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Comprehensive Physicochemical Profiling and Characterization of Neem Plant Leaf Extracts: Insights for Pharmaceutical & Biomedical Applications

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Abstract

This study presents a comprehensive physicochemical analysis of neem plant leaf extracts with a focus on their potential applications in pharmaceutical and biomedical contexts. Utilizing the soxhlet extraction method with n-hexane as the solvent, the study investigated the quantitative and qualitative composition of neem leaf extracts in reference to concentrations. The results revealed a diverse array of compounds, including cyanogenic glycoside, cardiac glycoside, tannin, steroids, phytate, flavone, oxalate, rutin, lunamarin, catechin, spatein, naringin, resveratrol, kaempferol, flavonones, epicatechin, and epihedrine, with notable concentrations. Further analyses indicated shared physicochemical properties, such as carboxyl and hydroxyl groups. Qualitative assessments affirmed the presence of flavonoid and phenolic compounds, while FTIR analysis confirmed the existence of carboxyl and hydroxyl groups. These findings emphasize the potential use of neem leaves as pharmaceutical raw materials due to their antioxidant-rich content. Additionally, the study explored the density, viscosity, saponification value, and foaming power of neem leaf extracts, providing insights into their industrial applicability. GC-MS analyses highlighted the presence of significant chemical compounds, with potential therapeutic implications. Mineral analysis demonstrated essential elements for human and animal nutrition. This study underscores neem plant leaves' multifaceted potential across pharmaceutical, herbal medicine, cosmetic, and functional food sectors. It lays a solid foundation for further research into the specific health benefits, offering valuable insights for harnessing neem leaves' potential in innovative products and treatments.

Keywords

Phytochemical, N-Hexane, Neem Leaves, FTIR, Pharmaceutical, Biomedicine, Biomedical, Antioxidant, Chemical, Herbal

1. Introduction

The neem (*Azadirachta indica* A. Juss) trees have been grown successfully in all parts of Nigeria. Neem has become a naturalized species in various parts of Nigeria. Neem tree occurs throughout Nigeria; its performance is quite good even in the harshest conditions. Most of the original plantations were carried out by the colonial officers along the railway and the Nile banks and then spread all over the country. The neem tree is noted for its drought resistance. Normally it thrives in areas with sub-humid conditions, with annual rainfall between 400 and 1200 mm. It can grow in regions with an annual rainfall below 400 mm, but in such a case it depends largely on groundwater. Neem can grow in many different types of soil, but it thrives best on well-drained deep, and sandy soils. It is a typical tropical/subtropical tree and exists at annual mean temperatures within 21˚C - 32˚C. It can tolerate temperatures below 40˚C. Temperature is one of the most important factors affecting seeds. Water uptake, gas diffusion, respiration, and other metabolic processes all proceed faster at higher temperatures. Germination is dependent on all these processes and thus is strongly affected by temperature [1]. In India, neem grows in the plains and in areas that reach an elevation of approximately 1850 m. In its introduced range, neem is cultivated from sea level up to 1500 m elevation. Neem is tolerant to most soil types including dry, stony, shallow soils, lateritic crusts, and highly leached sands and clay.

Seed propagation is the usual method of neem regeneration. Seeds normally do not store well over 6 months. Seeds should be cleaned with water to remove the casing. No pre-treatment is necessary, commonly planted in nursery seedbeds for 8 - 11 months. Neem seedlings, once established, do not need frequent watering or fertilizing. Mature fresh seeds germinate within 2 - 3 weeks with a germination percentage of 75% - 90%. Neem can also be propagated vegetatively by air layering, root and shoot cuttings, grafting, and cutting. Clonal propagation and micropropagation by somatic embryogenesis and organogenesis are also used. The term "provenance" is normally used in a broad sense, including all seed sources (natural and introduced) from anywhere in the world. Provenance testing of a tree species is important because it defines the genetic and environmental components of phenotypic variations that are associated with geographic locations.

Neem is known as *Azadirachta indica* in its botanical name and it belongs to the Meliaceae family. It is a common plant found throughout the world with many therapeutic benefits [2]. According to the research study by [3], Neem plants grow very well in regions with minimum rainfall. According to [4], plants

are composed of extensive ranges of phytochemical compositions known as secondary metabolites. [5], stated that these secondary metabolites can be used especially in pharmaceutical industries. The research study of [6], reviewed that these secondary metabolites can act as antioxidants by preventing cell damage which is caused by free radicals in the body and those associated with heart diseases. The research study of [6], further stated some secondary metabolites that act like antioxidants are flavonoids, carotenoids, and polyphenols. [5] [6] also stated that phytochemicals can act as hormones in the body and concluded in his research work that neem oil using ethanol solvent had the highest activity on *Candida albicans* when compared to other extracting solvents. Neem plants have been reported by many researchers to have medicinal uses such as cures for skin diseases fever and antiviral sicknesses. For instance, [7] stated that Neem leaves paste is used to treat smallpox and chicken pox. According to the research study by [8], the leaf juices are used to increase appetite and intestine worm removal. [9] also reported that the leaves extract has antimicrobial potency against dental pathogens. Finally, the review made by [10], on contemporary medicinal uses to humankind showed that neem leaves extract and its formulated product could be used in the treatment of AIDS, cancer, and other several body system disorder.

Neem leaf juice extract contains bioactive substances, for instance, phenolic compounds [11]. The following compounds have been found in Neem leaves plant juices: Steroids, tannins, flavonoids, saponins, amino acids, anthraquinones, triterpenoids, carbohydrates, glycosides, polyphenolics, Nimbin, and several others [12] [13], used immersion and Soxhlet methods technique to extract neem leaf juice. And it was concluded that the Soxhlet extraction method using n-hexane solvent yielded a better result than immersion.

In classical methods of optimization, the research works of [14] [15], stated that it is difficult to solve the complex interactions between the process variables and responses of the extraction since it can only handle the variation of one parameter at a time, while other factors are constant. They further stated that response surface methodology is one of the most, potable approach to design experiments statistically. Several researchers have extracted and determined the optimum conditions for the extraction of juice from neem plants using different extracting solvents. [16], optimized the extraction of phenolic compounds in neem leaves using surface response methodology, he stated that the optimum concentration, temperature, and time for the extraction of the total phenolic compound are 0.01 g/ml, 40.54˚C and 2.79 hours. [17], optimized the extraction of neem seed oil using a central composite design of surface responses, according to his research study, he stated that the optimum time and temperature for the extraction of oil yield, pH, iodine value, and saponification value are 6 hours, and 37.20˚C. [18] optimized and characterized neem seed oil extraction using the surface response method at a constant temperature of 60˚C and varying particle size and time, he later obtained the optimum yield, particle size, and time to be 49%, 1.39 mm, and 2 hours. The flavonoid content of neem leaves was opti-

mized using surface response methodology by [19], the work reviewed that the optimum temperature and extraction time for the flavonoid content of neem leaves are 80˚C and 80 minutes.

Having reviewed all these, it is observed that no work has been done on neem leaves to consider the effect of time and temperature yield and antioxidant compounds (tannin or amino acid or steroid) using response surface methodology which this work considers.

2. An Overview of Neem Plant Leaves

Neem is known to be a large flow plant of about 25 meters in length. Neem plants produce fruit upon maturity which is between the period of 4 - 5 years and its productivity is within 10 years [20]. The tree has a rough and grey bark, and the length of the leaves are up to 30 cm. [3] reported that Neem trees grow very well in countries with minimum rainfall. The neem plant is a useful resource for many purposes such as agriculture and medicine. Oil from neem seed is used for soap production, lubricant oil for engines, and edible oil after refining [21].

[22], reported that *Azadirachta indica* leaves and seed have a therapeutic role which could be a result of the rich source of antioxidant and other invaluable active compounds such as azadirachtin, salannin, nimbolinin, nimbidol, nimbin, quercetin, and nimbidin. Many other researchers have reported that its Neem leaf juices could be used for the treatment of cancer skin diseases, sexually transmitted diseases, hypolipidemic activities, antimicrobial activities, antibacterial activities, fever, diabetes, and several over human diseases. It is also used in preparing mosquito-repellent tablets [7] [9] [10] [23]. Neem and its ingredients play a vital role in the growth inhibition of several microorganisms such as bacteria, viruses, and pathogenic fungi [22].

3. Materials and Method

3.1. Sample Collection and Preparation

The mature neem leaves were harvested from a neem tree in International Secondary School Residential Quarters Uturu, in Abia State, Nigeria in the morning hours of the day. In a bid to retain the physiochemical components of the leaves, the sun drying method was used. The dirt on of the leaves was removed and sun-dried for a total of three days (72 hours). The drying was to achieve a constant moisture content, which also made the extraction process much easier.

After three days, the dried neem leaves were ground to reduce the particle size thereby increasing the surface area for easy extraction. After the leaves were grounded, they were kept in a sealed plastic bag at room temperature and protected from sunlight. The neem leaves particle size reduction was done with a mortar and pestle, the ground particle was sieved using a mesh into different sizes (0.42 mm, 1.41 mm, and 2.38 mm) of model BS 410, Endecott Ltd, London.

3.2. Neem Leaves Juice Extraction Using Solvent Soxhlet Extractor Technique

The neem leaves juices were extracted using the Soxhlet extraction method. Neem juice in the neem leaves powder was extracted at a temperature of 60˚C with n-hexane solvent using a heating mantle. This condition was designed to determine the optimum temperature and time for the optimum yield. The solvent used was n-hexane. The neem seed powder was packed inside a muslin cloth and placed in a thimble of Soxhlet extractor. A round bottom flask containing n-hexane was fixed to the end of the extractor and a condenser was tightly fixed at the bottom end of the extractor. The flask was heated at 60˚C with the use of an electric mantle. The solvent then vaporized and condensed into the evaporator. The extract moved directly into a round bottom flask. The process continues for the specified time. After extraction, the n-hexane solvent in the extracted juice was evaporated completely by using a rotary evaporator (model).

3.3. Extraction of Phytochemicals

1 g of sample was weighed and transferred in a test tube and 15 ml ethanol and 10 ml of 50% m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60˚C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separator funnel. The tube was washed multiple times with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water, and 3 ml of hexane, which were all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 ul of pyridine of which 200 ul was transferred to a vial for analysis.

3.4. Qualitative Analysis of Phytochemicals

Qualitative analyses were carried out using the methods of [24] [25] to ascertain the presence of different phytochemicals in the leaves before quantitative analysis are carried out.

3.5. Test for Alkaloids

Procedure: Pipette 1.0 ml of extract in a test tube, Pipette 5.0 ml of 2% HCl in a test tube and heat the test tube in a water bath (Memmert) for 10 minutes, Filter using Whatmann No 1 filter paper. Use the filtrate for the following tests.

Wagner's Reagent Test

Principle: Alkaloids under acidic condition and at room temperature reacts with iodine and potassium iodide to give a reddish-brown precipitate.

Reagent: Wagner's reagent: 2 g of iodide and 3 g of potassium iodide are weighed, mixed, and dissolved in 30 ml distilled water and made up to 100 ml with distilled water.

Procedure: Pipette 1.0 ml of filtrate in a test tube. Pipette 1.0 ml of Wagner's reagent in the test tube. Mix properly and observe for color change.

A reddish-brown precipitate indicates the presence of alkaloids.

Meyer's Reagent Test

Principle: Alkaloids under acidic condition and at room temperature reacts with mercuric chloride and potassium iodide to give a cream color or precipitate.

Reagents: Dissolve Meyer's reagent: 1.4 of mercuric chloride in 60 ml of distilled water and 4.5 g of potassium iodide in 20 ml of distilled water. The two solutions are mixed and diluted to 100 ml with distilled water.

Procedure: Pipette 1.0 ml of filtrate in a test tube. Pipette 1.0 ml of Meyer's reagent in the same test tube**.** Mix properly and observe for color change.

Cream color precipitate indicates the presence of alkaloids [24].

3.6. Test for Flavonoids

Ferric Chloride Test for Phenolic Nucleus:

Principle: Phenolic nucleus reacts with ferric chloride at room temperature to give greenish brown or black color or precipitate.

Reagent: 10% $FeCl_2$ (weigh 10.0 g $FeCl_2$ and dissolve in 100 ml of distilled water).

Procedure: Pipette 1.0 ml extract into a test tube. Pipette 1.0 ml 10% ferric chloride in the test tube. Mix and observe any change in color. Greenish brown or black color/precipitate is an indication of the presence of a phenolic nucleus.

Lead Acetate Test

Principle: Flavonoids at room temperature react with lead acetate to give a yellow color or precipitate.

Reagent: 10% lead acetate (weigh 10.0 g lead acetate and dissolve in 100 ml distilled water).

Procedure: Pipette 1.0 ml extract into a test tube. Pipette 1.0 ml of 10% lead acetate solution in the same test tube. Mix properly and observe color change or precipitate which indicates the presence of flavonoids.

Sodium Hydroxide Test

Principle: Flavonoids at room temperature and under alkaline pH form an observable precipitate.

Reagent: Dilute NaOH: (weigh 40 g of NaOH and dissolve in 1 liter of distilled water).

Procedure: Pipette 1.0 ml of extract into a test tube. Pipette 1.0 ml of dilute NaOH solution in the same test tube. Mix and observe any change in color. The formation of a precipitate indicates the presence of flavonoids [24].

3.7. Test for Tannins

Acid Test

Principle: Phlobotannins under acidic condition reacts with dilute HCl to give a red color or precipitate.

Reagent: 1% HCl, pipette 1.0 ml concentrated HCl and made up to 100 ml with distilled water.

Procedure: Add 3.0 ml of extract to 2.0 of 1% HCl. Observe the presence of red color or precipitate. This indicates the presence of phlorotannins.

Lead Acetate Test

Principle: phlorotannins react at room temperature with lead acetate to give a dark-blue to black precipitate.

Reagent: 5% lead acetate: weigh 5.0 g lead acetate and dissolve in 100 ml distilled water

Procedure: Pipette into a test tube, 2 ml of extract. Add 3 drops of 5% lead acetate solution to the extract. A dark blue to black precipitate indicates the presence of phlorotannins.

3.8. Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

3.9. Test for Cardiac Glycosides

One (1 ml) of the extract was added 10 cm^3 of 50% H_2SO_4 and was heated in boiling H_2O for 5 minutes. 10 cm^3 of Fehling's solution (5 cm^3 of each solution A and B) was added and boiled. A brick red precipitate indicating the presence of glycosides was observed.

3.10. Test for Terpenoids

Five (5 ml) of each extract was mixed in 2 ml of chloroform (Numex, India), and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inner face was formed to show positive results for the presence of terpenoids.

3.11. Test for Steroids

0.5 ml of the chloroform extract in a test tube was carefully added 1 ml concentrated H_2SO_4 to form a lower layer. Then, the color at the interface was observed and recorded.

3.12. Physicochemical Analysis

The following physicochemical analyses (density, viscosity, saponification value, iodine value, acid value, peroxide value, and free fatty acid) of the neem oil were carried out using the association of Official Analytical Chemists' methods [26].

3.13. Quantification by GC-FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromato-

graphy equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m × 250 um × 0.15 um) was used. The injector temperature was 280˚C with a spitless injection of 2ul of sample and a linear velocity of 30 cms^{-1} Helium 5.0 pas was the carrier gas with a flow rate of 40 $ml{\cdot}min^{-1}$. The oven operated initially at 200˚C, it was heated to 330˚C at a rate of $3˚C{\cdot}min^{-1}$ and was kept at this temperature for 5 min. the detector operated at a temperature of 320˚C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expresses in ug/g.

4. Results of Analysis

The results of the general analysis provide a comprehensive overview of the key characteristics and composition of the studied subject. Through systematic investigation and rigorous data collection, essential parameters were assessed, yielding valuable insights. This analysis encompassed a range of aspects, including physicochemical properties, elemental composition, and potential contaminants.

The data revealed notable findings in terms of elemental presence, where elements such as Calcium, Selenium, Molybdenum, Potassium, Sodium, Iron, Copper, and Zinc were identified and quantified. This composition holds significant implications for various applications, particularly in the biomedical and pharmaceutical sectors, where these elements play pivotal roles in physiological processes and product formulation.

Moreover, the absence of toxic heavy metals, such as Mercury, and the presence of trace amounts of Arsenic and Tin at levels below thresholds of concern offer reassurance regarding potential health risks associated with the studied subject.

The outcomes of this general analysis lay the foundation for subsequent in-depth investigations and application-specific studies. They provide a valuable baseline for further research, guiding formulation development, industrial processing, and regulatory compliance. As a result, these results contribute to an enhanced understanding of the subject's potential and limitations in diverse fields, ultimately fostering advancements in both scientific knowledge and practical applications.

5. Discussion

Extraction and characterization of juice from neem plant (leaves) was studied in this research work. It was studied to determine the phytochemical and physiochemical compositions of neem leaves and their usefulness to health. The rest of the discussion of the results obtained is stated under the following headings below.

5.1. Physicochemical Analysis of Neem Leaf Juice

The results of the physicochemical analysis of the neem oil extracted are shown

in Table 1. The density of the neem leave oil was measured at 25˚C and was measured to be 0.889 kg/m^3. Comparing the obtained density of neem leaves oil and the density of most vegetable oil, it lies between 0.8767 and 0.8811 kg/m^3. [18] explained that the denser the oil the higher energy it contains. The kinematic viscosity measures the flow resistance of the fuel. The value of viscosity obtained was 36.67 mm^2/s. The foaming power of oil defines its ability to be used in cosmetics industries. The saponification value of the neem leaves oil obtained was 210 mg·KOH/g.

5.2. GC-MS Quantitative Phytochemical Properties of Neem Leaves

The results of the quantitative phytochemical analysis of the neem oil extracted are shown in Table 2. A sensitive GC–MS was used for the identification of different chemical compositions in the crude extracts obtained from the neem leaves. The chemical compounds steroid, Cyanogenic glycoside, Rutin, Lunamarin, Catechin, Flavone, Epicatechin, and ephedrine were obtained at very high concentrations. The quantitative analysis results obtained indicated that neem leaves plant contain cyanogenic glycoside (10.8555 ppm), Cardiac glycoside (7.8854 ppm), Tannin (5.6680 ppm), Steroids (13.0406 ppm), phytate (7.1886 ppm), flavone (10.6777 ppm), oxalate (5.9552 ppm), rutin (13.5812 ppm), lunamarine (14.0509 ppm), catechin (10.1884 ppm), spatein (8.2814 ppm), naringin (9.3647 ppm), resveratrol (5.8222 ppm) kaempferol (9.0841 ppm), Flavonones (7.5546 ppm), epicatechin (33.9152 ppm), and epihedrine (32.4385 ppm). The results showed that epicatechin (33.9152 ppm), and epihedrine (32.4385 ppm) have the highest concentration, this could be a result of the extraction solvent used which can result in an increase in solubility of these components. All the major chemical ingredients obtained from different extracts are hydrocarbon, flavonoids, and phenolic derivatives. [27], explained that the major chemical ingredients have previously been reported from a number of other plant species.

5.3. Qualitative Phytochemical of Neem Leaves

According to the qualitative phytochemical analysis results in Table 3, the results obtained showed that alkaloid, flavonoid, Gardiac glycoside, phenolic, and steroid were found to be present in neem leaves. The qualitative result affirms the presence of these components of neem leaves according to the quantitative analysis results in Table 4. The presence of these phytochemicals in the extracts means that neem leaf juice can be used in herbal treatment. The neem leaves juice may serve culinary purposes and possess anti-inflammatory properties because of the presence of flavonoids in it [28]. Also, the presence of appreciable amounts of flavonoid and phenolic have been linked with good free radical scavenging activity of N. latifolia ethanolic extract which is an attribute of excellent antioxidants. This implies that the neem leaves extract in his study may possess more unique antioxidant properties when compared with the lemongrass oil extract [29]. The presence of terpenoids was not much present according to the

Table 1. Physicochemical characterization of Neem Leaf Juice.

Properties	Values
Density (kg/m^3) at 25˚C	0.889
Viscosity (mm^2/s)	36.67
Saponification value (mg·KOH/g)	210.00
Iodine value (g·I_2/100 g)	36.54
Acid value (mg·KOH/g)	2.88
Free fatty acid (% w/w)	5.11
Peroxide value (meqo_2/kg)	10.20

Table 2. Quantitative phytochemical analysis of Neem Leaf Juice.

Parameters	Composition (ppm)
Flavan-3-ol	3.8774
Tannin	5.6680
Cardiac glycoside	7.8854
Cyanogenic glycoside	10.8555
Flavonones	7.5546
Steroids	13.0406
Phytate	7.1886
Flavone	10.6777
Oxalate	5.9552

Table 3. Qualitative phytochemical analysis of Neem Leaves Juice.

Parameters	Observations	Inference
Alkaloid	Reddish brown ppt formed	++
Flavonoids	Formation of ppt	++
Tannin	Formation red brown ppt	+
Saponnin	Formation of light emulsion	+
Gardiac glycoside	Formation of brick red ppt	++
Steroid	Emulsion formed	++
Phenolic	Green-brown ppt	++
Resin	No colour change	+
Terpenoids	Slight reddish brown ppt form	+

Table 4. Quantitative phytochemical analysis of Neem Leaf Constituents.

Parameters	Composition (ppm)
Lunamarin	14.0509
Catechin	10.1884

Continued

Rutin	13.5812
Spatein	8.2814
Sapogenin	3.4794
Naringin	9.3647
Kaempferol	9.0841
Epicatechin	33.9152
Resveratrol	5.8222
Proanthocyanin	2.0734
Epihedrine	32.4385

qualitative results obtained. According to [30], the presence of terpenoids in leaves makes it possible for them useful for dietary purposes such as in the management of metabolic disorders which are induced by obesity. Also, this justifies the use of neem leaf extract for the treatment of diabetes in traditional medicine, [5]. Most of the phytochemical compositions obtained in the results from neem leaves such as alkaloids, steroids, flavonoids, tannins, saponin, and fatty acid were also identified in the research study of [31].

5.4. Phytochemical Analysis

Phytochemical analysis plays a pivotal role in understanding the chemical composition of plant extracts and their potential impacts on various industries and human health. This study presents a quantitative analysis of neem leaf juice, focusing on parameters such as Flavan-3-ol, Tannin, Cardiac glycoside, Cyanogenic glycoside, Flavonones, Steroids, Phytate, Flavone, and Oxalate (**Figure 1**). The implications of these phytochemical compositions on the biomedical, pharmaceutical, and human health sectors are listed below.

5.5. Effect on Biomedical Industry

The presence of Flavonones, Steroids, and other phytochemicals in neem leaf juice can have implications for the biomedical industry. Flavonones, for instance, possess antioxidant and anti-inflammatory properties that could be harnessed for developing novel therapeutic agents. Steroids are known for their diverse physiological effects, suggesting that neem leaf juice might serve as a resource for steroid-based drug development. Additionally, the detection of Cardiac glycosides raises the possibility of neem leaf juice contributing to cardiovascular drug research.

5.6. Impact on Pharmaceutical Industry

The quantitative analysis reveals the presence of Tannins, Cyanogenic glycosides, and Flavones in neem leaf juice. Tannins have astringent properties and could be utilized in the formulation of pharmaceutical products such as wound

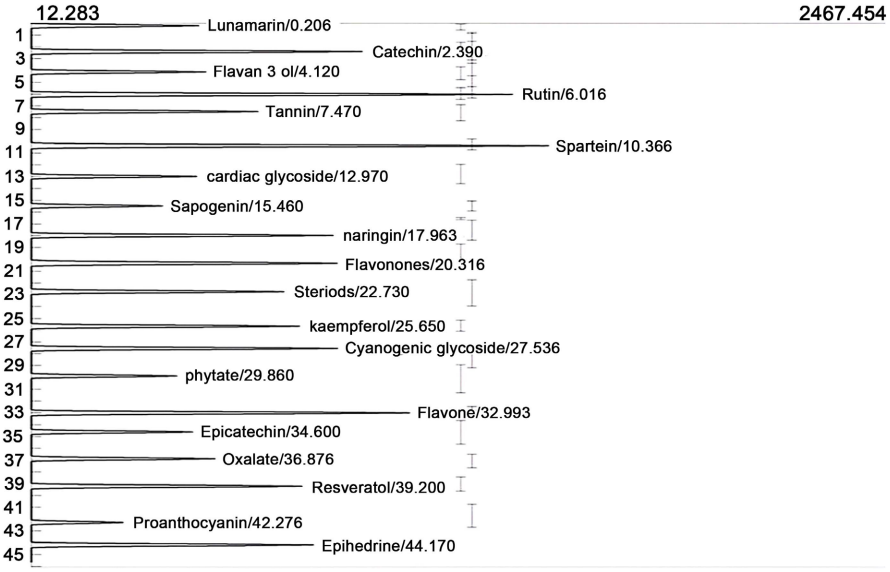

Figure 1. Visualization of GC-MS analysis outcomes for sample of Neem Leaves Juice.

healing agents and anti-diarrheal medications. Cyanogenic glycosides are potential precursors of hydrogen cyanide, requiring careful consideration in pharmaceutical applications. Flavones, known for their antioxidant and anti-cancer properties, may stimulate the development of new anticancer drugs or dietary supplements.

5.7. Relevance to Human Health

The phytochemical content of neem leaf juice has direct implications for human health. Flavan-3-ol, with its potential antioxidant effects, could contribute to cellular protection against oxidative stress. Cyanogenic glycosides' presence necessitates controlled consumption due to their toxicity. Conversely, the presence of Phytates and Oxalates might raise concerns regarding nutrient absorption and kidney stone formation in individuals with high neem leaf juice intake.

5.8. FTIR Analysis of Neem Leaves

The functional groups present in neem leaves ethanol extract were determined using FTIR analysis at different wavelengths of transmittance. The FTIR analysis of neem leaves results obtained are shown contained in **Figure 2**. The peaks observed within the range of 3833.018 - 2714.719 correspond to O-H and C-H stretching frequencies, respectively. This means that neem leaf extract contains some hydroxyl groups such as phenol, alkane, alkenes, and alcohol. The peak within 858.5226 - 2064.345 corresponds to the carboxyl (–C=O), so might be contributed to the presence of the methoxy group. The results obtained also correspond to the research work of [32].

5.9. Mineral Analysis of Neem Leaves

This discussion examines the heavy metal compositions identified in the analysis

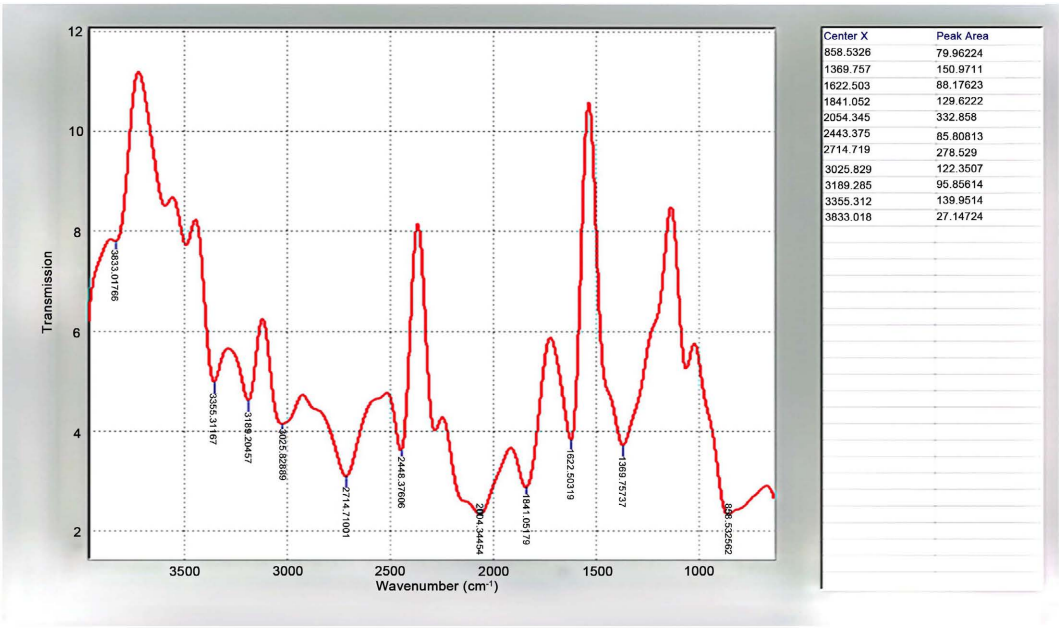

Figure 2. FTIR analysis findings for Neem Leaf Sample.

of neem leaves juice and their potential implications on various aspects, including biomedical, pharmaceutical, industrial applications, and human health. The concentrations of different heavy metals, such as Calcium, Selenium, Molybdenum, Potassium, Sodium, Iron, Copper, Mercury, Arsenic, Tin, Zinc, and Chromium, are presented in parts per million (ppm) in Table 5. The presence of heavy metals in natural products like neem leaves juice can have profound effects on both biological systems and industrial processes, necessitating careful consideration and assessment which are as follows.

6. Impact on Biomedical and Pharmaceutical Applications

The presence of Calcium, Selenium, Molybdenum, Potassium, Sodium, Iron, Copper, and Zinc in neem leaves juice could contribute to its potential biomedical applications. These elements play essential roles in various physiological processes and can enhance the therapeutic properties of the juice. For instance, Iron is critical for oxygen transport in the blood, while Copper is involved in enzymatic reactions. However, the concentrations of these metals must be within acceptable limits to avoid potential toxicity or adverse effects on human health.

In pharmaceutical applications, the heavy metal composition can influence the stability and efficacy of formulations containing neem leaves juice. Metal ions can interact with active pharmaceutical ingredients, altering their chemical properties and affecting the overall product quality. Therefore, understanding the concentrations of these heavy metals is crucial for maintaining consistent pharmaceutical product quality [33].

6.1. Industrial Implications

In the industrial context, the presence of heavy metals in neem leaves juice can

Table 5. Heavy metal compositions in the analysis of Neem Leaves Juice.

Parameters	Composition (ppm)
Calcium	4.034
Selenium	2.060
Molybdenum	0.107
Potassium	3.407
Sodium	4.187
Iron	5.004
Copper	8.002
Mercury	0.00
Arsenic	0.006
Tin	0.006
Zinc	5.765
Chromium ppm	0.438
Aluminum	0.023
Cadmium	0.045
Silver	0.102
Lead	0.065
Manganese	0.102
Cobalt ppm	0.012
Silicon	0.024

impact processing and manufacturing procedures. Elements like Chromium and Zinc may affect the extraction processes, altering the yield and purity of the final product. Additionally, the potential for heavy metal contamination in products derived from neem leaves could raise concerns about compliance with regulatory standards in various industries.

6.2. Effects on Human Health

The safety of neem leaves juice for human consumption relies on assessing the levels of toxic heavy metals such as Mercury, Arsenic, and Tin. These metals are known for their harmful effects even at trace concentrations. Monitoring and controlling the presence of these toxic metals are paramount to ensure that the consumption of neem leaves juice does not pose risks to human health.

7. Conclusions

In conclusion, the meticulous research conducted in this study has yielded an extensive body of knowledge concerning neem leaf extracts, shedding profound insights into both their phytochemical and physicochemical attributes. Through a rigorous process of comprehensive physicochemical analysis, fundamental

properties such as density, viscosity, saponification value, and foaming power have been unveiled. These revelations underscore the multifaceted potential applications of neem leaf juice across diverse industrial sectors.

The quantitative phytochemical analysis, employing advanced GC-MS techniques, has illuminated a myriad of chemical constituents present in neem leaf juice. Notably, notable concentrations of cyanogenic glycosides, cardiac glycosides, flavonoids, and steroids have been identified, emphasizing the intricate assortment of bioactive elements within neem leaves. This intricate composition suggests potential therapeutic avenues for addressing human health concerns.

Furthermore, the qualitative phytochemical analysis has corroborated the presence of alkaloids, flavonoids, cardiac glycosides, phenolics, and steroids, substantiating the prospects for both medicinal and culinary applications of neem leaf juice. The significant levels of flavonoids and phenolics suggest antioxidative potential, essential for counteracting oxidative stress and promoting overall wellness.

The insightful FTIR analysis has unveiled the underlying functional groups inherent in neem leaf extract, prominently featuring hydroxyl and carboxyl groups characteristic of specific bioactive compounds. This nuanced comprehension enhances our grasp of neem leaf chemistry, bolstering its potential roles in herbal therapies and functional foods.

Moreover, mineral analysis has accentuated the abundance of pivotal elements, including calcium, selenium, potassium, sodium, iron, copper, and zinc, within neem leaves. This nutritional profile underscores the capacity of neem leaf juice to augment health and vitality for both human and animal consumption.

In summation, this study makes a significant contribution to the existing reservoir of knowledge concerning neem leaves and their versatile potential in various sectors, spanning pharmaceuticals, herbal medicine, cosmetics, and functional foods. The profusion of bioactive compounds, phytochemicals, and essential minerals underscores the intrinsic worth of neem leaf juice as a remarkable natural resource with promising health-promoting attributes [34].

However, while these findings offer a robust foundation for further exploration into the therapeutic utilities of neem leaf juice, it is paramount to acknowledge the imperative of additional clinical investigations to validate its efficacy and safety for medical applications. Nevertheless, the thorough and systematic analysis presented in this study establishes a platform for unlocking the latent potential of neem leaves and their substantive contributions to human health and overall well-being.

8. Recommendations

Extraction remained a fundamental unit operation in ascertaining the compositions of leaves extracts, it is recommended:

1) That the optimization of the neem leaf extraction should be researched with different extracting solvents and temperatures using surface response methodology to determine the compositions of neem leaf juice.

2) The modeling of the extraction of neem leaf juice is to be carried out in order to determine the possible outcome of neem leaves juice on a higher scale.

9. Materials and Apparatus Utilized

Retort stand, 500 ml separation funnel, 250 ml and 150 ml beaker, Pipette, Test tube, electronic weighing balance, Water bath, Bunsen burner, 500 ml flat bottom flask, Mortar and Pestle, Sieve, Soxhlet extractor Apparatus, Heating mantle, BUCK M910 Gas chromatography, Distilled water, N-hexane, Sodium hydroxide, Hydrochloric acid, Lead acetate solution, Fehling's solution, Meyer's reagent, Wagner's reagent.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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