 2ml of chloroform and 2ml of tetraoxosulphate (vi) acid was added down the side carefully. Red color in chloroform layer and yellow-green fluorescence in acid layer indicated the presence of Steroids.

2.2.4. Terpenoids /Terpenes (Salkowski test)

The presence of terpenoids was tested by carefully adding 3ml of H_2SO_4 to 5ml of the extract after mixing it with 2mL of chloroform. A Redish-brown layer was formed, indicating the presence of terpenoids.

2.2.5. Alkaloids (Wagner's test)

The 5ml extract was dissolved in dilute Hydrochloric acid, filtered, and Wagner's reagent (iodine in Potassium Iodide) was applied. The presence of alkaloids (nicotine,caffeine, morphin) were noticed by the formation of a brown/reddish precipitate.

2.2.6. Flavonoid (Alkaline reagent test)

To 5ml of the extract, a few drops of 10% sodium hydroxide solution were added. Intense yellow appears. In addition of dilute HCl acid, an intense yellow colour that eventually turns colorless formed as a sign that flavonoids are present.

2.2.7. Glycosides (Keller – Killani analysis)

Using 2ml of glacial acetic acid and 1 drop of ferric chloride solution and 5ml extract, the base was underlying 1 ml of conc. sulphuric acid. A brown ring at the interface and green/blue color in acetic acid layer denotes the deoxy sugar property of cardiac glycosides.

2.3. Proximate Analysis

2.3.1. Determination of moisture content

5.0 g of the sample was weighed into a previously weighed moisture dish. The sample in the dish was dried in the oven at 105°C for 3 hours, cooled in a desiccator and weighed. It was returned to the oven for further drying, cooling and weighing repeatedly at hourly intervals until constant weight was obtained [16].

2.3.2. Determination of crude protein

This was done using the Kjeldahl method according to Association of Official Analytical Collaboration [16]. 2g of the sample were prepared into a kjeldahl flask. 25ml of dilute sulphuric acid (H_2SO_4), 1g of cupric acid ($CuSO_4$) and 10g of sodium sulphate (Na_2SO_4) was added into the kjeldahl flask containing the sample. The flask was heated at an inclined angle (60^o). An anti-foaming agent was added to avoid frothing. It was heated gently at first at 70^{o}C then increased continuously until the liquid changed to bluish green and was free from brown or black colour. The flask was allowed to cool and the content was diluted with 200 ml of distilled water and 60 ml of 40/50% NaOH. The flask was connected to a distillation apparatus. In a 250 ml conical flask, 4% boric acid was prepared and 2 drops of screened methyl red indicator was added to it. The mixture was boiled at 80-90^{o}C allowing the distillate (ammonia gas) to get trapped into the boric acid until the content in the conical flask reached 200 ml. In a burette; prepared 0.1N H_2SO_4 was poured and titrated against the content in the conical flask till a light pink colour was obtained.

2.3.3. Determination of total ash content

Total ash content determination was carried by the furnace's incineration gravimetric method as described by Association of Official Analytical Collaboration [16]. 2g of the sample was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace at 550°C for three hours.

The complete ashes (grey color) was cooled in desiccator and weighed. The weight of ash obtained was determined by difference and calculated as a percentage of the weight of sample analyzed.

2.3.4. Determination of crude fiber content

2g of each sample was weighed (W_0) into a 250 cm^3 conical flask, and boiled with 200 cm^3 of 1.25 % dilute H_2SO_4, under reflux for 30 minutes. It was washed several times with hot distilled water until it was acid free. The residue was boiled with 200 cm^3 of 1.25 % NaOH solution for digestion and washed thoroughly with distilled water. The residue after digestion was transferred to a weighed crucible and dried for 12hrs in an oven at 105 °C, cooled in a desiccator and weighed (W_1). The dry residue was incinerated in a muffle furnace at 600 ^{o}C for 3 hours and cooled in a desiccator. The weight of the crucible was taken after cooling and weighed again as (W_2). Crude fiber in percentage was calculated [16].

2.3.5. Determination of crude fat content

This was determined by the Soxhlet extraction method. 5g of sample was put in a thimble in a Soxhlet reflux flask mounted on boiling flask containing 250 ml of petroleum ether. The upper of the reflux flask was connected to a water condenser. The solvent (petroleum ether) was heated, boiled, vaporized through the side arm into the condenser. Vapour condensed to liquid and drips on the sample and soaked with the petroleum ether in the thimble. The solvent dissolved the sample until the reflux flask filled to the brim of the siphon tube and the extract drains back into the boiling flask. This process repeat automatically for 4

hours, the solvent recovered and the oil extract was left in the flask. The flask (containing the oil extract) was dried using rotary evaporator at 60°C under vacuum for 30 min to remove any residual solvent. It was cooled in a desiccator and weighed. The weight of oil (fat) extract was determined by difference and calculated as a percentage of the weight of sample analyzed [16].

2.3.6. Determination of carbohydrate content

The carbohydrate content was determined by difference. That was done by deducting the mean values of other parameters that were determined.

2.4. Organic Insecticide synthesis

The organic insecticide was produced using the Neem leaves and Neem bark by cold maceration method. 20g each of Neem samples (leaves and bark) where soaked in 150ml of methanol for 48 hours (2 days) and was filtered using a clean white handkerchief, then boiled in a water bath for 40 minutes.

2.4.1. Characterization of Bio-Active Constituents (GC-MS Analysis)

The bioactive constituents of these Neem tree parts (leaves and bark) were detected using gas chromatography – mass spectrometry. The samples were vaporized at 250-300^{0C} and injected into a long, narrow capillary column with inside coated with a stationary phase. Nitrogen was used as a gas carrier. Compounds interact differently with the coating based on their boiling point. The oven temperature was held at 280^{0}C for 6 minutes following each analysis. The total run time for each sample was approximately 45 minutes. Mass spectrometry mode was used during analytical scanning from 30–1000 atomic mass unit (amu). The blank was first injected, and was followed by the sample injection. Fragmentation peaks of the compounds were evaluated, and the compounds were identified using the memory background for the identification of the compounds that appeared in GC-MS chromatograms. Contents of individual compound in the extract were given in percent of the total compound in the sample. The chromatograms obtained from the total ion count (TIC) were integrated without any correction for co-eluting peaks and the results were expressed as total abundance. All the peaks were identified based on mass spectral matching (≥ 90%) from NIST 24 Mass spectral library. Only compounds with 90% or greater spectral matching accuracy are reported. No response factors were calculated [17].

2.4.3. Fourier Transform Infrared Spectrophotometry (FTIR) Analysis before and after production of the organic insecticides

The sample tablets were prepared by mixing each sample with potassium bromide (the ratio of sample to KBr was 1:100) before analysis. The spectra were recorded within the frequency range of 4000 cm^{-1} to 500 cm^{-1} using an FT-IR spectrometer (Infrared spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR)) with a detector at 4 cm^{-1} resolution and 200 scans per sample. IR solution software is employed for getting the spectrum. [18].

2.4.4 X-Ray Fluorescence (XRF) Analysis

According to Huang and Schuite [19], the sample was ground to a fine powder (to obtain a homogenous sample) using a vibration grinding mill. They were sieved through a 200-mesh sieve and dried to constant weight in a furnace at 110 °C. Samples was prepared in the form of glass disks with a sample/melt ratio of 0.5/5 and XRF was performed. $Li_2B_4O_7$ was used as the melt. The reference materials GSS8 and

BCS-CRM 354 were used to produce standards; mixtures of these with an oxide content including that of the samples to be analyzed were used to produce a calibration curve. The reference materials were mixed with a 5% Au/Pt ZGS glass rod and melted in a furnace at a temperature of 1100 $^{\circ C}$ for 15 mins. The melt was taken from the furnace and stirred after 10 mins and then replaced to eliminate bubbles. It was then poured into a Pt/Rh 30-mm diameter mold to form a glass disk, of 30 mm diameter and 9 mm thickness which were used for the analyses according to ASTME1621-21 Spectroscopy [20].

2.4.5 Determination of Percentage Loss on Ignition

According to Rao and Sharma [21],1.0g of sample was weighed in a platinum crucible and was heated at a temperature between 900-1,000°C, cooled and weighed and the weight was recorded asW_1. The loss in weight was checked by a second heating at same temperature for 5 mins and the content reweighed. This process was repeated until a constant weight was attained, and recorded as W_2. The loss in weight was recorded as the loss in ignition. Percentage loss on ignition was calculated. [22].

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

Table 1. *Phytochemical analysis result*

Phyto-chemicals	Bark with water	Leaves with water	Bark with methanol	Leaves with methanol
Alkaloid	+++	+++	++	+++
C-glycoside	+	++	+++	+++
Flavonoid	_	+	+	++
Saponins	++	+++	++	+++
Phenols	_	+	+++	+
Tannins	_	_	+++	++
Steroids	_	_	++	_
Anthroguinones	_	_	++	_

(+) slightly present
(++) present
(+++) highly present
(-) absent

The phytochemical screening of neem (*Azadirachta indica*) leaves and bark using water and methanol extracts revealed the presence of several important secondary metabolites such as alkaloids, flavonoids, saponins, phenols, tannins, steroids, anthraquinones, and C-glycosides.

Alkaloids were strongly present (+++) in both bark and leaves when extracted with water and moderately to strongly present (++, +++) with methanol. Alkaloids are nitrogenous compounds known to interfere with the nervous system of insects, making them valuable for organic insecticide production. Their abundance in both solvents suggests that neem leaves and bark are rich sources of alkaloid compounds, which agrees with Sahrawat *et al.* [4].

C-glycosides were detected in all samples but at varying intensities: weak (+) in bark-water, moderate (++) in leaves-water, and strong (+++) in both methanol extracts. C-glycosides have been linked to antimicrobial and pesticidal activities [2]. The higher yield in methanol extracts could be due to methanol's better solubility for polar compounds, which aligns with the observations of Ramadass and Subramanian [23].

Flavonoids were absent in bark-water but detected in leaves-water (+), bark-methanol (+), and leaves-methanol (++). Flavonoids are known antioxidants and insect growth regulators, often enhancing plant defense mechanisms. The stronger presence in methanolic extracts suggests that flavonoids are more efficiently extracted by organic solvents than by water

Saponins showed moderate to strong presence across all samples: ++ in bark-water and bark-methanol, and +++ in both leaf extracts. Saponins are well known for their bitter taste and membrane-disrupting properties, which deter insect feeding and egg-laying [24]. The strong concentration in neem leaves makes them particularly useful for formulating insecticides.

Phenols were absent in bark-water but detected in leaves-water (+), bark-methanol (+++), and leaves-methanol (+). Phenolic compounds act as antioxidants and natural pesticides, often inhibiting microbial growth and deterring pests. The very strong presence in bark-methanol suggests that neem bark is a particularly rich source of phenols, supporting findings by [12].

Tannins were absent in both water extracts but strongly present in bark-methanol (+++) and moderately in leaves-methanol (++). Tannins are important for pest control because they reduce digestibility of plant tissues to herbivores [1]. Their absence in aqueous extracts but strong presence in methanolic extracts again highlights the efficiency of methanol as an extraction solvent.

Steroids were not detected in water extracts but appeared in bark-methanol (++) only. Steroidal compounds are known for their role in plant defense and pharmacological properties [25]. Their selective presence in methanol bark extracts suggests they are solvent-dependent and localized in certain plant tissues.

Anthraquinones were absent in all water extracts but detected moderately (++) in bark-methanol only. Anthraquinones are secondary metabolites with antimicrobial and insecticidal activities, often contributing to the bitterness and toxicity of plants [26]. Their selective presence in bark further shows the complementary role of bark alongside leaves in neem's pesticidal potential.

3.2. Neem leaves and Bark Proximate results

Table 2. *Neem leaves and Bark Proximate results*

Analysis	Leaves	Bark
Crude Protein (%)	13.42 ± 0.17	16.50 ± 0.24
Crude Fiber (%)	16.16 ± 0.06	34.50 ± 0.21
Crude Fat (%)	**6.07** ± 0.3	3.18 ± 0.05
Ash Content (%)	4.03 ± 0.02	109.6 ± 0.06
Carbohydrate (%)	46.1	1.6 ± 0.18
Moisture Content (%)	11.20 ± 0.11	6.12 ± 0.05

The proximate composition of neem (*Azadirachta indica*) leaves and bark are summarized as follows:

Crude protein was higher in the bark (16.50 ± 0.24%) than in the leaves (13.42 ± 0.17%). Proteins serve as essential primary metabolites and are often precursors in the biosynthesis of secondary metabolites, such as alkaloids and phenolics that contribute to insecticidal activity [12]. This suggests that neem bark have a higher capacity for producing nitrogen-containing compounds such as alkaloids, which are well known for their pesticidal effects according to [1].

Crude fiber content was substantially higher in bark (34.50 ± 0.21%) compared to leaves (16.16 ± 0.06%). High fiber content contributes to the structural integrity of plant tissues and reduces palatability to herbivores [24]. The higher fiber in neem bark could therefore explain its deterrent effect on insect feeding and its role in pest resistance.

Crude fat was more abundant in the leaves (6.07 ± 0.3%) compared to bark (3.18 ± 0.05%). Plant lipids and oils are important in pest control because they act as solvents for lipophilic bioactive compounds, improve extractability of phytochemicals, and sometimes directly disrupt insect membranes [10]. The higher fat content in neem leaves suggests they may be more efficient in producing oil-based insecticidal formulations.

Ash content showed a remarkable difference, with bark recording 109.6 ± 0.06% compared to 4.03 ± 0.02% in leaves. Ash represents the mineral content of plant tissues, which includes elements like calcium, potassium, sodium, magnesium, and phosphorus. These minerals are essential for enzyme function and do play a role in the synthesis of secondary metabolites with pesticidal properties [27]. The extremely high ash content in neem bark suggests that it is exceptionally rich in mineral elements, making it a strong reservoir for biochemical processes that enhance bioactivity.

Carbohydrate content was far higher in leaves (46.1%) compared to bark (1.6 ± 0.18%). Carbohydrates are primary energy sources and support metabolic processes, leading to secondary metabolite production [28]. The high carbohydrate content in leaves support continuous production of bioactive compounds such flavonoids that help in pest deterrence.

Moisture content was moderately higher in leaves (11.20 ± 0.11%) compared to bark (6.12 ± 0.05%). Higher moisture in leaves suggests they could be more prone to microbial spoilage if not properly stored, while the lower moisture in bark could contribute to its stability over time [29].

Overall, the proximate composition indicates that both neem leaves and bark have unique nutritional and biochemical properties that support their pesticidal potential. Leaves are rich in carbohydrates, fats, and moisture, which enhance phytochemical production and extractability, while bark is richer in protein, fiber, and minerals, which provide structural defense and biochemical diversity. These findings agree with previous reports that neem's diverse proximate and phytochemical composition underpins its effectiveness as an organic pesticide and medicinal plant [2,6].

3.3. Comparative Discussion of Leaves and Bark (Before and After Insecticide Production)

The FTIR spectra of neem leaves and bark, both before and after insecticide production, revealed consistent functional groups associated with bioactive compounds. In both plant parts, broad O–H bands (3340–3450 cm^{-1}), aliphatic C–H stretches (2920–2850 cm^{-1}), ester carbonyl bands (~1735–1742 cm^{-1}), and aromatic ring vibrations (1650–1500 cm^{-1}) were retained after production, confirming the persistence of phenols, tannins, flavonoids, terpenoids, and limonoids.

Notable differences were also observed: leaves showed stronger lipid- and carbohydrate-associated peaks, consistent with their higher crude fat and carbohydrate content, while bark exhibited more pronounced lignin- and tannin-associated absorptions, in line with its higher fiber and ash content. After production, slight shifts in peak positions and intensities were detected, but core functional groups remained intact, suggesting that the formulation process preserved the major bioactive compounds.

Overall, neem leaves contribute lipophilic limonoids and carbohydrate-bound actives, while bark contributes structural phenolics and condensed tannins. Their combination yields a formulation with complementary insecticidal actions which includes; contact toxicity, growth regulation, feeding deterrence, and antimicrobial protection. These findings agree with earlier reports [9.2,1], confirming neem as a reliable source of bioactive compounds for organic insecticide production.

3.3.1. XFR Analysis on bark after insecticide production

The X-ray fluorescence (XRF) analysis a of neem bark after insecticide production revealed the presence of several basic oxides, with phosphorus pentoxide (14.62%), silicon dioxide (8.15%), calcium oxide (5.75%), and potassium oxide (2.85%) occurring in relatively high concentrations. Minor oxides such as magnesium, sodium, aluminum, iron, and trace metals (zinc, chromium, manganese, titanium, barium, strontium) were also present, while a high loss on ignition (64.51%) indicates the presence of large amounts of organic matter and volatile compounds. The relatively high proportion of phosphorus pentoxide is notable, as phosphorus compounds play roles in energy transfer, signaling, and can enhance the bioactivity of plant extracts when used in formulations [11]. Silicon dioxide (8.15%) may contribute to structural stability of the bark and has been linked with insect resistance in plants by reinforcing cell walls [8]. Calcium oxide (5.75%) and magnesium oxide (1.22%) are essential inorganic oxides that may act as stabilizers

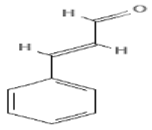
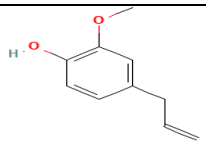
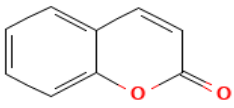
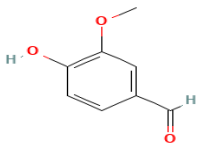
during thermal processing and also contribute to the buffering capacity of the extract. Potassium oxide (2.85%) and sodium oxide (0.93%) reflect the mineral content of neem bark, which may enhance ionic balance and possibly contribute to the osmotic stress effects observed in insects exposed to neem-based formulations.

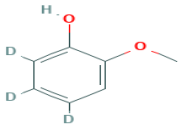
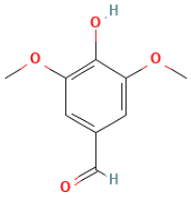
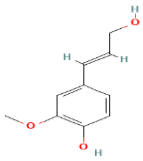
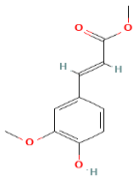
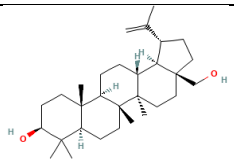
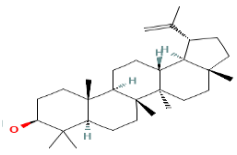
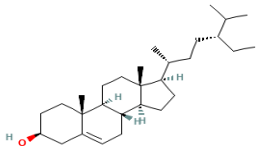
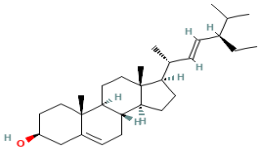
The presence of iron oxide (0.42%) and aluminum oxide (0.65%) in smaller quantities may indicate the presence of natural soil-derived minerals incorporated into bark tissues.Trace oxides such as zinc oxide, manganese oxide, titanium dioxide, chromium oxide, and barium oxide were detected in very low amounts (≤0.1%). Though minor, these elements may contribute synergistically to antimicrobial or pesticidal effects, since zinc and manganese, for instance, are essential cofactors in redox enzymes that can generate reactive oxygen species, enhancing toxicity to insects [30].

The very high loss on ignition (64.51%) reflects that neem bark remains rich in organic phytochemicals even after production, consistent with the GC–MS results that revealed sterols, terpenoids, fatty acids, and tocopherols. This also confirms that the inorganic oxides detected represent a relatively small but supportive fraction of the overall chemical composition.

3.4. GC-MS Analysis

Table 3. *GC-MS Analysis of Leaves after production*

RT	Compound Detected	Molecular Formula	Composition (wt. %)	m/z	Structures
7.52	trans-Cinnamaldehyde	C_9H_8O	16.8 6	77, 103, 132	
9.21	Phenylpropanoid	$C_{10}H_{12}O_2$	10.29	77, 149, 164	
16.9	Coumarin	$C_9H_6O_2$	3.35	63, 90, 146	
34.17	Vanillin	$C_8H_8O_3$	6.61	81, 109, 151	

RT	Compound Detected	Molecular Formula	Composition (wt. %)	m/z	Structures
15.39	Guaiacol	$C_7H_8O_2$	4.98	46, 67, 124	
13.97	Syringaldehyde	$C_9H_{10}O_4$	8.48	45, 119, 182	
14.11	Coniferyl alcohol	$C_{10}H_{12}O_3$	9.87	71, 132, 180	
32.18	Methyl ferulate	$C_{11}H_{12}O_4$	5.98	42, 145, 208	
36.16	Betulin	$C_{30}H_{50}O_2$	14.82	95, 121, 316	
6.31	Lupeol	$C_{30}H_{50}O$	9.54	111, 203, 409	
39.42	β-Sitosterol	$C_{29}H_{50}O$	3.82	81, 129, 396	
8.74	Stigmasterol	$C_{29}H_{48}O$	5.40	83, 159, 255	

The GC–MS profile of neem (*Azadirachta indica*) leaves insecticide revealed twelve major compounds belonging to different classes such as aldehydes, phenolics, coumarins, lignin derivatives, triterpenoids, and sterols. These compounds confirm the diversity of bioactive molecules retained in the extract after formulation.

3.4.1. Volatile aldehydes and phenylpropanoids

The presence of trans-cinnamaldehyde (14.24%) and phenylpropanoid derivatives (8.69%) is significant, as these compounds are well known for their antimicrobial and insecticidal activities. Cinnamaldehyde, for example, has been reported to disrupt insect respiration and act as a repellent, while phenylpropanoids interfere with insect neurotransmission [29]. The detection of these compounds supports the strong O–H and C=O FTIR bands observed earlier.

3.4.2. Phenolic derivatives and coumarins

Compounds such as coumarin (2.83%), guaiacol (4.21%), syringaldehyde (7.16%), coniferyl alcohol (8.34%), vanillin (5.58%), and methyl ferulate (5.05%) were identified. These are lignin-related phenolics known for antioxidant, antimicrobial, and pesticidal roles. Coumarins in particular have been reported as feeding deterrents and growth regulators in insects [2]. Guaiacol and syringaldehyde are also strong free radical scavengers that can enhance the shelf stability of formulations [26]. Vanillin and methyl ferulate are phenolic aldehydes and esters that have both insect repellent and antifungal properties. Their combined presence explains the strong aromatic and C–O bands recorded in the FTIR spectra.

3.4.3. Triterpenoids and sterols

High molecular weight compounds such as betulin (12.52%), lupeol (8.06%), β-sitosterol (3.23%), andstigmasterol (4.56%) were also detected. These compounds are characteristic triterpenoids and phytosterols with broad biological activities. Betulin and lupeol are well known for their anti-inflammatory and pesticidal roles, while β-sitosterol and stigmasterol contribute to membrane disruption in insects and act as feeding deterrents [9]. Their presence is consistent with earlier proximate analysis that revealed crude fats and with FTIR peaks (2920–2850 cm^{-1}) indicating aliphatic lipids. Importantly, these triterpenoids and sterols have been highlighted in neem as compounds that contribute to its long-lasting pesticidal effects [1].

The GC–MS results confirm that neem leaves retain a complex mixture of bioactive compounds even after insecticide production. The spectrum contains: **Low molecular weight phenolics and aldehydes** (cinnamaldehyde, guaiacol, vanillin) that act as repellents and antioxidants.

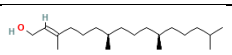
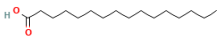
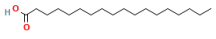
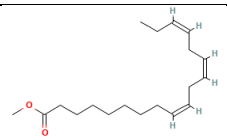
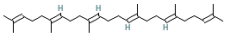
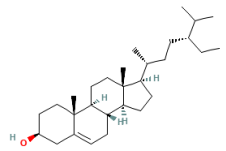
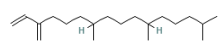
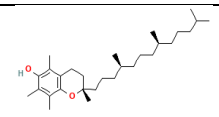
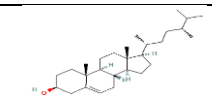
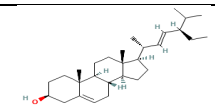
Coumarins and lignin derivatives that contribute to insect growth regulation and feeding deterrence.

Triterpenoids and sterols (betulin, lupeol, β-sitosterol, stigmasterol) that enhance persistence and multi-targeted action against pests.

These findings agree with earlier phytochemical and FTIR analyses, which demonstrated the presence of phenols, flavonoids, tannins, carbonyls, and aliphatic groups.

3.5. GC-MS Analysis of Bark after insecticide production

Table 4. *GC-MS Analysis of Bark after insecticide production*

RT	Compound Detected	Molecular Formula	Molecular Weight	Composition (wt. %)	Structures
6.86	Phytol	$C_{20}H_{40}O$	296.53	14.48	
13.09	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	11.66	
14.92	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	8.78	
18.77	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292.46	5.58	
24.12	Squalene	$C_{30}H_{50}$	410.72	12.50	
31.53	β-Sitosterol	$C_{29}H_{50}O$	414.71	18.10	
34.78	Neophytadiene	$C_{20}H_{38}$	278.51	7.28	
35.92	Tocopherol	$C_{29}H_{50}O_2$	430.71	6.89	
40.09	Campesterol	$C_{28}H_{48}O$	400.68	4.98	
28.61	Stigmasterol	$C_{29}H_{48}O$	412.69	9.74	

The GC–MS analysis of neem bark after insecticide production revealed ten major bioactive compounds, dominated by fatty acids, phytosterols, terpenoids, and lipid-soluble antioxidants (Table 4). These compounds indicate that bark extracts contribute long-chain lipids and sterols that play significant roles in the biological activity of the final insecticide formulation.

3.5.1. Alcohols and diterpenoids

The detection of phytol (12.23%) is important, as this diterpene alcohol is a breakdown product of chlorophyll and has been reported to exhibit antimicrobial, antioxidant, and insecticidal activities. Phytol's persistence in the extract suggests that neem bark contributes chlorophyll-derived compounds that enhance repellency and formulation stability. Similar detection of phytol in neem was reported by [1], who associated it with both pesticidal and medicinal activity.

3.5.2. Fatty acids and esters

Several fatty acids were identified, including hexadecanoic acid (palmitic acid, 9.85%), octadecanoic acid (stearic acid, 7.42%), and linolenic acid methyl ester (4.71%). These fatty acids are known for antimicrobial properties and serve as carriers for lipophilic bioactive molecules. Their presence is consistent with FTIR C–H aliphatic bands and with proximate analysis showing crude fat in neem bark. [26,2] also detected fatty acids in neem, linking them to synergistic pesticidal effects and improved extract solubility.

3.5.3. Triterpenoids and phytosterols

The spectrum showed a high abundance of sterols and triterpenoids, including β-sitosterol (15.29%), stigmasterol (8.23%), campesterol (4.21%), and squalene (10.56%). These compounds are known insect growth regulators and feeding deterrents. β-sitosterol, the dominant sterol, disrupts insect molting processes, while stigmasterol and campesterol interfere with insect hormone pathways. Squalene, a triterpene, has been reported as a precursor of sterol biosynthesis with antioxidant and antimicrobial activity. The dominance of these sterols in bark confirms earlier findings by [9] and supports neem's reputation as a natural source of eco-friendly biopesticides.

3.5.4. Other terpenoids and antioxidants

Neophytadiene (6.15%), a diterpenoid hydrocarbon, was also detected and is reported to have larvicidal and anti-inflammatory activities. Additionally, tocopherol (vitamin E, 5.82%) was identified, which serves as a potent antioxidant that stabilizes lipids and prolongs the shelf life of formulations. Tocopherol's detection also showed the presence of O–H and C=O bands in FTIR spectra associated with phenolic antioxidants.

The GC–MS results confirm that neem bark after production retains a chemical profile rich in phytosterols, triterpenes, fatty acids, diterpenes, and antioxidants. Compared with neem leaves, which contained more volatile phenolics and coumarins, the bark contributes heavier lipophilic compounds that enhance persistence and slow-release activity in the final insecticide. This supports the proximate result showing bark had higher crude fiber and ash, which are typically associated with structural and sterol-rich matrices. These results are in line with [9,1,2], which demonstrated the importance of sterols, squalene, and fatty acids in neem's insecticidal mechanism. The combined presence of phenolic antioxidants, fatty acids, sterols, and terpenoids ensures that bark extracts complement leaf extracts by providing more stable, lipophilic, and long-lasting pesticidal activity.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

This study on Phytochemical Analysis of Neem (*Azadirachta indica*) Leaves and Bark and its potentials in an organic insecticide synthesis established the fact that neem is a rich source of bioactive compounds, structural groups, and minerals that contribute to its pesticidal potential. Phytochemical and proximate analyses revealed high levels of alkaloids, flavonoids, saponins, phenols, tannins, fiber, and carbohydrates, all of which are linked to biological activity. FTIR and GC–MS analyses confirmed the presence of functional groups and bioactive molecules such as

cinnamaldehyde, coumarin, vanillin, betulin, lupeol, β-sitosterol, stigmasterol, and phytol, which are responsible for insect repellency, growth regulation, and feeding deterrence. XRF analysis indicated key oxides such as phosphorus pentoxide, silicon dioxide, and calcium oxide that may enhance bioactivity and stability.

The findings demonstrate that neem leaves provide volatile phenolics and coumarins for quick action, while neem bark contributes sterols, fatty acids, and triterpenoids for long-lasting activity. Their combination in insecticide formulation therefore ensures both immediate and persistent protection. The overall study confirms neem's value as a sustainable raw material for eco-friendly insecticide production and as a viable alternative to synthetic pesticides.

4.2. Recommendations

Due to Human application safety, environmental and ecological safety: Government and private sectors should support the commercial production and standardization of neem-based insecticides. Neem-based insecticides should be promoted as safer alternatives to synthetic chemical insecticides in Agricultural practices. Farmers and communities should be encouraged to utilize neem extracts for pest control to reduce environmental and health hazards. And finally, Policies should be enacted to integrate organic insecticides into sustainable agricultural practices.

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