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SUSTAINABLE TECHNIQUE FOR NEEM (*AZADIRACHTA INDICA*) SEED OIL EXTRACTION: OPTIMIZATION AND CHARACTERIZATION

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ABSTRACT: *N-hexane is a solvent that is frequently used in oil extraction processes. But there is enough data to demonstrate this solvent's harmful nature. In this study, the Soxhlet process was utilized to extract neem seed oil from neem seeds using a greener petroleum ether. We looked into how the temperature (25–125 °C) and time variation (30–50 min) affected the extraction process. When the temperature is held constant, it indicates a high yield (57.08%) at 150 minutes and a maximum yield (52%) at 125°C when the duration is held constant. An examination of the saturated and unsaturated fatty acids' makeup was done using the gas chromatography-mass spectrometry (GC-MS) method. The result of the GC-MS method showed a high proportion of unsaturated fatty acids (58.82%), which are important because they may provide health benefits when included in a balanced diet. Alkaloids, coumarins, terpenoids, saponins, steroids, flavonoids, and triterpenoids are all present, with the exception of tannins, according to the phytochemical analysis. This implies that the oil may have therapeutic value. The extracted oil exhibits the following characteristics: 3.53% moisture content, 1.70 mg KOH/g acid value, 0.81 density (g/cm3), 31.60 viscosity (mm2/s), 268 mg KOH/g saponification value, 3.71% ash content, and 119.8 mg I2/100g iodine value. These figures illustrate the oil's potential for use in a variety of multi-industrial applications; they are compliant with ASTM standards.*

KEYWORDS: Neem oil, Azadirachta Indica, Soxhlet Extractor, Sustainable Extraction, Oil Extraction.

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INTRODUCTION

The Neem tree, scientifically named *Azadirachta indica A. Juss*, is native to the Indian subcontinent and a member of the *Meliaceae* family of mahogany trees [1]. It grows well in tropical and semi-tropical climes and is quite adaptable to a wide range of habitats, including Nigeria. The Neem plant has been used historically for the manufacturing of industrial items and for the treatment of a wide range of human maladies in indigenous medicine. These parts of the plant include leaves, bark, flowers, fruits, seeds, and roots [2]. Among these parts, Neem seeds stand out for their remarkably high oil content compared to other parts of the tree. This oil finds extensive application as lubricants, insecticides, and medicinal compounds for addressing ailments such as diabetes, leprosy, and tuberculosis. Moreover, Neem seed oil serves as a key ingredient in the formulation of numerous skincare products, like body lotions, soaps, and facial packs, often blended with other natural components [2].

Neem seed extracts are inexpensive and eco-friendly, and they have the potential to protect crop species against fungal infestation, which will increase crop production and yield [3]. When compared to the bark and leaves alone, *Azadirachta Indica* may have more antibacterial and antifungal action due to the high amounts of azadirachtin, quercetin, and β-sitosterol, in addition to its antifungal qualities [3].

Green extraction emphasizes innovation in all facets of solid-liquid extraction processes and is based on the concepts of Green Chemistry and Green Engineering. This strategy focuses on using alternative solvents and renewable plant resources, producing co-products rather than trash, putting safer and more controllable processing techniques into place, and, in the end, creating non-denatured, biodegradable extracts free of pollutants. Because of the safety, environmental impact, and economic feasibility of using alternative solvents instead of harmful ones, this has become a popular trend among academic researchers and industrial practitioners [4].

There are several ways to extract neem oil from its seeds: solvent extraction, supercritical fluid extraction, and mechanical pressing. Of these, solvent extraction is the most widely used technique. The oil obtained by this method is usually less valuable due to its turbidity and high water and metal contents. Conversely, supercritical fluid extraction yields very high purity neem oil, but the associated operating and investment costs are very high. The first attempts to extract neem oil using supercritical CO_2 have yielded significant oil yields. However, because of the high operating expenses and specialized equipment needed, this method's widespread industrialization still faces obstacles [7]. Solvent extraction, on the other hand, has a number of advantages over supercritical fluid extraction, including higher yields and less turbidity than mechanical pressing and lower operating costs when compared to supercritical fluid extraction [5].

Due to its excellent oil selectivity, ease of evaporation, and comparatively low energy costs, n-hexane has historically been the primary solvent used in commercial oil extraction procedures over the past 50 years. However, there are serious health and environmental concerns associated with extended exposure to n-hexane and its possible leakage during industrial extraction and recovery procedures (Figure 1a) [6]. Thus, in line with the ideas of sustainable development, there is an increasing need for more environmentally friendly solvents to take the role of n-hexane in oil extraction procedures. For sustainable extraction, petroleum ether

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was utilized as an alternative to n-hexane in this research, offering a promising and safer extraction method (Figure 1b).

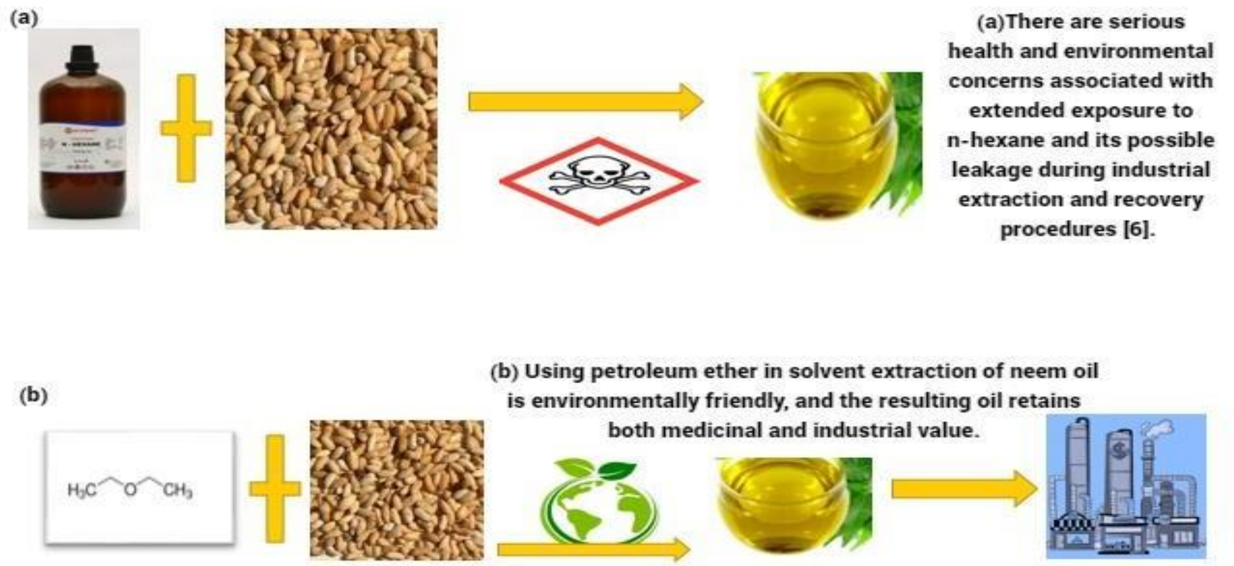

Figure 1: Petroleum Ether versus N-hexane Solvent Extraction

Considering the various applications for neem oil and its adaptability to alteration for various purposes, as previously mentioned, it is essential to investigate the mechanism of neem oil extraction, which greatly reduces extraction parameters while optimizing the quantity and quality of the oil. In this work, solvent extraction was utilized along with petroleum ether as a greener alternative to the dangerous n-hexane. Studies were conducted on the influence of process parameters such as temperature and extraction time. The oil's saturated and unsaturated contents were examined using gas chromatography-mass spectrometry. An alternative phytochemical screening and physicochemical study was performed. The overall goal is to optimize neem seed oil's industrial and medicinal potential while simultaneously fostering a shift towards sustainable alternatives to hazardous oil extraction practices.

MATERIALS AND METHOD

Extraction of Oil

The neem powder weighing 200 g was loaded into the thimble and inserted into the Soxhlet chamber. Meanwhile, a round bottom flask was filled with 1000 ml of petroleum ether and set up for the Soxhlet extractor. Subsequently, the distillation process commenced. Upon completion of the extraction process, both the solvent and the extractor were transferred onto a water bath to facilitate the evaporation of the solvent.

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Measurement of Output Parameters of Extraction Operation

- Determination of the percentage oil yield:

The calculation of the parentage oil yield followed the method outlined by [8].

Qualitative Analysis of the Phytochemical in the Extracted Oils

1.) ***Test for Tannins:*** The assessment of tannin content was conducted following the procedure described by [9]. Five grams of the extracted oil were placed into an empty 50 ml beaker, to which 3 drops of ferric chloride were added. The solution was observed, and its appearance remained largely unchanged, indicating the absence of tannins, as noted by [10].

2.) ***Test for Coumarins:*** Two milliliters of the aqueous extract from the extracted oil sample were mixed with three milliliters of 10% NaOH solution. The appearance of a yellow coloration in the solution indicated the presence of coumarins [10].

3.) ***Test for Alkaloids*****:** One milliliter of the extracted oil sample was mixed with one or two drops of Dragendorff's reagent (potassium bismuth iodide solution) in a test tube. According to [11], the presence of alkaloids was suggested by the production of a reddish-brown precipitate.

4.) ***Test for Terpenoids:*** The presence of terpenoids was assessed following the procedure outlined by [11]. Five milliliters of the extracted oil sample were combined with 2 ml of chloroform. Subsequently, 3 ml of concentrated H_2SO_4 were added carefully to form a layered structure. The appearance of a reddish-brown coloration in the inner phase indicated the presence of terpenoids.

5.) ***Test for Saponins*****:** Shaking approximately 1.5 grams of the extracted oil with water in a test tube resulted in persistent frothing, indicating initial indications of the presence of saponins, as described by [11].

6.) ***Test for Steroids:*** A small portion of the extract was dissolved in two milliliters of chloroform, and then concentrated H_2SO_4 was added to create a bottom layer. According to [9] and [10], the existence of a steroidal nucleus was indicated by the emergence of a reddish-brown hue close to the interface.

7.) ***Test for Flavonoids*****:** One technique was to mix two or three drops of concentrated H_2SO_4 with one milliliter of the extracted oil to detect the presence of flavonoids. Upon examination, the appearance of an orange to crimson red coloring suggested the presence of flavonoids [10].

8.) ***Test for Triterpenoids*****:** One milliliter of chloroform was used to dissolve ten grams of the extracted oil, and then two milliliters of concentrated H_2SO_4 were added. One milliliter of acetic anhydride was then added. According to [9], the presence of triterpenoids was suggested by the emergence of a golden-yellow hue at the bottom of the solution.

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Physicochemical Properties

The properties of neem seed oil, including specific gravity, density, iodine value, saponification value, and acid value, were analyzed in accordance with the methods outlined by the Association of Official Analytical Chemists (AOAC) (1990) and the American Society of Testing Materials (ASTM) (23, 24).

Fatty Acid Determination Using Gas Chromatography-Mass Spectrometry (GC-MS)

The composition of fatty acids in the neem seed oil extracted was analyzed using gas chromatography-mass spectrometry. Five different types of fatty acids were identified: oleic acid, palmitic acid, caprylic acid, stearic acid, and myristic acid.

RESULT AND DISCUSSION

Percentage Yield

The extraction of neem oil using greener petroleum ether resulted in a yield of 55%, which aligns with the average yield achieved using the hazardous n-hexane, as indicated by [11]. The higher yield obtained with n-hexane may be attributed to the absence of OH groups, which have been noted to adversely affect the extraction process of certain oilseeds, as discussed by [8].

Phytochemical Screening

As previously discussed, neem seed oil has been demonstrated to contain numerous active biological compounds. Table 1 presents the results of various tests conducted to determine whether the synthesized neem oil retained its essential compounds when n-hexane was replaced with petroleum ether. As depicted, coumarins, alkaloids, terpenoids, saponins, steroids,

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flavonoids, and triterpenoids were all detected in the extracted oil. The only compound found to be absent was tannins.

Table 1: Phytochemical Analysis of Neem Oil Using Petroleum Ether

S/N	Active Compound	Test Applied	Observation	Result
1.	Tannins	Ferric Chloride	Unchanged	Absent
2.	Coumarins	Sodium Hydroxide	Yellow Color	Present
3.	Alkaloid	Dragendorff	Redish-brown Precipitate	Present
4.	Terpenoids	Chloroform + H_2SO_4	Reddish-brown colouration	Present
5.	Saponins	Frothing Test	Persistent Frothing	Present
6.	Steroids	Salkowski Test	Brown-ring	Present
7.	Flavonoids	H_2SO_4	Orange to crimson red colouration	Present
8.	Triterpenoids	Chloroform + Acetic Anhydride	Golden-yellow Colour	Present

Physicochemical Parameters

Acid values serve as indicators of oxidative byproducts and corrosive free fatty acids, reflecting the degradation level of oil. This parameter is vital for assessing oil quality, as lower free fatty acid content typically denotes superior oil quality. Elevated acid values can lead to heightened corrosion within internal combustion engines and fuel supply systems [12]. The study herein revealed a notably low acid value of 1.70 (mg KOH/g), as illustrated in Table 2.

The oil's density was determined to be 0.81, falling within the established range of 0.8–0.9 for non-edible oils, as per prior research [13]. Density stands as a crucial parameter for oil suitability, necessitating adherence to predefined thresholds. Additionally, the oil exhibited a saponification value of 206.7 mg KOH/g, aligning with ASTM standards. Ash content, another significant variable reflecting oil quality, was measured at 2.1%. A lower ash content, such as the recorded value, signifies the potential for utilizing the extracted neem oil in biofuel production, as indicated in previous studies [14].

The iodine value serves as a gauge of the degree of unsaturation present in fats and oils, with higher values indicating increased unsaturation, as observed in previous research [13]. Oils are categorized based on their iodine values: those exceeding 120 are classified as drying oils,

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those falling within the range of 60–120 are deemed semi-drying oils, and those below 60 are categorized as non-drying oils. In this investigation, the iodine value of neem oil was measured at 119.8 mg I2/g, closely aligning with the ASTM D763 standard (120 mg I2/g) for dry oils. Consequently, this oil exhibits potential applications in the cosmetic industry, as dry oils are preferred for their rapid skin absorption without leaving a sticky residue, as corroborated by previous studies [15]. Furthermore, a higher saponification value indicates a greater proportion of fatty acids within the oil, suggesting its potential utility in soap production, as suggested by prior research [14].

Table 2: Physicochemical Screening of Neem Seed Oil Extracted Using Petroleum Ether

Parameter	Measured Value	ASTM 763 Standard
Moisture Content (%)	3.53	0.3-6.0
Acid Value (mg KOH/g)	1.70	1.5-2.4
Density (g/cm^3)	0.81	0.8-0.9
Viscosity (mm^2/s)	31.60	25-35.5
Saponification Value (mg KOH/g)	268	200-220
Ash Content (%)	3.71	1.5-4.5
Iodine Value (mg I_2/100g)	119.8	120

Effect of Extraction Time

The oil yield, expressed as a percentage, was found to be dependent on the extraction duration. Typically, an increase in extraction time resulted in a corresponding rise in oil yield, as depicted in Figure 2. However, it was noted that beyond 2.5 hours, there was no significant increase in oil yield, consistent with previous findings. Table 3 provides a detailed breakdown of the extraction times ranging from 30 minutes to 2.5 hours, demonstrating an increase in oil yield from 19.74% to 57.08% when using petroleum ether at a constant temperature. The relationship between extraction time and oil yield is visually represented in Figure 2, illustrating a direct proportionality between the two parameters.

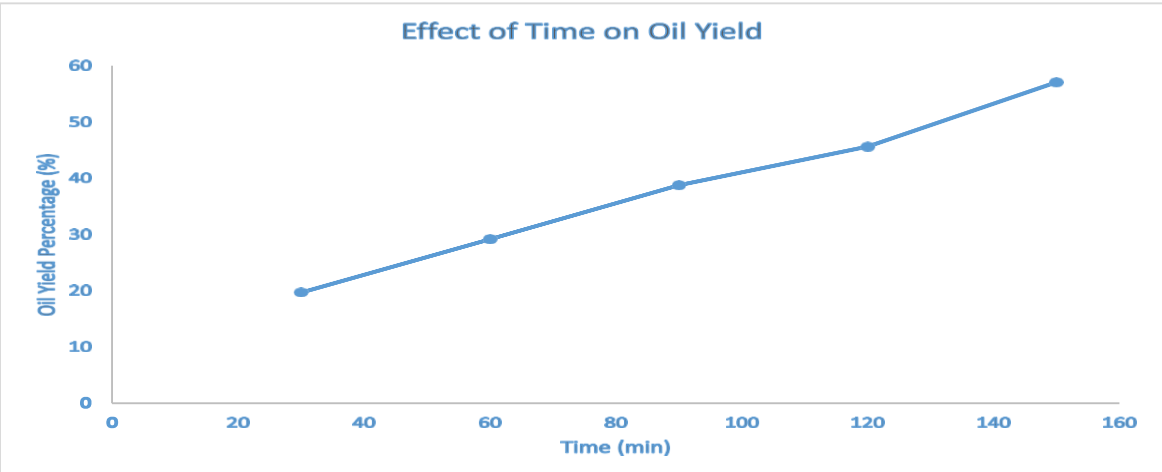

Figure 2: A Graph of Oil Yield Percentage with Time at Constant Temperature

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Table 3: Effect of Extraction Time on Oil Yield

Trial	Temperature (°C)	Time (min)	Oil Yield (%)
1	70°C	30	19.74
2	70°C	60	29.21
3	70°C	90	38.78
4	70°C	120	45.63
5	70°C	150	57.08

Effect of Extraction Temperature

Table 4 delineates the alteration in oil yield corresponding to varying temperatures. Within the temperature range of 25–125°C, there was a notable escalation in yield, ranging from 15.23% to 52%. Figure 3 visually illustrates this trend, indicating that higher temperatures generally result in increased oil yield. This phenomenon is attributed to the enhanced solubility of oils at elevated temperatures. Elevated temperatures lead to a reduction in solvent viscosity, alongside an increase in diffusivity and evaporation rate. Consequently, this augments the duration of contact between the solvent and the oil-bearing material, facilitating greater extraction efficiency [16].

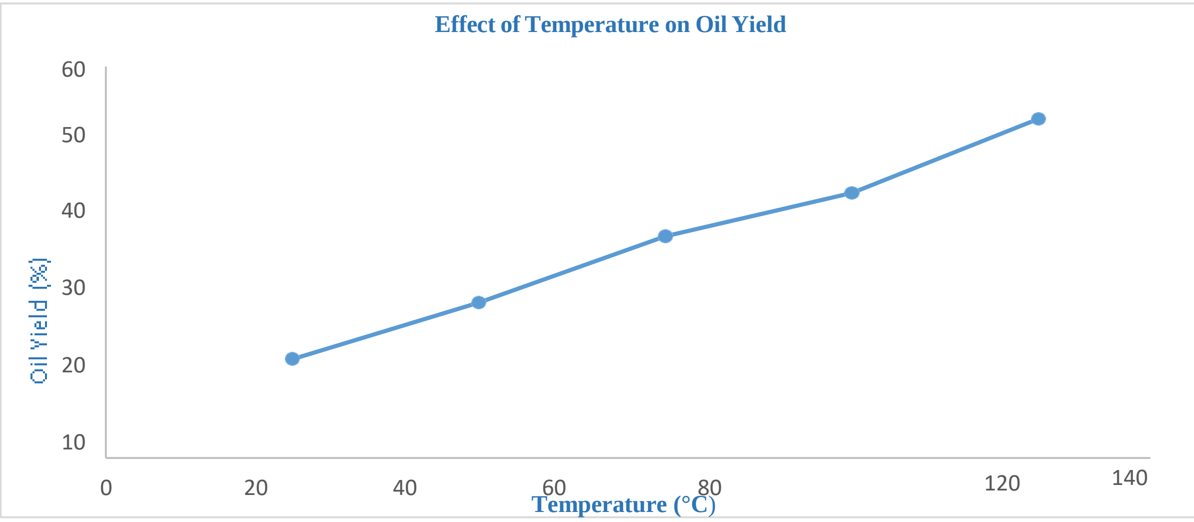

Figure 3: A Graph of Oil Yield Percentage with Temperature at Constant Time

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Table 4: Effect of Extraction Temperature on Oil Yield

Trial	Time (min)	Temperature (°C)	Oil Yield (%)
1	35	25	15.23
2	35	50	23.86
3	35	75	34.01
4	35	100	40.66
5	35	125	52

Saturated and Unsaturated Compositions of Fatty Acids in the Oil

The composition of fatty acids in the neem seed oil extracted using green petroleum ether was analyzed using gas chromatography-mass spectrometry. Five different types of fatty acids were identified. According to Table 5, the most abundant fatty acid was oleic acid (which is the unsaturated fatty acids), followed by palmitic acid, caprylic acid, stearic acid, and myristic acid, respectively. Among these fatty acids, only oleic is unsaturated and the rest are all saturated. The chromatography-mass spectrometry analysis revealed a significant presence of unsaturated fatty acids in the neem seed oil. Detailed information regarding the fatty acid composition and their structures can be found in the table below:

Table 5: GC-MS Result of Saturated and Unsaturated Composition of Fatty Acids in theOil

Fatty Acid	Formula	Composition (%)
Oleic acid	$C_{18}H_{34}O_2$	58.82
Palmitic acid	$C_{16}H_{32}O_2$	26.57
Caprylic acid	$C_8H_{16}O_2$	15.79
Stearic acid	$C_{18}H_{36}O_2$	0.005
Myristic acid	$C_{14}H_{28}O_2$	0.0055

Total Composition: **101.1905**
Total Unsaturated Fatty Acid: **58.82**

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CONCLUSION

The results of this study highlight the significance of using more environmentally friendly and sustainable extraction techniques, such as extracting neem seed oil using petroleum ether rather than N-hexane. Variations in temperature and duration in conjunction with the Soxhlet extraction method produced encouraging outcomes, with a maximum oil output recorded at 125°C and 150 minutes. Additionally, a substantial percentage of unsaturated fatty acids was found in the fatty acid composition analysis, indicating possible health advantages of consuming neem seed oil as part of a balanced diet. Numerous bioactive substances, such as alkaloids, coumarins, terpenoids, saponins, steroids, flavonoids, and triterpenoids, were found in the phytochemical examination. These molecules may be responsible for the extracted oil's medicinal qualities.

The extracted neem seed oil's good physical and chemical characteristics, which all meet ASTM criteria, were emphasized by the characterization process. These characteristics included low moisture content, acidity, density, viscosity, ash content, high saponification, and iodine values. These qualities highlight neem seed oil's potential for a wide range of industrial uses.

In conclusion, the use of more environmentally friendly extraction techniques in conjunction with thorough characterization has demonstrated the encouraging potential of neem seed oil as a renewable and adaptable resource with uses in a wide range of industries. Further research and development efforts are warranted to explore its full range of benefits and applications.

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CONFLICT OF INTEREST

The authors have reported no conflicts of interest.

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