

Comparison of Neem Leaf (*Azadirachta Indica* A. Juss) and Scent Leaf (*Ocimum Gratissimum*) Phytochemical and Nutritional Properties

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Abstract: 'Scent leaf' and Neem leaf' are commonly consumed vegetables in Southern Nigeria. *O. gratissimum* is used by the 'Igbos' of South East Nigeria to flavor soups and stews while it is popular with the 'Yorubas' of South West Nigeria for treatment of stomach disorders. The enormous use of these vegetables in the diet motivated the present study whereby nutritional compositions. Neem and Scent leaves were plucked from parent trees at farms within, Esa Oke, Osun State. The aqueous extract of plant was prepared by weighting 200g of the powder on a weighing balance and soaked in 500ml distilled water in one liter conical flask. Qualitative Phytochemical, Quantitative Phytochemical and Proximate analysis were determined using their various standard methods. Qualitative photochemical analysis of neem leaf shows the presence of saponins, tannins, flavonoids while alkaloids and phenol are absent. Scent leaf had saponins, tannins, flavonoids, alkaloids and phenol. Quantitative phytochemicals analysis shows the varied quantities of alkaloids, saponins, tannins, flavonoids, and phenols but quantity of tannin is not present in neem leaf. Proximate analysis was determined using their various standard methods. The results of proximate analysis revealed the presence of crude protein (18.5 ± 0.7 and 3.57 ± 0.50), crude fat (2.27 ± 0.37 and 2.67 ± 0.51), ash content (8.36 ± 1.08 and 5.80 ± 0.30), moisture content (14.57 ± 0.65 and 56.3 ± 4.20), Dry matter (91.0 ± 1.85 and 76.2 ± 5.33) and carbohydrate (0.00 ± 0.00 and 33.0 ± 1.57) each in neem and *O. gratissimum* respectively. There are higher values of phytochemicals and food components in *O. gratissimum* had phytochemical constituents and nutritional composition. These two vegetables are potential source of components for complementary medicine.

Keywords: Phytochemical, Neem leaf, Water leaf, Nutritional, Qualitative, Quantitative.

1. INTRODUCTION

Plants whose roots, stems, leaves, and seeds have therapeutic, tonic, and other pharmacological potential have been identified and known throughout human history as medicinal plants (Mgbemena and Amako, 2020). They are utilized as drugs, food additives, and nutritional supplements. The chemical components in some plants that generate specific physiological activities in the body are what give them medicinal significance. Phytochemicals are bioactive natural compounds found in plants, and they are among the chemical substances (Kolawole *et al.*, 2018).

Chemical substances in these plants mediate their effects on the human body through processes that are similar to those that are widely understood in conventional medications. As a result, the effects of herbal remedies on the body are similar to those of conventional drugs. This allows herbal treatments to be effective while also posing a risk of dangerous side effects (Tapsil *et al.*, 2006; Lai and Roy, 2004).

Because medicinal plants are known to contain some chemical compounds that can be utilized for treatment purposes or to make medications, the usage of herbs and the hunt for drugs and nutritional supplements derived from plants has increased in recent years (Sofowora, 1999)

Medicinal plants have an important part in people's health; in fact, most modern pharmaceuticals are derived from them (Odugbemi, 2006; WHO, 2004). Plant-based medicines have the advantages of being simple, efficacious, and having a broad spectrum of action. Knowledge of the chemical elements of plants is required for the discovery of therapeutic agents in plants as well as the discovery of new sources of economic materials such as essential oils, gums, precursors, and other materials for the synthesis of complicated chemical molecules (Erinle, 2012).

Pharmacologists, natural products and environmental chemists, botanists, microbiologists, and other researchers are scouring the globe for phytochemicals derived from plants that could be developed further for the treatment of various ailments and for new development possibilities.

Due to its strong aromatic fragrance, *Ocimum gratissimum* (Scent leaf) is a scented shrub with lime green fuzzy leaves that are used to flavor soup and spice cuisine (Oladosu-Ajahi et al., 2017). The essential oil in the leaves and stems of *Ocimum gratissimum* is the reason for its cultivation. The essential oil has antimicrobial effects (Health facts, 2015), and the leaves, when dried and burned, are used as insect repellents (Health facts, 2015). It is used in traditional medicine to treat diarrhea (Iwu, 1993), stomach ache, as a febrifuge, and as a component of anti-malaria treatments (Stasi et al., 2002), wound dressing, skin infection, conjunctivitis, and bronchitis. Fever and diaphoresis are treated with an infusion of the leaves known as "Ocimum tea." Children's sedatives are made from the roots. *Ocimum gratissimum* (scent leaf) is high in alkaloids, tannins, phytates, flavonoids, and oligosaccharides, according to phytochemical analysis (Ijeh et al., 2004).

Neem (*Azadirachta indica* A. Juss.) is an Indian medicinal tree belonging to the Meliaceae family. Because of its numerous biological qualities, the neem tree has found applications in agriculture, industry, medicine, cosmetics, and livestock production around the world (Girish and Shankara Bhat, 2008). Neem contains a wide range of medicinal compounds. azadirachtin is the most active component, followed by nimbin, nimbidin, nimbidol, nimbanene, nimbadiol, nimbolide, nimbiol, sodium nimbin, 6-desacetylnimbinene, gedunin, salannin, quercetin, ascorbic acid, amino acids, n-hexa (Alzohairy, 2016).

Antibacterial, antifungal, antimalarial, antiviral, insecticidal, larvicidal, nematocidal, immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antioxidant, antimutagenic, anticarcinogenic, antimutagenic, anticarcinogenic, and other biological activities have been demonstrated in neem constituents. Neem is appropriately known as the 'Village Pharmacy,' 'Doctor Tree,' 'Wonder Tree of India,' or 'The Bitter Gem,' because to its diverse properties (Girish and Shankara Bhat, 2008)

The WHO and other developing countries have renewed their interest in the use and importance of medicinal plants, resulting in increased efforts to document ethnomedical data of medicinal plants. Traditional herbalists do not keep records, and their knowledge is passed down verbally from generation to generation, leaving gaps in knowledge. All of this piqued the researchers' interest in the topic because more information on the phytochemicals and nutritional value of medicinal plants is needed. As a result, the study focused on the Neem (*Azadirachta Indica* A. Juss) plant and Scent (*Ocimum gratissimum*), two of the most widely used vegetables in Southern Nigeria.

2. METHODOLOGY

2.1 Sample collection and preparation

Neem and Scent leaves were collected from parent trees on farms in Esa Oke, Osun State, and transported to the Laboratory, Department of Science and Technology, Esa Oke. The leaves were cleaned and sliced after being washed to eliminate the dirt. The leaves were dried in the sun for seven days before being pulverized using an electric grinder to obtain a finer milled powder.

2.2. Aqueous Extraction

The aqueous extract of the plant was made by weighing 200 grams of powder on a weighing balance and soaking it in 500 milliliters of distilled water in a one-liter conical flask. It was agitated for 30 minutes before being set aside for 48 hours. The extract was filtered twice, once with a fine cloth and then again with filter paper (Whatman No, 1). The filtrate was put into a round bottom flask and dried in a water bath at 60°C by evaporation. The condensed extract was kept in tightly corked, labeled bottles and kept at 4°C in the refrigerator until needed.

2.3 Proximate Composition Analysis

Crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate were all measured in the freshly manufactured samples. The Association of Official Analytical Chemists had previously outlined how this was done (AOAC, 2002)

2.3.1 Crude Protein

The micro-Kjedahl method was used to accomplish this. Using copper sulphate as a catalyst, the nitrogen component of the protein in 5 g of the sample was transformed into ammonium sulphate by digestion with concentrated hydrogen tetraoxosulphate (VI) acid. The ammonia was collected in a boric acid double indicator solution, and nitrogen was measured using a normal hydrochloric acid titration until the end point was achieved. After that, a factor of 6.25 was used to calculate the amount of crude protein.

2.3.2 Crude Fat

5 g of plant materials, petroleum ether, and a Soxhlet extractor device were used to extract crude fat from the sample. The crude fat in the samples was calculated using the weight of the fat obtained after evaporating the petroleum ether from the extract, and this was stated as a percentage.

2.3.3 Ash Content

To remove organic components, five grams of the material were placed in a crucible and heated to 550°C. After cooling and weighing the crucible and its contents, the ash was calculated as a percentage of the dry weight of the samples.

2.3.4 Moisture

The ground sample was weighed exactly 5 g each and oven dried at a constant temperature of 70°C. After cold weighing, the amount of moisture in the sample was reported as a loss in weight.

2.3.5 Dry Matter

The fibre content of samples was determined using five grams of defatted samples extracted by acid digestion, filtration, and base digestion. At 550°C, the resultant leftovers were eventually ignited. Fibre content was then represented as a proportion of initial weight loss after ashing.

2.3.6 Carbohydrate

The difference between 100 and the total of crude protein, fat, ash, and fibre was then used to calculate the amount of carbohydrate in the sample.

2.4 Qualitative Analysis of the Phytochemicals

2.4.1 Test for Alkaloids

On a steam bath, about 0.5g of each extract was mixed with 5ml of 1% aqueous hydrochloric acid; 1ml of each filtrate was treated with a few drops of Mayer's reagent, and a second 1ml portion was treated similarly with Dragendorff's reagent. As preliminary proof of the presence of alkaloids in the extracts, turbidity or precipitation with either of these reagents was detected (Harborne, 1973).

2.4.2 Test for Flavonoids

A fraction of the aqueous filtrate of plant extract will be mixed with 5 ml of diluted ammonia solution, then concentrated sulphuric acid will be added. Flavonoids are present when there is a yellow coloration.

2.4.3. Test for Saponins

In a water bath, about 2 g of powdered sample will be cooked in 20 ml of distilled water, and the filtrate will be mixed with 5 ml of distilled water and rapidly shaken for a stable persistent foam. The foaming will be combined with three drops of olive oil and vigorously shaken. Saponins are present in the emulsion formation.

2.4.4. Test for phenols by ferric chloride test

A few drops of a neutral 5% ferric chloride solution were added to 50 mg of extract dissolved in 5 ml of distilled water for each sample. The presence of phenolic compounds was indicated by a dark green color.

2.4.5 Test for Tannins

In a test tube, about 0.5 gram of dried powdered sample was cooked in 20 ml of water and then filtered before adding a few drops of 0.1 percent ferric chloride. The presence of tannins is indicated by the presence of a brownish green color

2.5. Quantitative Analysis of the Phytochemicals

2.5.1. Estimation of Alkaloids (Harborne and Baxter, 1983)

In a 250 mL beaker, 5 g of the sample was weighed. 200 mL acetic acid in ethanol (10%) was added and let to stand for 4 hours. The extract was then filtered and concentrated to one quarter of its original volume in a water bath. To produce precipitation, concentrated ammonium hydroxide was applied to the extract drop by drop. The entire solution was allowed to settle, and the precipitate was collected and filtered after being washed with diluted ammonium hydroxide. The residual is the dried and weighed alkaloid..

$$\text{Formula} = B - A \times 100 / S$$

where,

B = Weight of Whatman filter paper.

A = Weight of Whatman filter paper, after drying.

S = Sample weight.

2.5.2 Estimation of Total Flavonoids

The volume will be made up to 100 ml with distilled water after 100 mg of tannic acid has been dissolved in a little amount of distilled water. By diluting the standard with distilled water, different concentrations of the standard were achieved. The solution's concentration was 100 mg/ml. At zero time, 0.5 ml of aqueous extract sample was diluted with 3.5 ml distilled water. The tubes were filled with 0.3 ml of 5% sodium nitrate. After five minutes, all of the tubes received 0.3 ml of 10% aluminum chloride. 2 ml of 1M sodium hydroxide was added to the mixture at the 6th minute. The contents of the reaction mixture were immediately diluted with 2.4 mL distilled water and properly stirred. The mixture's absorbance was immediately measured at 510 nm in comparison to a prepared blank. Total flavonoids were measured in mg per 100g of edible part using tannic acid as a reference ingredient.

2.5.3. Estimation of Saponins (Obadoni and Ochuko, 2001)

A conical flask containing 100 ml of 20% aqueous ethanol was filled with 20 g of sample. At roughly 55°C, the sample was heated for four hours in a hot water bath with constant stirring. The residue was re-extracted with another 200 mL of 20% ethanol after the mixture was filtered. Over a water bath at roughly 90°C, the combined extract was reduced to 40 mL. The concentrated solution was poured into a 250 mL separator funnel, along with 20 mL of diethyl ether, and rapidly shaken. The aqueous layer was saved, while the ether layer was discarded, and the purification procedure was repeated. The extract of n-butanol was then added in 60 mL. The extracted n-butanol will be rinsed twice with 10 mL aqueous sodium chloride. In a water bath, the rest of the solution was heated. The sample was dried to a consistent weight in the oven after evaporation. The percentage of saponins will be determined.

$$\text{Formula} = B - A \times 100 / S$$

where,

B = Weight of Whatmann filter paper.

A = Weight of Whatmann filter paper with sample.

S = Sample weight.

2.5.4. Estimation of Phenols

In the test tubes, 0.5 mL of freshly produced was used. All of the tubes received 8 mL of distilled water. Folin's Ciocalteu reagent (0.5 mL) was also added to all tubes. All of the tubes were kept in B.O.D for a 10-minute incubation period at 40°C. The sodium carbonate solution was then added to each test tube in a volume of 1 mL. After that, the tubes were put in the dark for one hour to incubate (Malick and Singh, 1980). At 660 nm, the color formed was spectrophotometrically read. Tannic acid was used to draw the standard curve. In a shimadzu UV-1650 spectrophotometer, O.D. was read at 660 nm for different amounts of tannic acid. The standard curve was used to compute the sample concentrations.

2.5.5 Estimation of Tannins

In 100 mL of distilled water, 100 milligrams of tannic acid was dissolved. With distilled water, 5 mL of stock solution was diluted to 100 mL. 1 mL contains 50 g of tannic acid.

Tannin Extraction: The powdered substance was weighed and placed to a 250 mL conical flask, along with 75 mL water. The flask was gently heated and cooked for 30 minutes before centrifuging for 20 minutes at 2,000 rpm and collecting the supernatant in a 100 ml volumetric flask to make up the volume. 1 mL of the sample extract was placed in a 100 mL volumetric flask with 75 mL of water. 5 mL folin denis reagent, 10 mL sodium carbonate solution, and 100 mL water were mixed together. Shake well. After 30 minutes, the absorbance was measured at 700 nm. Make a 1 + 4 dilution of the sample if the absorbance is more than 0.7. Instead of the sample, water was used to make a blank. With 100 mg of tannic acid, a typical graph was created (Robert, 1971). From the standard graph, the tannin content of the sample was estimated as tannic acid equivalents.

2.6. Statistical analysis

Data are expressed as mean \pm standard deviation (SD) of triplicates

3. RESULT AND DISCUSSION

3.1 Qualitative Photochemical properties of Neem and Scent leave

Table 1 shows the findings of the qualitative phytochemical analysis of neem and *Ocimum gratissimum* leaf extracts. The results demonstrate that alkaloids are present in *Ocimum gratissimum* leaves, but not in neem leaves. Flavanoids can be found in both leaf extracts. In Neem and fragrance leaves, saponins are considerably present (++). In addition, there contains phenolic neem and fragrance leaves. Tannins are only found in fragrance leaves, not neem leaves (Table 1).

Many alkaloids are used as drugs some of which include; nicotine, quinine, caffeine, cocaine and morphine. They have wide range of pharmacological activities such as antimalaria (quinine), anti-asthma (ephedrine), anticancer, analgesics, (morphine), caffeine in tea, coffee stimulate and balance the nervous system (Qui *et al.*, 2004).

Flavonoids are polyphenolic chemicals that contribute to a variety of natural colors. They have anti-viral and anti-allergic properties, according to reports. Quercetin is a flavonoid that can help with hay fever, eczema, and asthma (Prabhavathi *et al.*, 2016). The antioxidants in flavonoids may work in tandem with other phytochemicals in *V. amygdalina* and *O. gratissimum*'s leaves to provide the medicinal advantages. Steroids are used to create the body's immune systems and DNA. Corticosteroids are a type of steroid that is used to treat disorders. The results of this study's qualitative and quantitative phytochemical examination demonstrated that *O. gratissimum* contains a sufficient amount of secondary metabolite.

Saponins are responsible for a variety of biological effects in plants, including anti-inflammatory, anti-diabetic, anti-HIV, and anti-atherosclerosis. It aids in the maintenance of liver function, the reduction of blood cholesterol, and the prevention of peptic ulcers. As in ruminants, it was discovered to lower nutrient utilization and conversion efficiency (Kashiwada *et al.*, 2009; Banno *et al.*, 2004). Saponin's non-sugar portion possesses direct antioxidant properties, which may lead to various benefits in humans such as a lower risk of cancer and heart disease (Rhoades, 2009).

3.2 Quantitative Photochemical properties of Neem and Scent leaves

Quantitative Photochemical Properties of Neem and Scent Leaves are shown in Figure 1. When it came to alkaloids, Neem leaves had a greater content (3.12 ± 0.03) than fragrance leaves (2.27 ± 0.19). Scent leaves showed a higher concentration of flavanoids (2.05) than neem leaves (1.88 ± 0.02). Neem leaves have a high concentration of saponins (6.21 ± 0.06), whereas fragrance leaves have a very

low concentration (3.7 ± 0.21). The phenolic content of neem leaves (4.11 ± 0.02) is higher than that of fragrance leaves (0.73 ± 0.04). Only smell had a tannin content of 3.15 ± 0.61 , but there was no quantity for neem leaves.

3.3 Nutritional composition of Neem and Scent leaves

Crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate were all investigated in the study. Figure 2 shows the nutritional components of neem and scents leaves. The crude protein content of fragrance leaf (18.5 ± 0.7) is higher than neem leaf (3.57 ± 0.50). Scent leaf crude fat values (2.27 ± 0.37) are not as high as neem leaf crude fat values (2.67 ± 0.51). The ash content of the scent leaf was 8.36 ± 1.08 , while the ash content of the neem leaf was 5.80 ± 0.30 . The moisture content of scent leaf is very low (14.57 ± 0.65) compare to high values 56.3 ± 4.20 found in neem leaf. In this study, scent leaf had dry matter of 91.0 ± 1.85 while that of neem leaf is 33.0 ± 1.57 . The carbohydrate content of scent leaf (48.07 ± 3.26) is more than that of neem leaf (33.0 ± 1.57).

Table 1: Qualitative Phytochemical analysis of the aqueous extract of the Neem and Scent leaf

S/N	Phytochemicals	Neem leaf	Scent leaf
1	Alkaloids	-	+
2	Flavanoids	+	+
3	Saponin	++15	++
4	Phenolic	+	+
5	Tannin	-	+

Key (++) = moderately present (+) = present (-) = absent

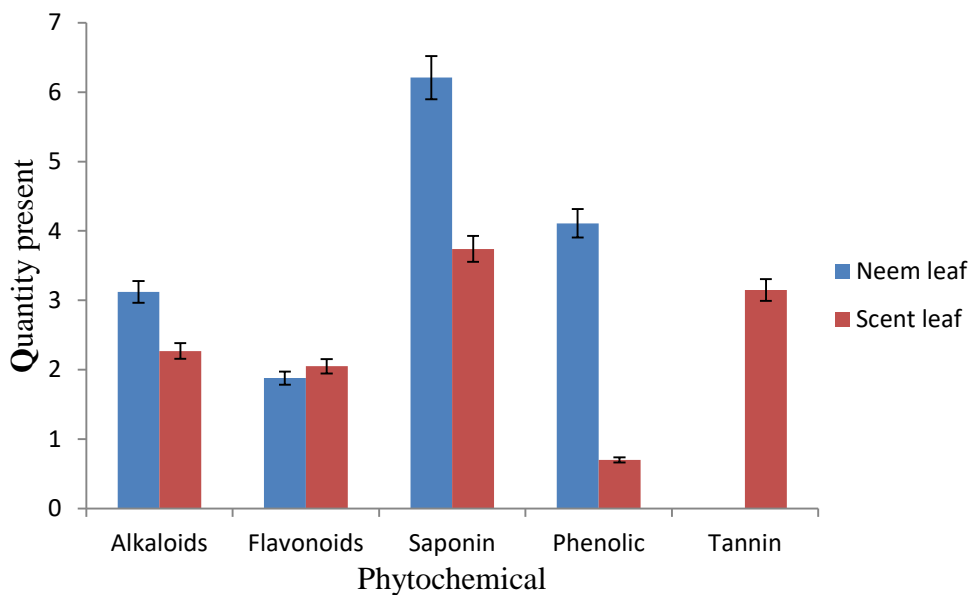


Figure 4.1: Quantitative Photochemical properties of Neem and Scent leaves

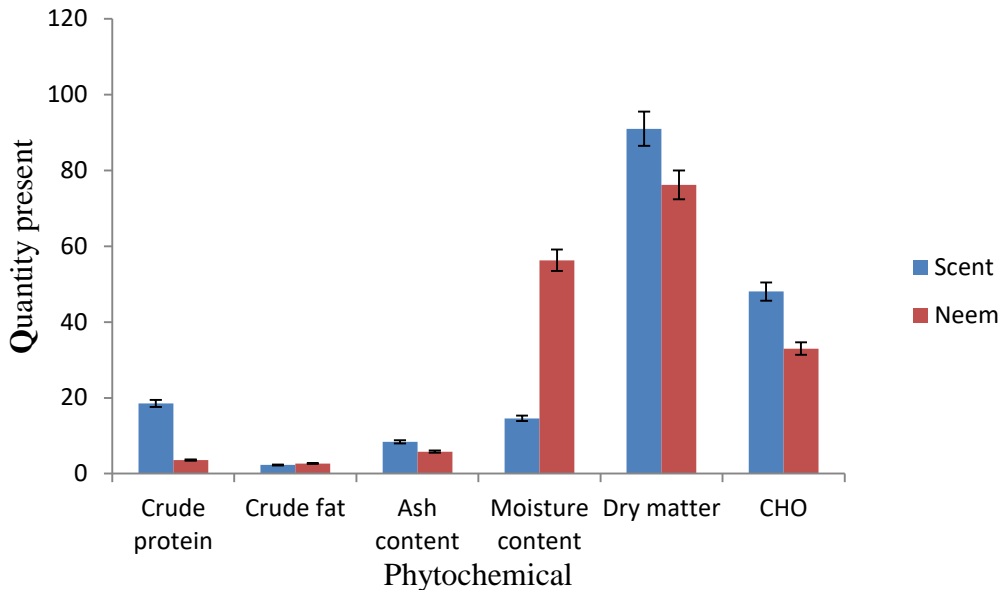


Figure 2: Nutritional composition of Neem and Scent leaves

4.1 CONCLUSION

Phytochemical study of neem leaves reveals the presence of saponins, tannins, and flavonoids, but no alkaloids or phenol. Saponins, tannins, flavonoids, alkaloids, and phenol are found in *Ocimum gratissimum*. Quantitative phytochemical study reveals varying amounts of alkaloids, saponins, tannins, flavonoids, and phenols in neem leaves, but no tannins.

Neem leaves have more alkaloids, saponin, and phenolic acids than fragrance leaves, although scent leaves contain more flavanoids and tannins than neem leaves. Moisture, protein, crude fat, crude fiber ash, and carbohydrates were detected by proximate analysis. Scent leaves have more crude protein, crude fats, dry matter, and carbohydrate content than neem leaves, although neem leaves have slightly higher crude fat than scent leaves. Both neem and fragrance leaves are highly suggested for use.

Protein present in increased amounts in fragrance leaf is employed to improve macromolecule penetration across cell membranes, demonstrating the need of using scent leaf extensively. Because of their therapeutic and nutritional properties, these two species are suggested for usage.

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