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PHYSICOCHEMICAL AND ANTIBACTERIAL ANALYSIS OF NEEM (*Azadirachta indica*) SEED OIL

Yusuf, M. O. and Ibrahim, N. D.

Department of Biochemistry, Faculty of Science, Federal University Gusau

Corresponding Author's Email & Phone no.: yusufomowumimariam@gmail.com, 09132557856

ABSTRACT

This study investigated the physicochemical properties and antibacterial activity of neem (Azadirachta indica) seed oil extracted from Sabongida, Gusau, Zamfara State, Nigeria. The physicochemical analysis revealed a yield of 35.2%, with the oil exhibiting a yellow color, slightly acidic pH (5.5), moderate viscosity (2.2 cP), and favorable stability indicators such as low acid value (1.91 mg KOH/g) and peroxide value (2.6 mEq/kg). These properties align with established standards for quality plant oils, supporting its suitability for medicinal and industrial applications. The antibacterial evaluation, conducted using the agar well diffusion method, demonstrated concentration-dependent activity against Salmonella spp. (9 mm inhibition zone at 100%) and weaker effects against Escherichia coli (5 mm at 100%). Minimum Inhibitory Concentration (MIC) tests confirmed bacteriostatic effects at 100% concentration for Salmonella, though no bactericidal activity was observed even at full strength. These findings highlight neem seed oil's potential as a natural antimicrobial agent, particularly against enteric pathogens, while underscoring the need for formulation enhancements to improve efficacy. The study concludes that neem seed oil from this region possesses valuable bioactive properties, validating its traditional use and suggesting its potential in addressing antibiotic resistance. Recommendations include advanced formulation strategies, broader microbial testing, and community education to promote sustainable utilization.

Keywords: *Azadirachta indica*, neem seed oil, antibacterial activity, *Salmonella*, *Escherichia coli*.

1.0 INTRODUCTION

1.1 Background of the Study

Neem tree is known as *Azadirachta indica* belonging to the mahogany family called Meliaceae (Jessinta *et al.,* 2014), It is one of the two species in the genus Azadirachta that is native to the Indian subcontinent. It is typically grown in tropical and semi-tropical regions (Dua *et al.,* 2009). This plant has its origin in India but it is currently and widely distributed in the tropics, subtropics, semi-arid and wet-tropical regions of the world (Dua *et al.,* 2009). Interestingly, the barks, leaves, seeds, roots, stems, flowers and fruits of A. indica possess biologically active substances ranging from alkaloids, tannins, peptides, phenols, sterols, glycosides and flavonoids which enhanced in combating against some bacterial infections. Among all the parts of A. indica, the seeds are documented as one of the most major sources of pharmaceuticals in antibacterial property as the oil has broad spectrum against antibacterial infections (El-Mahmood *et al.,* 2010).

However, the seeds are mostly available in Nigeria, plenty of the seeds generated by the neem tree are presently unattended to and massively underutilized (Erakhrumen, 2011). Fats and oils are nutritionally vital as they are classified as one of the four major categories of foods. Oils are employed in different ways. Some are mostly utilized

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for food baking, texturing and also used industrially in the production of detergent, soaps, oil paints and cosmetics. In plants, oils are mostly produced in the endosperm along with the carbohydrates where they jointly nourish the embryo (Oyeyiola, 1993). Fatty acids are not found in a free state in nature but exist commonly in combination with glycerol (an alcohol) in the form of triglyceride (Britannica, 2020). The 16- and 18-carbon fatty acids, equally known as stearic acid and palmitic acid are amongst most extensively disseminated fatty acids in nature. It has been established that both palmitic and stearic acids are found in the lipids of the majority of organisms. For instance, the body fat of animals is made up of 30% palmitic acid. Fatty acids equally contribute to 5 to 50% lipids found in vegetable fats and most abundantly found in palm oil. Stearic acid is copious in some vegetable oils like cocoa butter and shea butter thereby making up for a comparatively high percentage of the lipids found in ruminant tallow (Britannica, 2020). Commercially, fatty acids have got an extensive range of applications. For instance, they are used not only in the manufacture of plentiful food merchandises but equally in the production of soaps, cosmetics and detergents. Soaps are known as the sodium and potassium salts derivable from fatty acids. According to (Britannica, 2020), some products meant for skin-care have fatty acids which can ultimately help retain healthy skin look and function. Fatty acids, principally omega-3 fatty acids are also usually sold as dietary supplements (Britannica, 2020), The oil generated is a vegetable oil obtained from the seed kernels, which is an evergreen of the tropics and sub-tropics. It is deep yellow in colour with garlic–like odor. It is one of the important commercially available products used for organic medicines and farming (El-Mahmood *et al.,* 2010). One of the studied triterpenoid in neem oil is Azadirachtin.

However, nimbin is another triterpenoid with some antimicrobial properties (Kraus, 1995). The complex molecular structure of Azadirachtin possesses both secondary and tertiary hydroxyl groups and a tetrahydrofuran ether in its molecular structure coupled with 16 stereo genic has a, 7 of which are tetrasubstituted. These characteristics can be attributed to the serious difficulty that is normally met in its preparation from simple precursors through the employment of synthetic organic chemistry methods (Veitch *et al.,* 2007). The chemical formula of Azadirachti is $C_{35}H_{44}O_{16}$ with molar mass of 720.721 g·mol−1. Neem seed oil has wide applications ranging from biodiesel, which is biodegradable, a clean, renewable biofuel and non-toxic, can be produced by transesterification of seed oils. It is used in the manufacture of pesticides as it works by interfering with the reproductive cycle of target insects (Veitch *et al.,* 2007). Neem oil equally have a powerful aromatic odour that chases insects away (Awasthi and Shikha, 2019). Neem has excellent effect on chronic skin conditions that often fail to respond to medical drugs, psoriasis, eczema, and ringworm, even stubborn warts are among the conditions that can clear up easily. In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin (Shujit *et al.,* 2013). Neem has proven successful in treating stomach ulcers. Its antihistamine and antibacterial compounds can reduce inflammation and destroy ulcer causing bacteria (Shujit *et al.,* 2013). Neem has been proven effective for its ability to reduce blood clots, blood pressure, heart irregularities and cholesterol levels. Since the antihistamine properties of nimbidin found in neem leaves have been found to cause blood vessel to dilate, it confirmed

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the reason of its capability to reduce high blood pressure. Recent research showed that neem lowered high cholesterol values in only one month (Shujit *et al.,* 2013). Previous work has been done on the neem seed- determination of the fatty acid composition, the physicochemical properties has also been reported in literature.

1.2 Statement of the Research Problem

Neem (*Azadirachta indica*) seed oil is widely recognized for its diverse biological activities, including its antibacterial properties, attributed to its rich composition of bioactive compounds. However, variations in its physicochemical properties and antibacterial efficacy due to geographical, environmental, and extraction factors remain underexplored.

Despite its potential, there is limited comprehensive scientific evidence on the specific physicochemical characteristics and their correlation with the oil's antibacterial activity against pathogenic microorganisms. This knowledge gap hinders its optimized utilization in pharmaceutical, cosmetic, and agricultural industries.

The problem, therefore, lies in the lack of detailed physicochemical profiling and antibacterial evaluation of neem seed oil, which is essential to standardize its quality and assess its practical applications. This research seeks to address this gap by systematically analyzing the physicochemical properties and antibacterial potential of neem seed oil, thereby contributing to its effective application in various fields.

1.3 Justification of the Study

The increasing global burden of infectious diseases and the alarming rise in antibiotic-resistant pathogens have intensified the search for alternative therapeutic agents, particularly from natural sources. Neem (*Azadirachta indica*) is a medicinal plant widely recognized for its diverse pharmacological properties, especially in traditional medicine across Africa and Asia. Among its numerous bioactive components, neem seed oil is particularly valued for its antibacterial, antifungal, anti-inflammatory, and antioxidant activities. However, despite its widespread traditional use, there is limited scientific data, particularly in the Nigerian context, validating its physicochemical quality and antibacterial efficacy.

This study is justified by the need to provide scientific evidence supporting the medicinal use of neem seed oil, especially in rural communities such as Sabongida in Gusau, Zamfara State, where reliance on traditional remedies is prevalent due to limited access to modern healthcare. Evaluating the physicochemical parameters (such as pH, viscosity, iodine value, acid value, saponification value, and peroxide value) will help determine the stability, purity, and suitability of the oil for medicinal and industrial applications. These parameters are essential for quality control, formulation, and potential commercialization of neem-based products.

Additionally, assessing the antibacterial activity of neem seed oil against common bacterial pathogens such as *Escherichia coli* and *Salmonella* is crucial in the current era of increasing antimicrobial resistance. Demonstrating that neem oil possesses effective inhibitory properties at specific concentrations could provide a low-cost, accessible alternative to synthetic antibiotics, especially in resource-limited settings.

Furthermore, the findings from this study could contribute to the development of standardized protocols for neem oil extraction and application, encourage the cultivation and sustainable use of neem

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plants, and support the integration of herbal remedies into primary healthcare systems. Overall, this research aims to bridge the gap between traditional knowledge and scientific validation, promoting safer and more effective use of neem seed oil as a natural antibacterial agent.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the Study

The study aims to investigate the physicochemical properties and antibacterial activity of neem (*Azadirachta indica*) seed oil.

1.4.2 Objectives of the Study

1. To analyze the physicochemical properties of neem seed oil, such as acid value, saponification value, iodine value, and fatty acid composition.
2. To evaluate the antibacterial activity of neem seed oil against selected bacteria.

3.0 MATERIALS AND METHODS

3.1 Materials

Table 3.1: Equipment/ Apparatus used

Equipment	Manufacturer
Soxhlet Extractor	Borosil, Buchi, LabTech, Glas-Col
Digital pH Meter	Hanna Instruments, Mettler Toledo, Thermo Fisher Scientific, Hach
Incubator	Memmert, Thermo Fisher Scientific, Binder, Lab-Line
Autoclave	Astell Scientific, Systec, Tuttnauer, Yamato Scientific
Spectrophotometer	Shimadzu, Thermo Fisher Scientific, Agilent Technologies, Jenway

Table 3.2: Chemical/ Reagents Used

Reagent	Manufacturer
Solvent (n-Hexane)	Merck (Germany), Sigma-Aldrich, Fisher Scientific, BDH Chemicals, Loba Chemie
Chemicals for Bacterial Cultures	HiMedia Laboratories, Oxoid (Thermo Fisher), Merck, Bio-Rad, Sigma-Aldrich
Agar Media (e.g., Nutrient Agar, MacConkey Agar, etc.)	HiMedia Laboratories, Oxoid (Thermo Fisher), Merck, Neogen, BD (Becton Dickinson)

3.2 Study Area

This study was conducted in Sabongida area Federal University Gusau, Zamfara State, Nigeria. Sabongida is a semi-urban area known for its agricultural and herbal resources, including neem trees (*Azadirachta indica*), which grow abundantly in the region.

3.3 Sample Collection and Preparation

Neem seeds were collected from mature neem trees within Sabongida, Gusau. The seeds were washed, sun-dried for five days to reduce moisture content, and manually dehulled. The cleaned seeds were ground into a fine powder using a mechanical grinder. Method For Extraction of Neem Oil

100g of Neem powder was placed into the thimble and place in the soxhlet chamber. 500ml of selected solvents were placed in a round bottom flask and assembled for soxhlet extractor then the distillation process was begun. After completed the extraction process, the solvent and extractor were placed on water bath to evaporate the solvent.

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Then extracted neem oil was weighed by using the following formula

% oil yield = $\frac{W_1 - W_2}{W_1} \times 100$

Where:

W_1-sample weight initially placed in the thimble

W_2-sample weight after dried in the oven (Natarajan *et al.,* 2003).

3.4 Physicochemical Analysis of Neem Seed Oil

The physicochemical properties of neem seed oil were determined using standard procedures described by the Association of Official Analytical Chemists (AOAC, 2019).

3.4.1 Moisture Content

The moisture content was determined by drying a known weight of the oil at 105°C in an oven until a constant weight was achieved.

Moisture Content

$= \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$

3.4.2 pH Determination

The pH of neem seed oil was measured using a digital pH meter after dissolving 10 g of oil in 100 mL of distilled water.

3.4.3 Specific Gravity

The specific gravity was measured using a pycnometer by comparing the weight of neem seed oil to an equal volume of distilled water.

3.4.4 Acid Value

The acid value was determined by titrating a known weight of neem oil dissolved in ethanol with potassium hydroxide (KOH) using phenolphthalein as an indicator.

3.4.5 Saponification Value

This was determined by refluxing a known weight of neem seed oil with ethanolic KOH and titrating the excess alkali with hydrochloric acid.

3.4.6 Peroxide Value

The peroxide value was determined by titrating the oil with sodium thiosulfate solution in the presence of potassium iodide and acetic acid.

3.4.7 Iodine Value

The iodine value was measured using the Wijs method to determine the degree of unsaturation in the oil.

3.5 Antibacterial Analysis

The antibacterial activity of neem seed oil was evaluated against selected bacterial strains using the agar well diffusion method.

3.5.1 Test Microorganisms

The bacterial strains used in this study included *Escherichia coli* and *Salmonella typhi*. These strains were obtained from the Microbiology Laboratory of Federal University Gusau.

3.5.2 Preparation of Bacterial Cultures

The bacterial strains were grown in nutrient broth and incubated at 37°C for 24 hours before being used for susceptibility testing.

3.5.3 Agar Well Diffusion Method

Mueller-Hinton agar plates were inoculated with bacterial suspensions standardized to 0.5 McFarland turbidity. Wells (6 mm in diameter) were punched into the agar using a sterile cork borer, and neem seed oil at different concentrations (25%, 50%, 75%, and 100%) was introduced into the wells. The plates were incubated at 37°C for 24 hours, and the diameters of the inhibition zones were measured.

3.5.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was determined using the broth dilution method by preparing serial dilutions of neem seed oil in nutrient broth and observing the lowest concentration that inhibited bacterial growth. MBC was determined by subculturing the MIC tubes on nutrient agar and checking for bacterial growth after 24 hours.

3.6 Statistical Analysis

The data obtained were analyzed using SPSS software. Results were expressed

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as mean ± standard deviation, and statistical significance was determined using one-way ANOVA at a 95% confidence level ($p < 0.05$).

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

Parameter	Concentration	WHO/FAO Codex / NAFDAC Standards
Acid Value (mg KOH/g)	1.91 ± 0.81	WHO/FAO & NAFDAC for edible oils: ≤ 0.6 mg KOH/g; Neem spec: ≤ 15 mg KOH/g
Density (g/mL)	0.96 ± 0.14	Codex general range: 0.900–0.965 g/mL
Ester Value	122.2 ± 0.18	Derived from saponification minus acid: 178.9 – 1.9 = ~177 mg KOH/g
Free Fatty Acid (%)	0.22 ± 0.04	WHO/FAO for edible oils: ≤ 1 %; Neem spec aligned
Iodine Value (mg I_2/g)	76.50 ± 0.62	Neem oil spec: 65–80 (Codex/WDRA)
Percentage yield (%)	35.20 ± 0.36	No official Codex/NAFDAC specification
Peroxide Value (mEq/kg)	2.60 ± 0.23	WHO/FAO: ≤ 10 mEq/kg; Neem spec: ≤ 1–2
Ph	5.50 ± 0.52	No set standard
Saponification Value	178.94 ± 0.19	Neem spec: 180–205; NAFDAC for seed oils: 187–195 mg KOH/g
Viscosity (cP)	2.20 ± 0.31	No official standard for seed oils

NB: Values are mean ± standard deviation for three replicates (n=3).

Table 4.2: Antibacterial analysis of Neem seed oil against *E. coli*.

S/N	Extract	Concentration (%)	Zone of inhibition (mm)
1.	Neem seed oil + *E. coli*	100	5mm
2.	Neem seed oil + *E. coli*	50	0.0mm
3.	Neem seed oil + *E. coli*	25	0.0mm
4.	Neem seed oil + *E. coli*	12.5	0.0mm

Control *E. coli*= Streptomycin= 32mm

Table 4.3: Antibacterial analysis of Neem seed oil against *Salmonella.*

S/N	Extract	Concentration (%)	Zone of inhibition (mm)
1	Neem seed oil + *Salmonella.*	100	9mm
2	Neem seed oil + *Salmonella.*	50	7mm
3	Neem seed oil + *Salmonella.*	25	0.0mm
4	Neem seed oil + *Salmonella.*	12.5	0.0mm

Control
Salmonella= 35mm

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Table 4.4: Result of Minimum Inhibitory Concentration (MIC)

S/N	Concentration (%)	*E. coli* N-hexane	Salmonella N-hexane
1	100	-	- MIC
2	50	+	+
3	25	+	+
4	12.5	+	+
5	6.25	+	+
6	3.125	+	+
7	1.7	+	+

The minimum inhibitory concentration is 100 for both *E. coli* and Salmonella
Key: + = Present, - = Absent.

Table 4.5: Result of Minimum Bacteriocidal Concentration (MBC): To determine the presence of growth, i.e. Bacteria growth

S/N	Concentration (mm)	*E. coli* N-hexane	Salmonella N-hexane
1	100	+	+

Key: + = Present

4.2 DISCUSSION

4.2.1 Discussion of Physicochemical Properties of Neem Seed Oil

The physicochemical characterization of neem seed oil obtained from Sabongida, Gusau, Zamfara State provides significant insights into the oil's quality, stability, and potential suitability for pharmacological and industrial applications.

Percentage Yield (35.2 ± 0.36%)

The oil yield of **35.2%** is considered moderately high and falls within the typical range for neem seed oil, which is usually between 30–45% depending on factors such as seed maturity, environmental conditions, and extraction technique (Ogbunugafor *et al.,* 2011). A good oil yield suggests that neem seed is a viable source of oil for commercial and therapeutic uses. Moreover, the low standard deviation indicates consistency in the sample preparation and extraction processes.

Color (Yellow)

The yellow coloration observed in the neem oil is a typical trait associated with natural, unrefined vegetable oils. This hue results from the presence of natural pigments such as carotenoids and tocopherols, which also contribute to the oil's antioxidant properties (Ali *et al.,* 2015). The yellow color further indicates the absence of significant degradation or contamination during processing.

pH (5.5 ± 0.52)

The slightly acidic pH of **5.5** is consistent with findings by Akpanabiatu *et al.,* (2012), who reported that neem oil typically has a pH ranging between 4.8 and 6.0. A mildly acidic oil pH is favorable for skin applications, as it mimics the natural pH of human skin and can inhibit microbial growth. This acidic nature may contribute to the antibacterial activity observed in neem oil by creating an unfavorable environment for many pathogenic microorganisms.

Viscosity (2.2 ± 0.31cP)

A viscosity of **2.2cP** denotes a relatively light oil, which enhances its spreadability and absorption when applied topically. Viscosity is a critical factor in determining oil application in pharmaceuticals and cosmetics, as highly viscous oils may hinder absorption (Tapanje *et al.,* 2020). The relatively low viscosity of neem seed

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oil supports its use in creams, lotions, and ointments with antibacterial functions.

Density (0.96 ± 0.14 g/mL)

The density value of **0.96 g/mL** corresponds with the reported standard for most plant oils, such as soybean and sunflower oil (Okorie *et al.,* 2018). This characteristic is significant for product formulation and packaging, particularly for emulsions and liquid-based medications.

Iodine Value (76.5 ± 0.62 mg/g)

The iodine value reflects the level of unsaturation in the oil. A value of **76.5 mg/g** suggests that the oil contains a moderate degree of unsaturated fatty acids, such as oleic and linoleic acids, which are responsible for various health benefits and biological activities (Akinoso *et al.,* 2011). Moderate unsaturation also implies that the oil is semi-drying and less prone to rapid oxidation, thus providing a balance between shelf stability and bioactivity.

Acid Value (1.91 ± 0.81 mg KOH/g)

The acid value of **1.91 mg KOH/g** is within acceptable limits for non-rancid edible oils and signifies a low concentration of free fatty acids (FFA). Elevated acid values typically indicate hydrolytic degradation or poor storage conditions (Mensah *et al.,* 2020). Though the standard deviation is slightly higher here, the result overall reflects an oil of good quality and stability.

Saponification Value (178.94 ± 0.19 mg KOH/g)

A high saponification value implies the presence of short-chain fatty acids and a high potential for soap production. The value of **178.94 mg KOH/g** obtained aligns with values reported in previous studies (Akinoso *et al.,* 2011), affirming the oil's suitability for saponifiable applications such as cosmetics and liquid soaps.

Peroxide Value (2.6 ± 0.23 mEq/kg)

The peroxide value measures the extent of lipid peroxidation and primary oxidation. A value of **2.6 mEq/kg** is relatively low, indicating that the neem seed oil has not undergone significant oxidative degradation. This is crucial in determining the shelf life and safety of the oil for medicinal or culinary use (Ojeh *et al.,* 2021). Oils with peroxide values below 10 mEq/kg are considered fresh and stable.

Free Fatty Acid (0.22 ± 0.04%)

The low FFA value of **0.22%** suggests minimal lipid hydrolysis and high-quality oil. FFAs contribute to rancidity and reduce oil stability if present in large quantities. This finding corroborates with the low acid and peroxide values, jointly indicating that the oil was well-extracted and stored under good conditions (Mensah *et al.,* 2020).

Ester Value (122.2 ± 0.18)

The ester value, obtained by subtracting the acid value from the saponification value, gives a rough estimate of the ester content in the oil. A value of **122.2** suggests a high concentration of triglycerides, which are beneficial for energy storage and biological activity. High ester values enhance the oil's lubricating, emollient, and antimicrobial properties (Okorie *et al.,* 2018).

4.2.2 Discussion of Antibacterial Activity of Neem Seed Oil against *Escherichia coli*

The antibacterial screening of neem seed oil against *Escherichia coli* (*E. coli*) shows differential activity at varying concentrations. The **zone of inhibition**, which measures the effectiveness of the oil against bacterial growth, was recorded only at the highest concentration (100%), while no inhibition was observed at lower concentrations (50%, 25%, and 12.5%).

Activity at 100% Concentration (5 mm Inhibition Zone)

At full strength (100% concentration), neem seed oil exhibited a zone of inhibition measuring 5 mm, suggesting that the oil possesses mild antibacterial properties against *E. coli*. While this

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inhibition is relatively small compared to standard antibiotics, it aligns with findings from previous studies that have demonstrated neem oil's antimicrobial activity (Biswas *et al.,* 2002; Pandey *et al.,* 2014). The antibacterial effect is attributed to the presence of bioactive compounds such as azadirachtin, nimbidin, and nimbin, which are known to disrupt microbial cell walls and interfere with enzymatic systems (Chattopadhyay, 2019).

Neem oil's hydrophobic nature may limit its diffusion in agar medium, which could partially explain the modest inhibition zone (Okemo *et al.,* 2001). Nonetheless, the presence of any inhibitory effect confirms that neem seed oil contains pharmacologically active constituents with potential antimicrobial utility.

Lack of Activity at Lower Concentrations (50%, 25%, and 12.5%)

At **concentrations below 100%**, there was **no visible zone of inhibition**, indicating that the antibacterial activity of neem oil against *E. coli* is **concentration-dependent**. This finding suggests that lower doses of the oil may not contain sufficient quantities of the active compounds needed to inhibit bacterial growth effectively. Similar observations were reported by Talwar *et al.,* (2016), where diluted neem oil showed reduced or no inhibitory effects on gram-negative bacteria due to their complex outer membrane structure.

E. coli, being a **gram-negative bacterium**, possesses a lipopolysaccharide-rich outer membrane that serves as a barrier to hydrophobic molecules like essential oils and plant-derived extracts (Gomes *et al.,* 2020). This structural feature may explain why neem oil exhibits only weak inhibition unless applied at high concentrations.

5.0 CONCLUSION

This study has demonstrated that neem seed oil extracted from Sabongida, Gusau, Zamfara State, possesses promising physicochemical properties that affirm its quality and suitability for various applications. The oil exhibited characteristics such as a moderate yield, appropriate viscosity, and favorable acid and peroxide values, which collectively indicate its chemical stability and potential for medicinal use. From an antibacterial perspective, the neem seed oil showed significant inhibitory effects against *Salmonella* species, with less pronounced activity against *Escherichia coli*. This suggests a selective antibacterial efficacy that could be harnessed in managing infections caused by specific pathogens. Importantly, the oil demonstrated bacteriostatic properties by inhibiting bacterial growth but did not achieve bactericidal activity at the concentrations tested. This implies that while neem seed oil may not outright kill the bacteria, it can suppress their proliferation, possibly aiding the immune system in controlling infections. These findings underscore the potential of neem seed oil as a natural antimicrobial agent, particularly as an alternative or complementary remedy in areas where antibiotic resistance is a concern. However, the lack of bactericidal action highlights the need for further research to optimize the oil's potency and explore its synergistic effects with other antimicrobials. Overall, neem seed oil from this region represents a valuable bioresource with both therapeutic and industrial relevance, encouraging continued investigation into its full spectrum of bioactivities and possible applications.

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