**EXTRACTION AND CRYSTALIZATION OF OIL FROM Azadirachta indica** **(NEEM) SEED**

# TITLE PAGE

**BY**

**ABANG FAVOUR BENJURE**

**(ST/SLT/ND/23/110)**

**AMABEL IRMIYA**

**(ST/SLT/ND/23/161)**

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF SCIENCE AND TECHNOLOGY, FEDERAL POLYTECHNIC MUBI, ADAMAWA STATE.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN SCIENCE LABORATORY TECHNOLOGY**

**AUGUST, 2025**

# DECLARATION

We hereby declare that the work in this project titled **“EXTRACTION AND CRYSTALIZATION OF OIL FROM Azadirachta indica** **(NEEM) SEED”** was performed by us under the supervision of Dr. Suleiman Bala. The information derived from literatures has been duly acknowledged in the text and a list of references provided. The work embodied in this project is original and had not been submitted in part or in full for any other diploma or certificate of this or any other institution.

ABANG FAVOUR BENJURE ...………………………

(ST/SLT/ND/23/110) Signature/Date

AMABEL IRMIYA ...……………..…………

(ST/SLT/ND/23/161) Signature/Date

# APPROVAL PAGE

This project titled **“EXTRACTION AND CRYSTALIZATION OF OIL FROM Azadirachta indica** **(NEEM) SEED”** meets the regulations governing the award of National Diploma (ND) in Science Laboratory Technology, Federal Polytechnic Mubi, Adamawa State

Dr. Suleiman Bala ..…………………

(Project Supervisor) Sign/Date

Dr. Muhammed Buba ..…………………

(Project Coordinator) Sign/Date

Mr. Ishaku H. Midala ..…………………

(Head of Department) Sign/Date

………………………………… ...………………..

(External Examiner) Sign/Date

# DEDICATION

This project work is dedicated to Almighty God for his enabling strength he bestowed on us during the course of this project work. Also, our gratitude goes to our lovely parents for their never-ending support and encouragement during the course of this research work.

# ACKNOWLEDGEMENTS

We want to acknowledge Almighty God for his infinite mercy and protection throughout our academic activities. And for the understanding in achieving our academic success.

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# ABSTRACT

*This study focuses on the extraction and characterization of oil from Azadirachta indica (neem) seeds collected from Mubi North Local Government Area of Adamawa State, Nigeria. Given the increasing demand for natural and sustainable alternatives in various industries, neem oil presents significant potential due to its rich composition of bioactive and industrially useful compounds. The research involved the extraction of neem seed oil using the Soxhlet method and the evaluation of its physical and chemical properties, including specific density, pH, colour, odour, percentage yield, acid value, iodine value, and saponification value. Results showed a high extraction yield of 83.3%, a specific density of 0.912 g/cm³, a pH of 6, and a yellow, fruity-scented oil. Chemically, the oil exhibited a low acid value (3.73 mg KOH/g), a moderate iodine value (125.20 mg I₂/100g), and a high saponification value (190.46 mg KOH/g), indicating good stability, unsaturation, and suitability for applications such as soap making, cosmetics, and bio-pesticide formulation. The findings suggest that neem seed oil from Mubi North is of high quality and can serve as a viable alternative to imported industrial oils. This study supports the economic and environmental benefits of promoting local neem oil production and calls for further research and investment in its industrial exploitation.*

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Neem (Azadirachta indica) is a fast-growing tree native to the Indian subcontinent and has been widely naturalized in tropical and semi-tropical regions around the world, including Nigeria. In many parts of Africa, including Nigeria, neem trees are commonly found and cultivated due to their wide range of medicinal, agricultural, and industrial applications (Adewumi *et al.,* 2021). The seed of the neem tree contains oil that is rich in bioactive compounds, making it valuable in the pharmaceutical, cosmetic, and agricultural industries.

Neem seed oil is typically extracted using mechanical pressing or solvent extraction methods, with hexane being the most common solvent. The oil is known to possess a variety of biological properties, including antibacterial, antifungal, antiviral, and insecticidal activities (Okoh *et al.,* 2023). A key physicochemical parameter used to characterize neem oil is its **iodine value**, which reflects the degree of unsaturation in the fatty acids present in the oil. The iodine value is significant because it influences the oil’s drying properties, shelf life, and potential industrial applications.

Neem seed oil has been widely recognized for its unique composition of bioactive compounds such as *azadirachtin*, *nimbin*, and *salannin*, which contribute to its insecticidal and therapeutic properties. These compounds are of interest not only in traditional medicine but also in organic farming and environmentally friendly pest control. The presence of unsaturated fatty acids like oleic, linoleic, and palmitic acids also gives the oil potential for use in cosmetics, soap making, and as a base oil for pharmaceutical preparations (Ibrahim *et al.,* 2022). Given the increasing global shift towards natural and sustainable alternatives, the relevance of neem oil in industrial applications is gaining more attention.

In many rural areas across northern Nigeria, including Mubi North, neem trees are commonly found along roadsides, in compounds, and in farm boundaries. However, the commercial and scientific exploitation of the seeds remains limited due to a lack of technical knowledge and data on the quality of the oil. The determination of the iodine value of neem seed oil is especially important because it offers a clear indication of the oil's unsaturation level, which affects its drying capabilities and storage stability (Yahaya *et al.,* 2023). Oils with high iodine values are more prone to oxidation, making them suitable for surface coating applications, while oils with lower iodine values are more stable and better suited for food and medicinal uses.

Furthermore, Nigeria’s dependence on imported industrial oils places a burden on the economy and local industries. If neem oil from regions like Mubi North can be demonstrated to have favorable properties for industrial use, it can serve as a local substitute for imported oils. This would not only conserve foreign exchange but also promote agro-industrial development, reduce rural unemployment, and enhance the value chain for neem products. Local oil extraction and small-scale processing could also provide a source of income for women and youths in the area, contributing to poverty alleviation and rural development (Abdullahi & Musa, 2023).

Another important factor is the role of standardization in promoting the use of natural oils. Without accurate data such as iodine values and other physicochemical properties, local oils cannot compete effectively in national or international markets. As such, establishing reliable data for neem oil produced in specific localities such as Mubi North is crucial for quality control and marketing purposes. The extraction method also plays a significant role in determining the quality of the oil. Soxhlet extraction, for instance, tends to yield oil with higher purity compared to traditional cold pressing, though it may involve more chemical input (Umar *et al.,* 2022).

In Mubi North Local Government Area of Adamawa State, neem trees grow abundantly, but their potential, especially the oil derived from the seeds, remains underutilized. Given the increasing demand for natural and sustainable resources, exploring the quality and potential uses of neem seed oil in this region becomes important for both economic and environmental reasons.

## 1.2Statement of the Problem

Despite the wide availability of neem trees in Mubi North, the full potential of neem seed oil has not been exploited, particularly with respect to its physicochemical properties like the iodine value. The absence of local data and the lack of awareness about the value of this oil hinder its commercial utilization. This study seeks to fill the gap by examining the extraction method and analyzing the iodine value of neem seed oil obtained from Mubi North. Understanding these parameters will provide useful insights for potential industrial applications and promote the local use and commercialization of neem oil.

## 1.3 Aim and Objectives of the Study

To extract and determine the iodine value of neem seed oil found in Mubi North Local Government Area.

1. To extract oil from neem seeds using the Soxhlet extraction method.
2. To determine the physical properties which include odour, colour, pH, percentage yield, specific density, and chemical properties of the oil which include iodine value, saponification value, acid value.

## 1.4Significance of the Study

The findings from this study will contribute to the scientific understanding of the chemical properties of neem seed oil indigenous to Mubi North. It will also highlight the economic potential of neem oil in industrial applications such as in soap making, pharmaceuticals, and bio-pesticides. Moreover, this study will encourage local cultivation and processing of neem seeds as a means of boosting income generation and sustainable development.

## 1.4 Scope of the Study

This study focuses on the extraction and analysis of the iodine value of neem seed oil specifically collected from various locations in Mubi North Local Government Area, Adamawa State. The study is limited to laboratory-based analysis of the oil’s iodine value and does not cover other parameters such as saponification value or acid value.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 History of Neem

On the Indian sub-continent, the neem tree has been used for more than 4,500 years. The earliest documentation of Neem mentions the fruit, seeds, oil, leaves, roots and bark for their advantageous medicinal properties. In the first millennium BC the Neem tree was called the "Sarva Roga Nivarini" (= one that could cure all ailments and ills). The Indian physicians Charaka in 2nd century AD and Susruta (4th century AD), whose books provided the foundation of the Indian system of natural treatment, the Ayurveda, also mention the tree and its medical use (Buzzle, 2012).

With the advent of Europeans on the Indian subcontinent, the religious practices around the neem tree were stigmatized as heathen practice and over time most practical uses were abandoned. However, at the beginning of this century the neem tree was still highly esteemed by Indian emigrants and they took it along to the places where they settled. Thus, the neem tree was introduced in places like Australia, Eastand sub-Sahelian Africa, South East Asia, and South America. In Indian agriculture, neem cake (the remains from the oil production out of neem seeds) was in use as a fertilizer and pesticide in sugar cane fields up to the 1930s. With the end of the colonial’s era, interest in the neem tree was on the rise again. Pioneering work in the possible commercial use of Neem oil and cake had been done by the Indian Institute of Science in Bangalore as early as the 1920’s. Recalling the insecticidal properties of Neem, researchers began programs in the early sixties to identify the active principles and screen them against major crop pests (Iloveindia, 2012).

## 2.2 Description of Neem Tree

Neem is truly a tree with roots firmly embedded in the cultures of its people. For 2000 years in India, Neem twigs have been chewed on to clean teeth, Neem leaf juice applied on skin to treat disorders, Neem tea drunken as a tonic, and Neem leaves placed in the home to ward away bugs (Muñoz-Valenzuela, 2017).

Its fruit (about the size of an olive) are eaten raw or cooked, and young twigs and flowers are sometimes eaten as vegetables. The fruit is also a major food source for birds and bats (they eat the pulp, not the seed), among others. The gum (resin), which is colorless, sticky, and malodorous, is also a high-protein food additive used in Southeast Asia, known as “neem glue”. Even the leaves are a source of food; they are used as fodder in the dry season. Neem also has important fuel uses: the wood is used as firewood and to produce excellent-grade charcoal, and the oil is used as lamp oil throughout India. The timber, although it has a rough grain and does not polish well, is used locally to make furniture. Its popularity in being used to make furniture is partly due to its insect repellent properties, for insects are deterred from coming near the furniture or the items inside. The wood is also popular for fencing and construction. In addition, the tree bark has 12 % to 14 % tannins, which makes it a good source for tannin chemicals. Neem has a well-developed root system that can extract nutrients from lower soil levels, making it an important agent in erosion control because it is virtually drought-resistant. As such it is useful as a dune fixation tree (Muñoz-Valenzuela, 2017). Farmers in India use neem cake (the residue left after extracting oil from the seeds) as an organic manure and soil amendment; it enhances the efficiency of nitrogen fertilizers by reducing the rate of nitrification and hampering pests such as nematodes, fungi, and insects (Muñoz-Valenzuela, 2017).

## 2.3 Botanical Description

The Neem tree (*Azadirachta indica*), a member of the Meliaceae family (Mahogany family) (Muñoz-Valenzuela, 2017), is also called the Indian neem tree, Indian lilac, and Margosa tree. Neem populations are heterogeneous in all respects, owing greatly to differences in soil and climate. The trees themselves are known to have genetic variation in height, branching type, leaf form, and color (Muñoz-Valenzuela, 2017).

Height generally ranges from 15 to 25 m, or even 30m with limbs of 15 m in length. Neem has a large, round crown of about 10 m (maximum 20 m) in diameter. These foliage proportions provide for shade nearly year-round (Murugan, 2012). Shiny dark green leaves are innately compound (leaflets attached in two rows to the main vein, as in Figure 2). The 10 to 12 serrated leaflets on each leaf are 7cm long by 2.5cm wide (MuñozValenzuela, 2017) and the leaf blade is glabrous. When damaged, the leaves emit a garlic odor.



Figure 2.1: Neem leaves (MuñozValenzuela, 2017).

White flowers are found as inflorescences with joined sepals. The fruit is yellow, fleshy, about 1 to 2 cm long, and has one (infrequently, two) seeds.



Figure 2.2: Neem flower and Fruit (MuñozValenzuela, 2017).

Like other characteristics of the tree population, the fruit form, size, weight, kernel proportion, composition, and oil content and quality are also varied (Muñoz-Valenzuela 2007).

Reproductive Biology: Neem first produces white fragrant blossoms, then develops hard green fruits which are bitter to the taste. Subsequently, the flesh (pericarp) softens and the fruit changes color to yellow. The ripe yellow fruit is sweet. From the beginning of the flowering stage to the seed falling (after the fruit shrivels away), is 27 weeks. At four or five years old, neem can produce flowers and fruit, but only after 10 to 12 years will it produce economically viable seed quantities (Muñoz-Valenzuela, 2017). A mature tree produces 30 to 50 kg fruit annually (Neem Foundation), or even as much as 50 to 100 kg of fruit per year. It is pollinated by insects such as honeybees. Neem may be self-incompatible, as some isolated trees do not set fruit (Muñoz-Valenzuela, 2017).

The flowering and fruiting seasons vary, depending on the region and time of year. For example, in Thailand neem produces flowers and fruits year-round, whereas in East Africa, with its defined dry and wet seasons, it does not (Muñoz-Valenzuela, 2017).

### 2.4 Importance of Neem Tree

Over thousands of years, Neem has been used by hundreds of millions of people and no hazards have been documented for normal dosages (Klaus, 2016). Every part of this fascinating tree has been used, from ancient to modern times, to treat hundreds of different maladies. While it is still revered in India for its superior healing properties, recent investigation has dramatically increased worldwide interest in Neem and many products are now manufactured using this miraculous herb. More than any other Indian herb, Neem proved useful in helping the body resist diseases and restore the proper balance to the body’s systems.

Neem and its parts are available in powdered form which is put to many uses in industries ranging from cosmetics to oral care, from agriculture to medicine. Neem powder is used in agriculture to protect plants from insects and pest; it can also be applied as organic manure. It is also used in veterinary medicine to cure worms, intestinal problems and other internal as well as external infections (Palani, 2012).

### 2.5 Medicinal uses of neem

Neem (*A. indica*) is a divine tree mainly cultivated in Indian subcontinent and it is commonly known as neem. All the parts of *A. indica* tree is commonly used in traditional Indian medicine for household remedy against various human diseases (Anyaehie, 2019). Indian people have long revered the neem tree (*A. indica*). For centuries, millions have cleaned their teeth with neem twigs, smeared skin disorders with neem leaf juice, taken neem tea as a tonic, and placed neem leaves in their beds, books, grain bins, cupboards, and closets to keep away troublesome bugs. The tree has relieved so many different pains, fevers, infections, and other complaints so that it has been called “the village pharmacy.” In rural India, peoples often used water decoction of neem leaves for the prevention and treatment of various ailments. Research undertaken in the University of Nigeria showed the medicinal properties of fractionated acetone/water neem leaf extract. Tests conducted at the King Institute of Preventive Medicine, Chennai in December 2012 found that the Siddha neem preparation brought down symptoms and speeded up the recovery of patients affected by dengue (Anyaehie, 2019).

To those millions in India, neem has miraculous powers, and now scientists around the world are beginning to think they may be right. Two decades of researches have revealed promising results in so many disciplines that this obscure species may be of enormous benefit to countries both poor and rich. Even some of the most cautious researchers are saying that “Neem deserves to be called a wonder plant”.

### 2.5.1 Antibacterial activity

Recent research shows the isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. The study of Zhong *et al.* (2013), showed an antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. Elavarasu *et al.* (2012), studied in vitro anti-plaque microbial activity of neem oil.

## 2.5.2 Antiviral

Galhardi, Yamamoto and Sinha (2012), studied the in vitro antiviral property of Azadirachta indica polysaccharides for poliovirus. The study of Saha *et al.* (2012), showed water extracted polysaccharides from A. indica leaves with anti-bovine herpes virus type 1 (BoHV-1) activity. The research of Xu *et al.* (2012), showed the in vitro antiviral activity of neem seed kernel extracts against duck plague virus. Tiwari *et al.* (2014), showed the in vitro antiviral activity of neem (A. indica L.) bark extract against herpes simplex virus type-1 infection.

### 2.5.3 Sexually transmitted disease

Few researchers have focused on neem efficacy in treating sexually transmitted diseases. The reports that have been completed are overwhelmingly positive. Recent research of Shokeen Bala and Tandon (2019), showed the evaluation of the activity of 16 medicinal plants against Neisseria gonorrhoeae.

### 2.5.4 Neem and the immune system

Thoh *et al.* (2010), studied that azadirachtin interacts with the tumor necrosis factor (TNF) binding domain of its receptors and inhibits TNF induced biological responses.

### 2.5.5 Anti-inflammatory activity

The study of Alam *et al.* (2012), showed the anti-inflammatory activity of epoxyazadiradione against macrophage migration inhibitory factor. Thoh *et al.* (2010), found that azadirachtin interacts with retinoic acid receptors and inhibits retinoic acid-mediated biological responses.

### 2.5.6 Antioxidant effect

Manikandan et al. researched that antioxidant and protective effects of active neem leaf fractions against hydrogen peroxide induced oxidative damage to pBR322 DNA and red blood cells.

### 2.5.7 Anticarcinogenic activity

Chatterjee *et al.* (2010), showed that identification of a sulfon oquinovosyldiacylglyceride from A. indica and studies on its cytotoxic activity and DNA binding properties. Perumal *et al.* (2012), studied ethanolic neem (*A. indica A. Juss*) leaf extract induced apoptosis and inhibits the IGF signaling pathway in breast cancer cell lines. Aravindan *et al.* (2013), showed that molecular basis of ‘hypoxic’ breast cancer cell radio-sensitization with phytochemicals. Induction of apoptosis in human breast cancer cells by nimbolide were carried out by Srivastava *et al.* (2012), showed that neem oil limonoids induces p53-independent apoptosis and autophagy. A review of the anticancer biology of Azadirachta indica was carried out by Veeraraghavan et al. (2011), the research showed the effect of neem leaf extract on protein-regulated cell death/ radiosensitization in pancreatic cancer cells. Mahapatra *et al.* (2011), showed novel molecular targets of Azadirachta indica associated with inhibition of tumor growth in prostate cancer.

## 2.5.8 Skin diseases

Neem has a remarkable effect on chronic skin conditions. Acne, psoriasis, eczema, ringworm and even stubborn warts are among the conditions that can clear up easily when high quality, organic neem oil is used. Neem oil and leaves has been used in Siddha medicine for the treatment of skin diseases (Thas, 2018). In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin. 9.9. Antisnake venom activity Ashis et al. studied a snake venom phospholipase A2 (PLA2) inhibitor (AIPLAI) was isolated from leaves of A. indica (neem) and the mechanism of PLA2 inhibition by AIPLAI in vitro condition was also studied (Ashis, Robin and Debashree, 2018).

## 2.5.10 Digestive disorders

Neem is generally accepted in the ayurvedic medical tradition as a therapy for ulcers and other types of gastric discomfort. Neem promotes a healthy digestive system by protecting the stomach, aiding in elimination and removing toxins and harmful bacteria. Bandyopadhyay et al. studied the neem bark extract of gastroprotective effect (Bandyopadhyay, 2014).

## 2.5.11 Parasitic diseases

Historically, neem has been used to rid the body of all forms of parasites. Neem quickly kills external and internal parasites. Neem extracts have hormone mimics that interfere with the life cycle of parasites, inhibit their ability to feed and prevent the eggs from hatching. Abdel *et al.* (2012), studied the efficacy of a single treatment of head lice with a neem seed extract. Luong *et al.* (2012), found that neem leaf slurry is a sustainable, natural product and anopheline larvicide in west African Villages.

## 2.6 Extraction of Essential Oil

## 2.6.1 Essential oils

Essential oils from neem trees are the volatile, organic constituents of plant matter and contribute to both medicinal and agricultural usage. These oils were termed essential because they were thought to represent the very essence in pharmaceutical, cosmetic as well as agricultural purposes. Volatile oils are chemically complex mixtures, often containing in excess of hundreds of individual components. Unlike Neem oil, most Essential oils have one to several major components which impart the characteristic odour and taste such as sweet and spicy, but Neem oil has bitter taste. However, there are also many minor constituents which also play their part in producing the final product (Abdullah, 2019).

Chemically, the essential oils are a complex and highly variable mixture of constituents that belong to two groups: terpenoids and aromatic compounds. The name terpene is derived from the English word “Turpentine” (Guenther, 2015). The terpenes are the unsaturated hydrocarbons which have a distinct architectural and chemical relation to the simple isoprene Molecule.

Chemical analysis of essential oils is generally performed using gas chromatography (GC) (qualitative analysis) and gas chromatography –mass spectrometry (GC/MS) (quantitative analysis). Identification of the main components is carried out by the comparison of both the GC retention times and the MS data against those of the reference standards, Kovats retention indices (KI) and comparison with previous literature (Adams, 2017).

## 2.6.2. Sources and Isolation of essential oils

Essential oils are isolated from different aromatic plants across the world where they are esteemed as an imperative component of the native medicine systems. These essential oils can be produced in almost all plant organs such as flowers, buds, stems, leaves, fruits, seeds and roots etc. These are accumulated in secretary cells, cavities, channels, and epidermic cells (Burt, 2014). Almost all odoriferous plants contain essential oils. The raw material from which essential oils are manufactured may be fresh, partially dehydrated or dried (Ozcan, 2013).

The extraction of the essential oil depends mainly on the rate of diffusion of the oil through the plant tissues to an exposed surface from where the oil can be removed by a number of processes. There are different methods, depending upon the stability of the oil, for the extraction of the oil from the plant materials. The essential oils obtained by steam distillation or by cold-pressed are generally preferred for food and pharmacological applications.

Due to the bactericidal and fungicidal properties of essential oils, their pharmaceutical and food uses are becoming increasing important as alternatives to synthetic chemical products to protect the ecological equilibrium (Burt, 2014). The extracted oils can vary in quality, quantity and in the chemical composition depending upon the agro climate, plant organ, age and vegetative cycle stage (Masotti *et al.,* 2013).

The complexity of the essential oils is a real challenge for determining their reliable and accurate compositional data. The rapid advances in spectroscopic and chromatographic techniques have totally changed the picture of chemical study of essential oils. Many techniques have been used for studying the chemical profiles of essential oil e.g. IR-spectroscopy, UV-spectroscopy, NMR spectroscopy and gas chromatography. The increasing importance of essential oils in various domains of human activities including pharmacy, cosmetics, aromatherapy, and food and beverages industry has prompted an extensive need of reliable methods for analyses of essential oils. The combination of gas chromatography and mass spectrometry (GC-MS) allows rapid and reliable identification of essential oils components.

The yield and the quality of the essential oil are considerably affected by processing methods used for their handling and storage. The essential oils are enclosed in oil glands present in the cellular structure of the plant materials. Although essential oils may be produced from an endemic population, there can be several reasons why the composition and thus, the essential oil quality from aromatic plants might differ greatly. Genetic, physiological and environmental factors as well as processing conditions may play an important role while defining the chemistry and chemical composition of essential oils.

## 2.6.3 Methods of essential oil extraction

Quality of oil depends on the type of extraction. Manufacturing of neem oil includes the collection of raw materials for the extraction and selection of extraction method.

## 2.6.3.1 Traditional methods of extracting Neem oil

Neem oil can be extracted traditionally at home using cold pressed extraction by hand and around 100 to 150 mgs of oil for every 1 kilogram of neem seed. To press neem oil by hand, the kernels of the neem seed should be crushed in a mill or pound in a mortar. Add a small amount of water until the mixture forms a firm paste that can be kneaded. Knead the paste until oil drops form on the surface and press firmly to extract the oil. The kneading and pressing should be continued in turn until the maximum amount of oil is removed. The oil content of the seed kernel is about 45%, even though preparation of the oil at home possible, but this traditional method of processing Neem oil was not effective on percent yield.

## 2.6.3.2. Recent methods of Extraction

Extraction of oil from Neem seeds can be performed using three different methods: mechanical extraction, solvent extraction, and supercritical fluid extraction.

**A) Mechanical extraction**

Common method used to extract the Neem oil from the seed, since this method is effective for seed contain 30-70 % oil. The mechanical extraction has several advantages compared to the other methods, such as simple equipment and low investment, low operating cost, and the oil does not undergo solvent separation process, etc. Usually the quality and quantity of the oil obtained by mechanical extraction process are affected by various operating conditions such as pretreatment of the Neem seeds, extraction pressure, and storage condition. Effect of extraction condition on quality of oil has been investigated in several studies for wide variations of material, including conophor nut, olive, jojoba, and groundnut, and peanut kernel oil. The changes of oil quality during storage also have been investigated for numerous materials, such as soybean, peanut kernel, sunflower, olive, and fish oil.

Mechanical extraction of Neem seeds was performed using hydraulic pressing equipment (ENERPAC RC-256 and P-39). Untreated seed particles were pressed with various pressures to determine the optimum pressure. Pressure was started at 2000 psi as the oil started to flow out of the seedbed, and stopped at 6000 psi since the oil yield relatively constant at the pressure above 6000 psi. Mechanical extraction was performed for 25 minutes when the oil has stopped flowing out. Oil yield measurement was conducted using mass balance. Mechanical extraction is the most widely used method to extract Neem oil from Neem seed. However, the oil produced with this method usually has a low price, since it’s turbid and contains a significant amount of water and metals contents.

**B) Solvent Extraction**

Solvent Extraction is a process which involves extracting oil from oil-bearing materials by treating it with a low boiler solvent as opposed to extracting the oils by mechanical pressing methods (such as expellers, hydraulic presses, etc.) The solvent extraction method recovers almost all the oils and leaves behind only 0.5 % to 0.7 % residual oil in the raw material. In the case of mechanical pressing the residual oil left in the oil cake may be anywhere from 6 % to 14 %. The solvent extraction method can be applied directly to any low oil content raw materials. It can also be used to extract pre-pressed oil cakes obtained from high oil content materials. Because of the high percentage of recovered oil, solvent extraction has become the most popular method of extraction of oils and fats.

The process solvent extraction is basically a process of diffusion of a solvent into oil-bearing cells of the raw material resulting in a solution of the oil in solvent. Various solvents can be used for extraction. However, after extensive research and consideration of various factors, such as commercial economics, edibility of the various products obtained from extraction, physical properties of the solvent especially its low boiling point etc. food grade n-hexane is considered to be the best and it is exclusively used for the purpose. In a nutshell, the extraction process consists of treating the raw material with hexane and recovering the oil by distillation of the resulting solution of oil in n-hexane called miscella. Evaporation and condensation from the distillation of miscella recovers the n-hexane absorbed in the material. The n-hexane thus recovered is reused for extraction. The low boiling point of hexane (67 °C / 152 °F) and the high solubility of oils and fats in it are the properties exploited in the solvent extraction process.

**C) Two stage extraction process**

These two stages extraction process is a mechanical extraction followed by solvent extraction. As raw material for neem-based extract processing, Neem seeds (*Azadirachta indica*) is used. A certain kilogram of dried Neem seeds was used in the process. The seeds firstly decorticated to obtain the seed kernel, then crushed and finally pressed to separate neem oil using mechanical extraction. By moving-bed contacting extraction technique, defatted neem cake will be extracted with solvent in an agitated-extraction vessel. After decantation of crude cake in mixing-settling tank, the neem solution is drained out, then filtered and evaporated until a specific volume; the so-called concentrated solvent-neem-based extract of the oil attains its quality. After quality measurement, the concentrate could be formulated for specific purpose as different commercial grade. Eventually, the product will be bottled and shipped to the consumer.

**D) Supercritical fluid extraction**

The state of a substance is called supercritical fluid (SCF) when both temperature and pressure exceed the critical point values. Extractions with supercritical fluid (SCF) solvents have emerged in recent years as highly promising environmentally benign technologies for the production of natural extracts with high potency of active ingredients – such as flavours, fragrances, spice oils and oleoresins, natural colours, nutraceuticals or herbal medicines – for the food, cosmetics, and pharmaceutical industries. Supercritical carbon dioxide (SCCO2) at near-ambient temperatures is the most desirable SCF solvent for extraction of natural products today as it is non-toxic, inexpensive, non-flammable, and non-polluting. Its near-ambient critical temperature (31.1 oC) makes it ideally suitable for thermally labile natural products. It is generally regarded as safe (GRAS) and yields contamination-free, tailor-made extracts having superior organoleptic profile and enhanced shelf life. The supercritical fluid extraction (SCFE) technique ensures high consistency and reliability in the quality and safety of the bioactive heat-sensitive botanical products because it does not alter the delicate balance of bioactivity of natural molecules (Mukhopadhyay, 2018).

## 2.6.4 Factors affecting essential oil accumulation

Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances, it is difficult to segregate these factors from each other, since many are interdependent and influence one another (Terblanche, 2010). These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage (Marotti 2014).

## 2.6.4.1 Seasonal and maturity variations

These two factors are interlinked with each other, because the specific ontogenic growth stage will differ as the season progresses. There are variations in the chemical profile of essential oils from various plants collected during different seasons. The essential oils yields varied considerably from month-to-month and was also influenced by the micro-environment (sun or shade) in which the plant was growing. Results obtained by Badi (2014) also indicated that timing of harvest is critical to both yield and oil composition.

## 2.6.4.2 Geographical variation

There are many reports in the literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions (Uribe- Hernandez, 2012). Chalchat *et al.* (2015), reported variations in the yield and chemical profile of essential oils, collected from different geographical locations, respectively. Such differences could be linked to the varied soil textures and possible adaption response of different populations, resulting in different chemical products being formed, without morphological differences being observed in the plants. (Hussain *et al*., 2018).

Altitude seems to be another important environmental factor influencing the essential oil content and chemical composition. Climatic factors such as heat and drought were also related to the essential oil profiles obtained (Uribe-Hermandez *et al.,* 2012). Moreover, the preference of the plant for these conditions suggest that genetic make-up of the plant, rather than the soil-type in which it is growing, should have a greater influence on the chemical profile of the oil produced (Abdullah, 2019).

## 2.6.4.3 Genetic variation

Genotype is typically defined as “the genetic make-up of an organism, as characterized by its physical appearance or phenotype”, while chemotype is generally defined as “a group of organisms that produce the same chemical profile for a particular class of secondary metabolites”. Variations in chemical profiles were observed from oils produced from specimens from the same population and location, demonstrating the presence of different chemotypes within this species. Genetic makeup of the plant is one of the most important contributors to their essential oil composition.

## 2.6.4.4 Other factors affecting yield and composition of essential oil

Other factors which affect the growing plants thus leading to variations in oil yield and composition, include part of plant used; post- harvest drying; length of exposure to sunlight; availability of water, height above sea level, plant density, time of sowing and the presence of fungal diseases and insects. The oil composition and yield may also change as a result of the harvesting methods used, the isolation techniques employed, the moisture content of the plants at the time of harvest and the prevailing extraction conditions (Abdullah, 2019).

Postharvest drying of material is an accepted practice in the production of essential oils. Drying methods include exposure to natural air in the shade, sun-drying, as well as drying by blowing warm air over the material. Postharvest drying is thought to improve oil yield and accelerate distillation, by improving heat transfer, in addition to providing increased loading capacity, due to loss of plant moisture. Further advantages include the reduction of microbial growth and the inhibition of some biochemical reactions in dried material. However, some amount of the oil may be lost during such post-harvest treatment due to volatilization and mechanical damage to oil glands. Essential oil components (including terpenoids) are usually present in the free form, but may also be bound to sugar moieties, usually mono- or disaccharides (Abdullah, 2019).

## 2.6.1 Chemical composition of neem seed oil

Neem elaborates a vast array of biologically active compounds which are chemically diverse and structurally complex. Neem chemistry dates back to 1880-90 when influenced by its folk-lore medicinal values, the chemist took up the isolation of active principle from its seed and other parts. Siddiqui was the first to report the isolation of three products viz. nimbin, nimbidin and nimbinin from its oil. The neem constituent belonging to chemically diverse classes have been divided into two major sections: (a) Isoprenoids & (b) Non-Isoprinoids. The later category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acids, aliphatic compounds etc.

**CHAPTER THREE**

**Material and methods**

### 3.1 Materials

Conical flask (250cm3), Beaker 230cm3, Soxhlet extraction apparatus, hot plate, pH meter, Neem seed samples, N-Hexane (NH), Potassium hydroxide (KOH), Iodine (I2), Distilled water (H2O), Hydrochloric acid (HCl), Potassium iodide (KI).

**3.2 Collection of samples**

Fresh seeds of Neem Plant will be collected from Mubi Local Government Area of Adamawa State, Nigeria and taken to the Chemical Science Laboratory.

**3.3 Sample preparation**

In the preparation of neem seed, neem seed will be collected from neem tree in front of the Department of Science Laboratory Technology, Federal Polytechnic, Mubi. Then, they were dried in shaded area for 3 weeks. After that which it will be ground to get fine powder using an agate mortar and pestle. All samples will be prepared at the Laboratory and analysed by using EDX-7000 spectrometer using fundamental parameter (FP balance) method located at the Materials Science Laboratory, Adamawa State University, Mubi.

## 3.4 Sample treatment

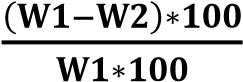
The outer layer of the epicarp were pealed and all the neem seed collected will be subjected to sun-drying. It will be completely dried out; the seed will be carefully cracked open using a cleaned motar and pistle to remove the hard endocarp. This expose bares the inner most neem seed that will be carefully collected for oil extraction using Soxhlet method as described by (Natarajan *et al.,* 2003)

**3.5 Extraction**

100 g of the sample will be placed in the thimble and about 300 ml of normal hexane will be poured into the round bottom flask. The apparatus will be heated at 69.9 °C and allowed for 2hrs, 4hrs, 6hrs and 8hrs continuous extraction using Soxhlet apparatus. The experiment was repeated for different particle size with one replica (Kebede *et al.,* 2022).

##### **3.6 Determination of the yield of Neem oil extracted**

At the end, the cake will be weighed and dried in the oven at 100 0C until the constant weight is attained and the percentage of oil extracted was determined as:

Percentage yield =

Where:

W1=Sample weight initially placed in the thimble

W2= sample weight after dried in the oven.

##### **3.7 Determination of pH Value**

100 g of the sample will be poured into a clean dry 25 ml beaker and 13ml of hot distilled water will be added to the sample in the beaker and stirred slowly. It will be then cooled in a cold-water bath to 25 °C. The pH electrode will be standardized with buffer solution and the electrode immersed into the sample and the pH value will be read and recorded (Adepoju & Ojo, 2020).

###### **3.8 Determination of Saponification Value**

Indicator method will be used as specified by ISO 3657 (1988). 100g of the sample will be weighed into a conical flask; 25 ml of 0.5 N Ethanolic potassium hydroxide will be then added. The content which will be constantly stirred and allowed to boil gently for 30min. A reflux condenser will be placed on the flask containing the mixture. Few drops of phenolphthalein indicator will be added to the warm solution and then titrated with 0.5M HCl to the end point until the pink colour of the indicator just disappeared. The same procedure will be used for other samples and blank (Ibrahim et al., 2022). The expression for saponification value (S.V.) is given by:

SV =

Where

V2 = the volume of the solution used for blank test

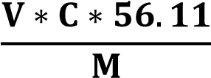
V1 = the volume of the solution used for determination S.V

N = Actual normality of the HCl used

M = Mass of the sample.

###### **3.9 Determination of Acid Value**

25 ml of Toluene and 25 ml of ethanol will be mixed in a 250 ml beaker. The resulting mixture will be added to 100 g of oil in a 250 ml conical flask and few drops of phenolphthalein will be added to the mixture. The mixture will be titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1M KOH (V0) was noted. The Acid value will be calculated as (Ogbonna *et al.,* 2021):

= 

**W**

Where;

V =Volume of potassium hydroxide (ml),

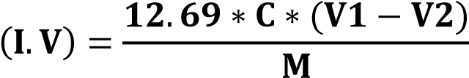
C = Concentration of potassium hydroxide,

56.11 =Molecular weight of potassium hydroxide,

M= sample weight

## 3.10 Determination of Iodine value

The method specified by ISO 3961 (1989) was used. 0.4 gm of the sample will be weighed into a conical flask and 20 ml of carbon tetra chloride was added to dissolve the oil. Then 25 ml of Dam’s reagent was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours and 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125 ml of water will be added using a measuring cylinder. The content will be titrated with 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator will be added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples (Nwachukwu *et al.,* 2023). The iodine value (I.V) is given by the expression:



Where:

C = Concentration of sodium thiosulphate used;

V1 = Volume of sodium thiosulphate used for blank;

V2 = Volume of sodium thiosulphate used for determination,

M = Mass of the sample.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Results

## 4.1.1 Result of Physical Properties of Neem seed oil

|  |  |
| --- | --- |
| **Test** | **Value** |
| Odour | Fruity odour/smell |
| Specific density (g/cm3) | 0.912 |
| pH value (oC) | 6 |
| Color | Yellow |
| Crystalizing oil (g) | 10 |
| Percentage yield (%) | 83.3 |

Table 4.1.1: The neem seed oil had a fruity odour, a specific density of 0.912 g/cm³, and a slightly acidic pH of 6, indicating good quality and stability. Its yellow color reflects natural pigments, while the crystallizing oil weight was 10 g. The extraction yield of 83.3% shows high efficiency.

## 4.1.2 Results of Chemical Properties of Neem Oil

|  |  |
| --- | --- |
| **Test** | **Value** |
| Acid value (mg/KOH/g) | 3.73 |
| Iodine value (mg/L) | 125.20 |
| Saponification (mg/KOH/g) | 190.46 |

Table 4.1.2: The oil’s acid value (3.73 mg KOH/g) indicates low free fatty acids and good stability. The iodine value (125.20 mg/L) shows moderate unsaturation, while the saponification value (190.46 mg KOH/g) suggests suitability for soap and cosmetic production.

## 4.2 Discussion

The results of the physical and chemical analyses of neem (*Azadirachta indica*) seed oil obtained in this study indicate that the oil is of high quality and suitable for various industrial and medicinal applications. The fruity odour observed aligns with reports by Yusuf *et al.* (2021), who stated that freshly extracted neem oil typically retains a mild fruity or nutty scent due to its natural volatile compounds. This suggests that the extraction process used in the current study preserved the oil’s volatile components and minimized degradation. The specific density of 0.912 g/cm³ falls within the standard range of 0.910–0.920 g/cm³ reported by Adejumo *et al.* (2022), further confirming its purity and absence of significant adulteration. The slightly acidic pH (6) also corresponds with findings by (Muhammad *et al.*, 2023), who observed that neem oil’s mildly acidic nature contributes to its oxidative stability and antimicrobial properties.

The yield of 83.3% obtained in this study is relatively high compared to the 76–80% range reported by (Eze & Okoro, 2021), indicating that the extraction method was efficient. Factors such as seed freshness, moisture content, and solvent extraction efficiency could have contributed to the high yield recorded. The yellow coloration is characteristic of carotenoid pigments present in neem oil (Olayemi *et al.,* 2020) and indicates that minimal bleaching or pigment degradation occurred during processing.

In terms of chemical properties, the acid value of 3.73 mg KOH/g is well below the maximum limit of 5 mg KOH/g recommended for edible and cosmetic oils (FAO/WHO, 2021), suggesting low free fatty acid content and good storage stability. This finding is consistent with the work of (Hassan *et al.,* 2022), who also reported acid values between 3.5 and 4.0 mg KOH/g for high-grade neem oil extracted under controlled conditions. The iodine value (125.20 mg/L) obtained in this study falls within the range of 120–130 mg/L reported by (Olufemi *et al.,* 2020), indicating a moderate degree of unsaturation. This level of unsaturation makes the oil resistant to rapid oxidation while retaining its bioactive properties, making it suitable for medicinal and cosmetic uses.

The saponification value of 190.46 mg KOH/g suggests a predominance of medium- to short-chain fatty acids, making the oil ideal for soap-making and other saponified products. This observation is in agreement with the findings of Adejumo et al. (2022), who reported similar values and highlighted that higher saponification values are linked to the presence of fatty acids that yield hard, high-lather soaps. Furthermore, the values obtained in this study corroborate earlier work by Adebayo *et al.* (2021), who emphasized the importance of neem oil’s fatty acid composition in determining its versatility in pharmaceutical and agricultural formulations.

Overall, the present findings are consistent with existing literature on neem oil quality, confirming that the oil produced in this study meets recognized physical and chemical standards. The results reinforce neem oil’s potential in applications ranging from bio-pesticides to skincare products, and they demonstrate the importance of extraction techniques in determining oil quality. The high yield, low acid value, and favorable saponification and iodine values obtained suggest that the current processing approach could be scaled for commercial use without compromising quality.

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

The findings of this study demonstrate that neem seed oil extracted under the applied method possesses excellent physical and chemical qualities. The high extraction yield, low acid value, moderate iodine value, and favorable saponification value all suggest that the oil is stable, nutritionally beneficial, and industrially valuable.

The results reinforce the versatility of neem oil, supporting its use in soap production, cosmetics, traditional medicine, and as a bio-pesticide. Furthermore, the relatively low free fatty acid content indicates a longer shelf life and reduced susceptibility to rancidity, making it more marketable and economically viable.

Overall, this study contributes to the growing body of evidence that neem oil, when properly extracted, meets the standards required for both domestic and industrial applications.

## 5.2 Recommendations

Based on the findings of this research, the following recommendations are made:

1. Given the high quality observed, local industries should be encouraged to invest in neem oil production for use in cosmetics, herbal medicine, and bio-pesticide formulations.
2. Regulatory bodies should set and enforce quality standards for neem oil production to ensure consistency, safety, and suitability for intended applications.
3. Further studies should focus on enhancing neem oil’s bioactive properties through blending with other plant oils for specialized purposes, such as therapeutic skin products or fortified agricultural inputs.
4. Farmers should be educated on neem seed handling, storage, and pre-processing methods to minimize contamination and degradation of oil quality before extraction.

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# APPENDIX I

**ACID VALUE**

Weight of sample taken (W) = 2g

Volume of HCl used (V2) = 3.73cm3

Normality of KOH = 0.1mldm3

Mass equivalent of KOH (MC) = 56.1

Calculation;

=

=

= 3.73mgKOH/g

**IODINE VALUE**

Iodine value (mgKOH/g) =

Where;

V1 = volume of NPa2S2O3 used (cm3)

V2 = volume of Na2S2O3 used for test sample (cm3)

N = Normality of Na2S2O3 used (0.1N)

Therefore;

I.V =

= =

=

= 125.19954

= 125.20mg/L

**SAPONIFICATION VALUE**

SV =

=

=

= 190.4595

= 190.46 mg/KOH/g