PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *Lantana camara* Linn. LEAF

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc. Hons) IN BIOCHEMISTRY

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CERTIFICATION

We the undersigned, certify that **DELE EMMANUEL** with matriculation number **LSC1906466** carried out this project work in partial fulfillment of the requirements for the award of Bachelor of Science (**B.Sc**. Hons) degree in Biochemistry, in the Department of Biochemistry.

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EXTERNAL EXAMINER DATE.

DEDICATION

This project is dedicated to my dearest mother, Mrs. Asaba Olayemi. Your love, support, and guidance inspired me to pursue my dreams. Though you are no longer with me, your memory lives on in my heart. Thank you for being my support system. Keep restimg on.

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ABSTRACT

Standard analytical methods were utilized to investigate the nutritional composition and phytochemical content of *Lantana camara* Linn. leaves in order to uncover the plant's potential bioactive compounds and nutritional profile.. The qualitative phytochemical screening of the aqueous extract of *Lantana camara* Linn. leaves revealed the presence of various compounds such as tannins, flavonoids, alkaloids, cardiac glycosides, saponins, steroids, terpenoids, phlobatannins, coumarins, and proteins, with tannins being particularly abundant. However, phenols, anthraquinones, emodins, and anthocyanins were not detected. The composition of the ethanol extract showed variations, with flavonoids, alkaloids, tannins, cardiac glycosides, saponins, steroids, terpenoids, phlobatannins, coumarins, and proteins present in moderate amounts, while phenols, anthraquinones, emodins, and anthocyanins were still absent. The medicinal properties of *Lantana camara* Linn. are influenced by the presence and levels of these secondary metabolites. Proximate analysis revealed that *Lantana camara* Linn. leaves are rich in carbohydrates (26.59± 1.86%), have a high moisture content (18.00 ± 1.63%), crude protein (18.41 ± 0.02%), crude fats (17.33 ± 0.14%), crude fiber (10.00 ± 0.82%), and ash (9.67 ± 0.27%). The significant presence of carbohydrates, protein, crude lipids, and fiber in the leaves may contribute to their nutritional value.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Lantana camara Linn., a flowering plant from the Verbenaceae family, is widely distributed and known for its beauty and traditional medicinal uses. It is commonly referred to as Wild Sage, Curse of India, Curse of Barbados, Lantana. This species displays a wide range of appearances and medicinal properties across its various strains and varieties (Singh *et al*., 2020).

In India, *Lantana camara* Linn was introduced before the 19th century and has since established itself in regions with moderate to high summer rainfall and well-drained sloping sites (Khan *et al*., 2019). Despite its invasive nature in certain ecosystems, *Lantana camara* Linn continues to be of scientific interest due to its potential medicinal value.

Traditional medicinal systems have long recognized the therapeutic properties of *Lantana camara* Linn utilizing different parts of the plant to treat various ailments, including gastrointestinal disorders and respiratory infections (Rathod *et al*., 2018). Recent scientific studies have further supported these claims, highlighting the pharmacological potential of *Lantana camara* Linn. extracts in modern medicine (Singh *et al*., 2020).

Although the medicinal properties of *Lantana camara* Linn have been extensively studied, there is a lack of research focusing on the proximate and phytochemical composition of its leaves. Understanding the nutritional and bioactive constituents of *Lantana camara* Linn leaves is essential for uncovering its therapeutic potential and promoting sustainable utilization.

Therefore, this study aims to comprehensively analyze the proximate and phytochemical composition of *Lantana camara* Linn. leaves. By characterizing the nutritional and bioactive components of the leaves, we aim to provide valuable insights into their potential health benefits and contribute to the scientific understanding of this culturally significant plant.

1.1.1 STATEMENT OF PROBLEM

Lantana camara Linn. leaves are well-known for their wide range of chemical components, which may include different vitamins, minerals, and other plant compounds with nutritional and medicinal importance. The main objective of this research is to extensively examine the proximate analysis and phytochemical constituents of *Lantana camara* Linn. leaves and extracts. By doing so, we hope to gain a deeper insight into its nutritional value and potential medicinal properties.

1.1.2 AIM AND OBJECTIVE OF THE STUDY

The main objective of this research is to carry out a thorough analysis of the proximate and phytochemical properties of *Lantana camara* Linn. leaves. This will involve examining the nutritional composition and identifying the potential bioactive compounds found in the plant.

1.1.3 SPECIFIC OBJECTIVES OF THE STUDY

The study has the following precise aims:

1. Proximate analysis of the leaf of *Lantana camara* Linn.
2. Phytochemical screening (qualitative) of the leaf of *Lantana camara* Linn.

1.2 LITERATURE REVIEW

1.2.1 Scientific Classification of *Lantana camara Linn.*

Domain……………………………………………Eukaryota

Kingdom…………………………………………. Plantae

Phylum……………………………………………Tracheophyta

Subphylum………………………………………..Angiospermae

Class……………………………………………… Magnoliopsida

Order………………………………………………Lamiales

Family…………………………………………….Verbenaceae

Genus……………………………………………. *Lantanas*

1.2.2 Nomenclature

Scientific Name: *Lantana camara* Linn.

Common Name(s): Wild Sage, Curse of India, Curse of Barbados, Lantana

Nigerian Name(s): *Anya nnunu* (Igbo), *Ewonadele* (Yoruba), *Kimbamahalba* (Hausa).

1.2.3 Description

Lantana camara Linn. also known as lantana or wild sage, is a flowering shrub that belongs to the Verbenaceae family. It is native to tropical regions and is characterized by its clusters of small, fragrant flowers and serrated leaves. This plant is highly valued for its decorative beauty and has

successfully adapted and spread to different parts of the world due to its ability to thrive in various environments (Deb, 2005).

In terms of its physical appearance, *Lantana camara* Linn. typically grows as a dense shrub, reaching heights of up to two meters. Its leaves are either oval or elliptical in shape and are arranged opposite each other along the stems. When crushed, these leaves emit a distinct aroma. The flowers of *Lantana camara* Linn. are small, tubular, and are arranged in rounded clusters called umbels. These flowers come in a wide range of colors, including red, orange, yellow, and pink, which attract pollinators like butterflies and bees (Singh *et al*., 2020).

Ecologically, *Lantana camara* Linn has shown remarkable adaptability, thriving in various habitats such as forests, grasslands, and even disturbed areas like roadsides and wastelands. Its ability to colonize diverse environments can be attributed to its rapid growth, abundant seed production, and allelopathic effects, which inhibit the growth of competing plant species (Parepa *et al*., 2013).

Moreover, *Lantana camara* Linn. possesses a complex chemical composition, containing a diverse range of phytochemicals such as alkaloids, flavonoids, phenolics, terpenoids, and tannins. These bioactive compounds contribute to the plant's medicinal properties and ecological interactions, making it a subject of interest in both traditional medicine and scientific research (Rathod *et al*., 2018).

1.2.4 Habitat

Lantana camara Linn. an adaptable plant species originally from the Americas, has successfully established itself worldwide and exhibits invasive characteristics (Sharma *et al*., 2019). Flourishing in warm regions with considerable rainfall, it effectively adjusts to various

environments including tropical forests, savannas, and disturbed areas like roadsides. Despite its invasive nature, *Lantana camara* Linn. contributes to ecosystem restoration and soil stabilization owing to its resilient root system (Sharma *et al*., 2019).

1.2.5 Ecological Conditions

Lantana camara Linn. flourishes in tropical regions characterized by warm temperatures and ample rainfall. It displays impressive versatility in adapting to various soil types and conditions (Sharma *et al*., 2019).

1.2.5 Propagation

Lantana camara Linn. can be propagated through various methods, including seeds, stem cuttings, and layering. Seeds are the most common method of propagation and can be collected from mature fruits. After harvesting, the seeds should be cleaned and soaked in water for 24 hours to soften the seed coat and improve germination rates (Mishra *et al*., 2017).

Once soaked, the seeds can be sown in trays filled with well-draining potting mix and covered lightly with soil. The trays should be placed in a warm, sunny location with regular watering to ensure optimal germination. Seedlings typically emerge within 1-2 weeks and can be transplanted into individual pots or directly into the garden once they have developed several sets of true leaves.

Stem cuttings serve as an alternative and efficient means of propagation, especially for generating consistent plants possessing desirable characteristics. It is advisable to extract cuttings from robust and healthy plants, subsequently treating them with a rooting hormone to encourage the growth of roots. During the growing season, it is recommended to obtain stem cuttings measuring

approximately 10 to 15 cm in length and plant them in a potting mix that facilitates proper drainage (Bhore *et al*., 2017).

Layering is a propagation technique recommended by Bhore *et al*., (2017) for *Lantana camara* Linn. It requires bending a low-lying branch towards the ground, making a small cut, and then covering it with soil until roots form. This method has been proven to be successful in creating new plants of *Lantana camara* Linn. (Bhore *et al*., 2017).

Lantana camara.

Leaves are opposite and decussate.

Hairs on the midrib underneath.

Hairs on small branchlets.

Ovate leaves have small teeth on the edge and the veins are depressed on the upper surface.

Hairs on the upper surface.

Plate 1: The leaves of *Lantana camara* Linn.

Source: Google

Plate 2: Flowers of *Lantana camara* Linn.

Source: Google

1.2.6 Phytochemistry

The chemical composition of *Lantana camara* Linn. Leaves consist of alkaloids, flavonoids, phenolics, terpenoids, and tannins, each having unique pharmacological properties (Singh *et al*., 2020; Rathod *et al*., 2018). Alkaloids, including lantanine, lantadene A, and lantadene B, display antimicrobial, antimalarial, and antifungal effects. Flavonoids such as luteolin and quercetin exhibit antioxidant, anti-inflammatory, and anticancer activities, while phenolic compounds like gallic acid and caffeic acid contribute to antioxidant and antimicrobial effects. Terpenoids, such as α-pinene and limonene, provide aromatic qualities and insecticidal properties, and tannins possess astringent characteristics, potentially contributing to medicinal applications.

Traditional Use

Lantana camara Linn, a plant with a rich history in traditional medicine across different cultures, possesses a wide range of medicinal benefits. In Ayurveda and Siddha practices in India, it has been traditionally used to treat gastrointestinal disorders, respiratory infections, skin ailments, and fever (Rathod *et al*., 2018). In African and South American traditional medicine, *Lantana camara* Linn. is highly regarded for its antimicrobial properties and is often employed to address wounds, infections, and parasitic infestations (Singh *et al*., 2020). Additionally, its anti-inflammatory effects make it a popular remedy for skin conditions like eczema and dermatitis. These traditional uses underscore the plant's extensive therapeutic potential and cultural importance.

Antimicrobial Properties

Lantana camara Linn. possesses notable antimicrobial properties, as indicated by several research studies. A study by Mendes *et al*. (2019) highlighted the antimicrobial effectiveness of *Lantana camara* Linn extracts against a diverse array of harmful microorganisms such as bacteria and fungi.

These results imply the promising role of the plant as a natural antimicrobial remedy for combating infectious ailments.

Anti-inflammatory Activity

Lantana camara Linn. is recognized for its remarkable anti-inflammatory characteristics, which render it a crucial element in traditional medicinal practices. Research indicates that *Lantana camara* Linn. extracts display substantial suppression of inflammatory agents like prostaglandins and cytokines, thereby enhancing its anti-inflammatory properties (Kumar *et al*., 2019). These results imply that *Lantana camara* Linn. could potentially serve as a natural solution for inflammatory ailments.

Antioxidant Activity

Lantana camara Linn. showcases impressive antioxidant characteristics, as proven by research conducted by Sharma *et al*. (2018). Their study showcased the plant's efficacy in combating oxidative stress and effectively scavenging free radicals. This emphasizes the potential of *Lantana camara* Linn. as a versatile and easily accessible source of antioxidants for a range of applications, including medicinal and ecological uses.

Anticancer Effects

A study conducted by Batista *et al*. (2018) has shown that *Lantana camara* Linn. has the potential to exhibit anticancer effects. The research conducted by them has demonstrated that extracts from *Lantana camara* Linn. can induce cytotoxicity in various human cancer cell lines, including those linked to breast, lung, and colon cancers. These findings indicate that the plant could be a valuable natural source of compounds with anticancer properties, which calls for additional research and development.

Insecticidal Effect

Singh *et al*. (2020) conducted a study revealing that *Lantana camara* Linn. exhibits notable insecticidal characteristics. The research highlighted the effectiveness of *Lantana camara* Linn. extracts in deterring and managing insect pests. These results indicate the promise of *Lantana camara* Linn. as a sustainable option for pest management in agriculture.

Antifungal Activity

Lantana camara Linn. exhibits potent antifungal properties, disrupting fungal growth by interfering with essential cellular processes like cell wall synthesis and membrane integrity (Silva *et al*., 2019). Its efficacy against various fungal species, including Candida albicans and Trichophyton mentagrophytes, suggests its potential as a natural remedy for fungal infections in both humans and animals. Additionally, synergistic effects with conventional antifungal drugs enhance treatment outcomes, highlighting its therapeutic promise (Silva *et al*., 2019).

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 PLANT SAMPLE

Fresh leaves of *Lantana camara* Linn. collected in February, 2024 from a fallowed farm at Eweka Street, Off Uwelu Road, Uwelu, Egor L.G.A., Edo State. Fresh parts of the plants were identified prior to analysis by Prof. H.A. Akinnibosun at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State and a **Voucher Number**: UBH-L367 issued.

2.1.2 REAGENTS

The reagents used in this study include:

Hydrochloric acid (Loba Chemie Pvt Ltd, India)

Ferric chloride (Loba Chemie Pvt Ltd, India)

Wagner's reagent (Loba Chemie Pvt Ltd, India)

Acetic anhydride (Loba Chemie Pvt Ltd, India)

Sodium hydroxide (Loba Chemie Pvt Ltd, India)

Ammonium hydroxide (Loba Chemie Pvt Ltd, India)

Ferric chloride (Loba Chemie Pvt Ltd, India)

Sodium hydroxide (Loba Chemie Pvt Ltd, India)

Sulphuric acid (Loba Chemie Pvt Ltd, India)

Chloroform (Loba Chemie Pvt Ltd, India)

Potassium permanganate (Loba Chemie Pvt Ltd, India)

Petroleum ether (Loba Chemie Pvt Ltd, India)

Ethanol (Loba Chemie Pvt Ltd, India)

2.1.3 EQUIPMENT/ APPARATUS

The apparatus used in this study include:

Electronic compact scale/ Weighing balance (Atom-A110C, China)

Soxhlet apparatus (Hanon Lab., China)

Heating mantle (Witeg Labortechnik, Korea)

Micro-Kjeldahl digestion flask (Pyrex, Nigeria)

Digester (Hanon Lab., China)

UV/Visible spectrophotometer (Bruker Physik, Germany)

Muffle furnace (Kejia furnace, China)

Beakers (Pyrex, Nigeria)

Conical flasks (Pyrex, Nigeria)

Standard flask (Pyrex, Nigeria)

2.2 METHODS.

2.2.1 PREPARATION OF SAMPLES

The freshly collected leaves of *Lantana camara* Linn. were separated by hand from twigs, washed for 10 minutes under a continuous stream of running tap water and then rinsed with ionized water. Thereafter, the plant samples were spread out in the shade to dry for 15 days, thereafter it was grinded and assayed.

2.2.2 EXTRACTION OF SAMPLES FOR PHYTOCHEMICAL SCREENING.

10g of sample was weighed and transferred into an electric blender and 100mL of distilled water was added and then blended for 30minutes. The mixture was transferred into a clean, dry sample bottle and allowed to stand for 72hours. After 72hours, the mixture was then filtered into another clean, dry sample bottle which was adequately labeled and corked. (Jimoh *et al*., 2010).

2.2.3 PROXIMATE ANALYSIS

Ash Content Determination (AOAC 1990)

Principle: The ash content determination method involves subjecting the sample to high temperatures in a muffle furnace. This process decomposes all the organic components, leaving behind only the inorganic matter in the crucible. Therefore, the final product obtained after this procedure represents the inorganic content of the sample.

Procedure:

An oven-dried crucible was initially dried and weighed. Exactly 1g of the sample was added to the crucible and labelled accordingly. The crucible with the sample was transferred into a muffle furnace with the temperature set at 550˚C. It was allowed to remain in the furnace for 3 hours. The crucible with the sample was removed from the muffle furnace after the specified time and it was

placed in a desiccator for 30 minutes to cool down. After it was cooled, the crucible with the sample was weighed and labelled accordingly.

Calculation

$$\% \text{ Ash} = \frac{\text{Loss in Ash Weight (g)}}{\text{Weight of Sample (g)}} \times 100$$

Moisture Content Determination (AOAC 2002)

Principle: Moisture content determination involves measuring the proportion of water in a sample. The sample is initially weighed, then dried to remove moisture, and re-weighed. The moisture content is calculated as the percentage of weight loss due to moisture removal.

Procedure: A porcelain crucible was dried and weighed, then it was recorded as W1 (g). Exactly 2g of the sample was added to the crucible to obtain a weight recorded as W2 (g). The crucible was then dried in an oven continuously. The dried sample was constantly re-weighed at 10 minutes intervals until a constant weight C (g) was obtained after which the crucible was removed from the oven and cooled. The moisture content was calculated as shown the equation below.

Calculation

$$\% \text{ Moisture} = \frac{Weight\ Loss\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

Weight loss= [(W_2–W_1) (g) — (W_2–C)] (g); and

Weight of sample= W_2–W_1 (g)

Where: W_1= Dried crucible (g), W_2= Dried crucible + sample (g), C= Constant weight (g).

Crude Fibre Determination (AOAC 1980)

Principle: The process of removing moisture content involves drying the sample in an oven. Additionally, the lipid content is eliminated by extracting it with an organic solvent, specifically Petroleum ether, using a soxthled extractor.

Procedure: Exactly 1g of the sample was carefully transferred into a 250mL conical flask.

Subsequently, 100mL of 1.25% NaOH solution was gently boiled for a duration of 30 minutes.

The resulting mixture was then filtered through filter paper using suction with the aid of a buncher funnel. The filter paper was thoroughly rinsed with distilled water, and the separated materials were returned to the flask using a spatula.

The residue remaining on the filter paper was carefully scraped off with a spatula and added drop by drop to ensure that all remnants were rinsed into the crucible.

The crucible, containing the sample, was then placed in an oven at a temperature of 105°C for a period of 2 hours. Subsequently, it was transferred to a desiccator and allowed to cool for 30 minutes before being weighed.

Following this, the crucible, along with the sample, was transferred to a muffle furnace and subjected to a temperature of 300°C for 2 hours. Once again, it was transferred to a desiccator and allowed to cool for 30 minutes before being weighed.

Calculation

$$\text{\% Crude fibre} = \frac{Oven\ extract - muffle\ extract\ (g)}{Weight\ of\ Sample\ (g)} \times 100$$

Where: Oven extract = weight after drying(g) - weight of crucible(g),

Muffle extract = weight after extraction(g)-weight before extraction(g).

Crude Fat Determination (AOAC 2002)

Principle: The determination of crude fat content involves the extraction of fat from the sample using a solvent, and the extracted fat is then weighed to determine the crude fat content.

Procedure:

Exactly 1g of sample was placed into a 250mL conical flask, 100mL of 1.25% NaOH solution was gently boiled for 30 minutes. The mixture was filtered through filter paper using suction with a buncher funnel, thoroughly rinsed with distilled water, and the components were separated back into the flask using a spatula.

The residue was scraped from the filter paper with a spatula and added drop by drop to rinse any remaining residue into the crucible. The crucible was then placed in an oven at 105°C for 2 hours, followed by cooling in a desiccator for 30 minutes before being weighed. The crucible with the sample was then transferred to a muffle furnace at 300°C for 2 hours, cooled in a desiccator for 30 minutes, and weighed.

Calculation

$$\%\ \text{Fat} = \frac{loss\ in\ Fat\ (g)}{Weight\ of\ Sample\ (g)} \times 100$$

Where: loss in Fat= initial fat content of the sample - fat content of the residue

Crude Protein Determination by Kjeldahl method (AOAC 1984)

Principle: Kjeldahl's method is a widely recognized technique for determining the total nitrogen content of a sample by using a conversion factor. The sample is digested in sulphuric acid, with

$CuSO_4$ and $FeSO_4$ acting as catalysts. During this digestion process, nitrogen (N) is converted to ammonia. This method is considered the standard for analyzing protein content in various organic materials.

Procedure:

Exactly 1g of the sample was weighed and transferred to a conical flask. Then, 25mL of sulfuric acid, along with a mixed catalyst, was added to the flask. Gradual heating commenced, starting with low heat for 15 minutes, followed by medium heat for 30 minutes, and finally increasing the heat until digestion was complete. The flask was rotated intermittently until the digestion became clear. After cooling, the digest was made up to 100mL. In a 50mL conical flask, 5mL of 2% boric acid was placed to trap the ammonia vapor from the digest. Three drops of a pre-prepared mixed indicator solution were added. Subsequently, 10mL of the digested sample was pipetted into a distillation flask, and then 15mL of 40% NaOH and 30mL of distilled water were added. Finally, 25mL was distilled into the receiving flask.

Calculation

$$\%\ \mathrm{N} = \frac{\mathrm{Na\ x\ Va(mL)\ x\ 0.014\ x\ 100\ x\ 100}}{Weight\ of\ Sample\ x(g) \times 100}$$

$$\%\ \text{Crude Protein} = \%\mathrm{N} \times 6.25$$

Where N_a= Normality of acid= 2x Molarity (18) = 36

V_a= Volume of acid,

0.014= Conversion factor to convert nitrogen content to protein,

N= Total nitrogen content in the sample,

6.25= Conversion factor to convert nitrogen content to protein.

Estimation of Total Carbohydrate

The total carbohydrate content in the diet samples was determined by subtracting the combined percentages of crude protein, crude fat, moisture, fiber, and ash from 100.

Calculation

Total carbohydrates = 100 — (% ash + % moisture + % crude fibre + % crude protein)

PHYTOCHEMICAL SCREENING

The Phytochemical examinations of the plant extracts were carried out using standard methods as employed by Tiwari *et al*., 2011, with little modification. The two extracts (namely, aqueous and ethanol) were subjected to same condition during this examination.

1. **Detection of Alkaloids**

Principle of Wagner's Test: The Wagner test is based on the principle that the K^+ metal ion creates a covalent coordinate bond with nitrogen in the alkaloid, resulting in the formation of a yellow precipitate of potassium-alkaloid complex. The presence of alkaloids in the Wagner test is confirmed by the development of a brownish to yellowish precipitate. (Altemimi *et al*., 2017).

Procedure: During the execution of the Wagner test, 2mL of the plant extract was introduced into a test tube. Subsequently, Wagner's reagent, which comprises of a solution of iodine in potassium iodide, was cautiously added drop by drop to the test tube. The occurrence of a yellow precipitate within a brief span of time signified a positive outcome for alkaloids.

2. **Detection of Tannins**

Principle: The detection of tannins is based on their interaction with specific chemical reagents, leading to the formation of colored complexes or precipitates. Tannins, which are polyphenolic compounds present in plants, can undergo complexation reactions with certain metal ions or proteins, enabling their detection. One widely employed method for tannin detection involves the reaction between tannins and iron (III) ions (Fe^{3+}), resulting in the development of a blue-black or greenish-black color. This reaction occurs due to the formation of coordination complexes between the phenolic hydroxyl groups of tannins and the iron ions, leading to the characteristic change in color. (Falcão and Araújo, 2011)

Procedure: To 1.0 mL of the extract, 2.0 mL of H_2O and a few drops of ferric chloride were added. Formation of a blue-black or greenish-black color precipitate indicated the presence of tannins.

3. **Detection of Phenols**

Principle: When compounds containing a phenol group, such as enols, hydroxamic acids, sulfinic acids, and oximes, are combined with aqueous ferric chloride ($FeCl_3$), they exhibit a range of colors including blue, violet, purple, green, or red-brown. This color change occurs due to the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by phenols present in the plant sample (Dai and Mumper, 2010).

Procedure: To determine the presence of phenols, 2.0 mL of the plant extract was treated with 2-3 drops of a 10% ferric chloride solution. The formation of a dark green color indicated the presence of phenols in the extract.

4. Detection of Saponins

Principle: Saponins exhibit surfactant characteristics. Agitating a solution containing saponins introduces air into the solution. The surfactant nature of saponins decreases the surface tension of water, enabling air to be trapped within the solution. Consequently, a stable froth or foam is produced on the solution's surface (Vincken *et al*., 2007).

Procedure: The froth test procedure involved diluting 1.0mL of the extract with distilled water, followed by heating and vigorous shaking. The formation of a 1cm layer of foam signified the existence of saponins. To confirm the presence of saponins, 3 drops of olive oil were added and shaken vigorously to observe emulsion.

5. Detection of Flavonoids

Principle: The detection of flavonoids, specifically flavones and flavonols, can be achieved using the dilute ammonia principle. This method relies on the reaction between flavonoids and a solution of dilute ammonia, which leads to the creation of color complexes that are yellow or orange-yellow in appearance.

When flavonoids interact with dilute ammonia, especially in the presence of oxygen from the atmosphere, a distinct color transformation takes place. This color change is often observed as a yellow or orange-yellow hue within the reaction mixture.

The underlying principle of this detection technique involves the formation of chelate complexes between the flavonoid molecules and the ammonia molecules. Flavonoids possess multiple hydroxyl groups that can function as ligands, allowing them to form coordination complexes with metal ions or basic molecules such as ammonia. As a result of this complexation, the characteristic color change became evident when dilute ammonia is present. (Sofowara. 1993).

Procedure: The extract was combined with 5.0mL of dilute ammonia solution and then 1.0mL of concentrated tetraoxosulphate (VI) acid was introduced. The emergence of a yellow coloration, which subsequently vanished upon standing, indicated the existence of flavonoids.

6. Detection of Steroids

Principle: Certain steroids, particularly those containing conjugated double bonds in their structure, have the ability to undergo a chemical reaction referred to as the "unsaturation test" or "Liebermann-Burchard test" when subjected to a combination of acetic acid and sulfuric acid. Acetic acid, being a weak acid, is utilized to establish an acidic environment, while sulfuric acid, a strong acid, is cautiously introduced into the solution. The degree of color alteration observed during this reaction can vary depending on the specific steroid being examined and its concentration. The emergence of a reddish-brown hue at the junction is particularly noteworthy as it serves as an indication of the presence of steroids in the sample (Nath *et al*., 1946).

Procedure: The presence of steroids was indicated by the development of a reddish-brown color at the junction after adding 2.0 mL of acetic anhydride, 1.0 mL of the extract, and 2.0 mL of H_2SO_4.

7. Detection of Terpenoids

Principle: When chloroform ($CHCl_3$) is introduced to a sample containing terpenoids, it aids in the extraction of lipophilic compounds, such as terpenoids, from the sample. Intense agitation helps in separating the chloroform layer from the aqueous layer, which harbors other water-soluble compounds. The addition of sulfuric acid results in the creation of an acidic environment within the mixture. If terpenoids are present in the sample, they undergo a chemical transformation upon interaction with sulfuric acid. This chemical reaction frequently results in the manifestation of diverse hues, which may vary from reddish-brown to violet or blue, contingent upon the specific terpenoid compound present (Siddiqui *et al*., 2009).

Procedure: The plant sample extract (2mL) was combined with chloroform (2mL) and concentrated H_2SO_4 (3mL) was added cautiously to create a distinct layer. The appearance of a deep red/brown coloration at the interface confirmed the presence of terpenoids in the sample.

8. Detection of Phlobatannins:

Principle: The identification of phlobatannins through the use of hydrochloric acid (HCl) and heat is centered on the distinct alteration in color that occurs when phlobatannins interact with HCl at increased temperatures. Phlobatannins are a category of tannins present in specific plants, and their identification hinges on their chemical characteristics, particularly their response to acids and heat. Throughout the process, the colorless or lightly-colored phlobatannins are transformed into red-colored substances, resulting in a noticeable alteration in color within the reaction mixture. This change in color serves as an indication of the presence of phlobatannins in the plant extract or solution. (Ejikeme *et al*., 2014).

Procedure: Positive test was observed when 2mL of extract was subjected to boiling with 2mL of 1% aqueous hydrochloric acid, resulting in the formation of a red precipitate.

9. **Detection of Coumarins:** The identification of coumarins through the utilization of sodium hydroxide (NaOH) is rooted in the distinct alteration in color that occurs when coumarins interact with a NaOH solution. Coumarins, which are a group of organic compounds naturally occurring in specific plants, are identified based on their chemical characteristics, particularly their reaction with alkaline substances like NaOH.

   The underlying principle of this detection technique involves the creation of yellow-hued compounds as a result of the reaction between coumarins and NaOH. Coumarins possess a lactone ring structure that can be hydrolyzed in the presence of bases. Upon exposure to NaOH, the lactone ring within coumarins is broken, leading to the generation of a carboxylate ion and a phenolic compound. (López-Castillo *et al*., 2013).

   Procedure: The identification of coumarins through the utilization of sodium hydroxide (NaOH) entails the addition of a NaOH solution to a plant extract, followed by the observation of a yellow coloration, which served as an indication of the existence of coumarins.

10. **Detection of Emodins:** The detection of emodins using ammonium hydroxide (NH_4OH) and chloroform ($CHCl_3$) is based on a fundamental principle. This principle involves the creation of a colored complex when emodins react with ammonium hydroxide in the presence of chloroform. Emodins are a type of compound called anthraquinones, which can form complexes with specific reagents, resulting in distinct color changes. By subjecting emodins to a chemical reaction with ammonium hydroxide and chloroform, a

colored complex is formed. This complex can be visually observed or measured using spectrophotometry. (Falcão and Araújo, 2011).

Procedure: Combining 2mL of the plant extract with 2mL of NH_4OH and 3mL of chloroform leads to the development of a characteristic pink-to-red colouration.

11. **Detection of Protein:** The identification of proteins through the use of sulfuric acid (H_2SO_4) relies on the concept of protein denaturation and precipitation. Upon exposure to concentrated sulfuric acid, proteins undergo denaturation as the acid disrupts their molecular structure, causing them to unfold and lose their original conformation. This process reveals hydrophobic regions within the protein, resulting in the aggregation and precipitation of insoluble protein complexes.

The underlying principle of this detection technique is the creation of a white or cloudy precipitate following the treatment of proteins with concentrated sulfuric acid. The presence of proteins in the solution is indicated by the formation of this precipitate. (Ali *et al*., 2019).

Procedure: The presence of protein in the plant extract was confirmed by observing the formation of a white precipitate upon adding 1mL of concentrated sulfuric acid to 1mL of the extract.

12. **Detection of Anthraquinones:**

Principle: The identification of anthraquinones through the use of chloroform and ammonia is based on the formation of colored complexes. When anthraquinones interact with ammonia in the presence of chloroform, distinct colors are produced, allowing for easy recognition. This method is frequently utilized in phytochemical analysis to determine the existence of anthraquinones in plant extracts (Pati *et al*., 2016).

Procedure: To initiate the process, 2mL of chloroform was introduced into the mixture. Following this, 2.5 mL of NH_3 (10%) was added into the 1mL of the extract. The presence of a pink, violet, or red coloration within the solution signified a positive outcome.

13. Detection Cardiac Glycosides:

Principle: The detection of cardiac glycosides through the use of glacial acetic acid is based on specific chemical reactions that result in the development of distinct color changes. Cardiac glycosides, which are a group of compounds present in particular plants, are identified based on their unique chemical properties.

In this method of detection, cardiac glycosides react with glacial acetic acid, leading to the creation of colored compounds. When cardiac glycosides are exposed to glacial acetic acid and then heated, they undergo particular chemical reactions that give rise to the formation of colored solutions or precipitates. (Nanna *et al.,* 2013).

Procedure: 1mL of the plant extract was treated with glacial acetic acid, followed by the addition of one drop of ferric chloride solution and 1mL of concentrated H_2SO_4, the presence of a browning interface or a blue-coloured solution signified a favorable result.

14. Detection of Anthocyanins:

Principle: The identification of anthocyanins through the utilization of hydrochloric acid (HCl) and ammonia (NH_3) is based on the fundamental concept of specific chemical reactions that produce distinct alterations in colour. Anthocyanins are pigments that are soluble in water and are responsible for the vibrant red, purple, and blue hues observed in numerous fruits, flowers, and vegetables.

The underlying principle of this detection technique involves the interaction between anthocyanins and HCl and NH_3, which leads to the creation of various pigmented

compounds. When anthocyanins are subjected to HCl, they undergo a reversible reaction that is dependent on the pH, referred to as "flavylium cation equilibria." This reaction induces a modification in the colour of the anthocyanin solution, often transitioning from red or purple to a more orange or pink shade.

Following this, the addition of NH_3 to the acidified anthocyanin solution serves to neutralize the acid and shift the equilibrium back towards the original coloured form of the anthocyanin. Consequently, the original red, purple, or blue colour of the anthocyanin solution is restored. (Njoku and Obi 2009).

Procedure: exactly 2mL of the extract was mixed with 2mL of HCL and NH_3. The presence of anthocyanin resulted in the formation of a pinkish-red to bluish-violet colouration.

2.2.7 STATISTICAL ANALYSIS

All experimental data were expressed as mean ± SEM and were statiscally analyzed using one way analysis of varience (ANOVA) by least significance difference (LSD) test values were considered significant at $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 RESULTS

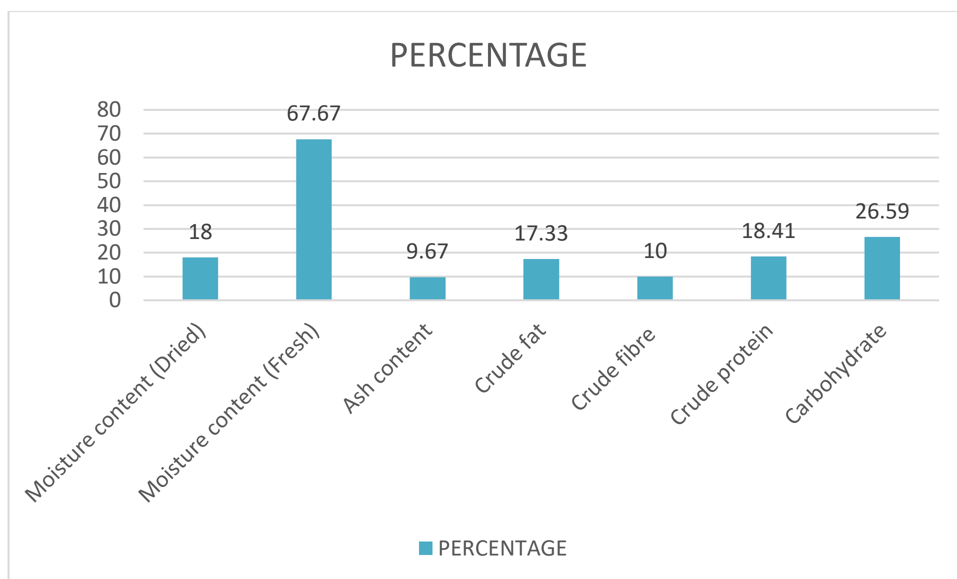
3.1.1 Proximate Composition

Table 3.1 shows the proximate composition values of the *Lantana camara* Linn. leaf samples. The results shows that *Lantana camara* Linn. is a good source of carbohydrate, fibre, fat, and crude protein.

Table 3.1: Proximate Composition of *Lantana camara* Linn.

S/N	PARAMETER	VALUES (%)
1	Moisture (Fresh Sample)	67.67 ± 0.67
	Moisture (Dried Sample)	18.00 ± 1.63
2	Ash	9.67 ± 0.27
3	Crude Fat	17.33 ± 0.14
4	Crude Fibre	10.00 ± 0.82
5	Crude Protein	18.40 ± 0.02
6	Carbohydrate	26.59 ± 1.86

Results are expressed as mean ± standard error of mean (S.E.M.) of 3 determinations.



3.1.3 Phytochemical Content

The phytochemical content of the *Lantana camara* Linn. leaf extract (extracted with water) revealed that tannins were in abundance, while flavonoids, alkaloids, cardiac glycosides, saponins, steroids, terpenoids, phlobatannins, coumarins, and proteins were modera`tely present. Phenols, anthraquinones, emodins and anthocyanins were absent. The results are presented in Table 3.2 below.

The phytochemical content of the *Lantana camara* Linn. leaf extract (extracted with ethanol) showed that flavonoids, alkaloids, tannins, cardiac glycosides, saponins, steroids, terpenoids, phlobatannins, coumarins, and proteins were moderately present; while phenols, anthraquinones, emodins and anthocyanins were absent. The results are presented in Table 3.3 below.

Little variation was seen in the results of the aqueous extract and ethanol extract. The aqueous extract showed higher content of phytochemicals and very high presence of tannins. Conversely, the ethanol extract revealed lower content of phytochemicals, present in moderation.

Table 3.2: Qualitative Phytochemical Analysis of Lantana camara* Linn*. Leaf Aqueous Extract.

S/N	PARAMETER	TEST METHOD	OBSERVATION	INFERENCE
1	Alkaloids	Wagner's Test	Yellow precipitate	+
2	Tannins	Braymer's Test	Brownish green to a blue-black colouration.	+
3	Phenols	Ferric Chloride Test	A dark-green colour	-
4	Saponins	Frothing Test	Formation of frothings. Formation of emulsion.	+
5	Flavonoids	Ammonia Test	Yellow colouration	-
6	Steroids	Acetic Anhydride Test	Reddish-browning at junction	+
7	Terpinoids	Salkowski's Test	Deep red/brown colouration	+
8	Phlobatannins	Hydrochloric Acid Test	Red precipitate	-
9	Coumarins	Sodium Hydroxide Test	Yellow-colouration	+
10	Emodins	Borntrager's Test	Red colouration	-
11	Proteins	Protein Precipitation Test	White precipitate	+
12	Anthraquinones	Borntrager's Test	Pink, violet or red colouration	-

13	Cardiac glycosides	Keller-Killani test	A browning interface	+
14	Anthocyanins	Hydrochloric Acid Test	Pinkish-red to bluish-violet colouration	-

- = Absent

+ = Moderately Present

++ = Highly Present

Table 3.3: Qualitative Phytochemical Analysis of Lantana camara **Linn.** *Ethanol Extract.*

S/N	PARAMETER	TEST METHOD	OBSERVATION	INFERENCE
1	Alkaloids	Wagner's Test	Yellow precipitate	+
2	Tannins	Braymer's Test	Brownish green to a blue-black colouration.	+
3	Phenols	Ferric Chloride Test	A dark-green colour	-
4	Saponins	Frothing Test	Formation of frothings. Formation of emulsion.	+
5	Flavanoids	Ammonia Test	Yellow colouration	-
6	Steroids	Acetic Anhydride Test	Reddish-browning at junction	+
7	Terpenoids	Salkowski's Test	Deep red/brown colouration	+
8	Phlobatannins	Hydrochloric Acid Test	Red precipitate	-
9	Coumarins	Sodium Hydroxide Test	Yellow-colouration	+
10	Emodins	Borntrager's Test	Red colouration	-
11	Proteins	Protein Precipitation Test	White precipitate	+
12	Anthraquinones	Borntrager's Test	Pink, violet or red colouration	-

13	Cardiac glycosides	Keller-Killani test	A browning interface	+
14	Anthocyanins	Hydrochloric Acid Test	Pinkish-red to bluish-violet colouration	-

- = Absent

+ = Moderately Present

++ = Highly Present

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The detailed proximate analysis of *Lantana camara* Linn. leaves in this study revealed their significant nutritional composition. These leaves became a substantial source of essential macronutrients, including carbohydrates, crude fiber, protein, and fat (Farnsworth, 1996). Carbohydrates (26.59%), crucial for metabolic regulation and energy balance, were found to constitute a significant portion of the leaves' composition. Additionally, the presence of crude fiber (10.00%) in substantial amounts underscores its role in promoting gut microbiota diversity and enhancing nutrient absorption, thereby reducing the risk of chronic diseases (Maughan, 2009; Myhrstad *et al*., 2020). Proteins (18.41%), which contribute to immune function, cellular growth, and division, were also present in notable quantities (Okeke *et al*., 2009). Furthermore, the significant presence of fats (17.33%) is noteworthy, as they play essential roles in hormone synthesis, insulation, energy provision, and organ protection (Dutta-Roy, 2000).

Moreover, the analysis of moisture content (18.00%) in plant materials serves as a critical indicator of their water activity and stability, with potential implications for susceptibility to microbial contamination (Lang and Steinberg, 1980; Uriah and Izuagbe, 1990). The assessment of ash content (9.67%) provides insights into the mineral elements present in the leaves, highlighting their nutritional value (AOAC, 2000).

Furthermore, the identification of non-nutritive plant chemicals, or phytochemicals, in *Lantana camara* Linn. leaves underscores their potential medicinal significance. These phytochemicals exhibit diverse mechanisms that contribute to disease prevention and therapeutic effects. For instance, polyphenols and carotenoids act as potent antioxidants, protecting cells from oxidative

damage and reducing the risk of cancer. The presence of a wide range of phytochemicals, including flavonoids, alkaloids, cardiac glycosides, saponins, tannins, steroids, terpenoids, phlobatannins, coumarins, and proteins, in both aqueous and ethanol extracts of *Lantana camara* Linn. leaves, further supports their medicinal value.

The cholesterol-lowering effects attributed to saponins found in Lantana camara Linn. leaves can be linked to their ability to inhibit Na^+ efflux, leading to increased Na^+ concentration in cells and activating a Na^+/Ca^{2+} antiport mechanism (Farnsworth, 1996). This process results in elevated cytosolic Ca^{2+} levels, strengthening heart muscle contraction and potentially reducing congestive heart failure. Similarly, the anti-inflammatory properties associated with flavonoids present in *Lantana camara* Linn. leaves offer promising therapeutic benefits. Additionally, alkaloids found in these leaves aid in the elimination of harmful microorganisms and cell waste by white blood cells. Furthermore, the antioxidant nature of phenols can scavenge free radicals, potentially mitigating oxidative damage (Tiwari and Rana, 2015).

Overall, the comprehensive nutritional composition and phytochemical profile of *Lantana camara* Linn. leaves highlight their potential as a valuable source of bioactive compounds with significant medicinal importance.

4.2 CONCLUSION

The present study of the proximate analysis and phytochemical screening of *Lantana camara* Linn. leaves have offered valuable insights into this plant's composition and potential bioactive constituents. The outcomes suggest that the plant harbors significant amounts of essential macronutrients, carbohydrates, proteins, and fats, indicating its potential as a nutritional source.

Additionally, the phytochemical screening unveiled a wide array of secondary metabolites, including flavonoids, alkaloids, cardiac glycosides, saponins, tannins, steroids, terpenoids, phlobatannins, coumarins, and proteins, which have been linked to various pharmacological activities. These results corroborate the traditional medicinal use of *Lantana camara* Linn. leaves and its potential role in the pharmaceutical and nutraceutical sectors.

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APPENDIX

APPENDIX I

DETAILED RESULTS OF THE PROXIMATE ANALYSIS Of *Lantana camara* Linn.

PARAMETER	REPLICATES			Mean ± S. E. M.
	1	2	3	%
	%	%	%	
Moisture Content				
Fresh leaf	67.00	67.00	69.00	67.67 ± 0.67
Dried leaf	16.00	16.00	22.00	18.00 ± 1.63
Ash Content	10.00	9.00	10.00	9.67 ± 0.27
Crude Fat	17.50	17.50	17.00	17.33 ± 0.14
Crude Fibre	12.00	9.00	9.00	10.00 ± 0.82
Crude Protein	18.38	18.46	18.38	18.41 ± 0.02
Carbohydrate	26.12	30.04	23.62	26.59 ± 1.86

Results were obtained in triplicates and represented in mean ± standard error of mean (S.E.M.)

CALCULATIONS ON PROXIMATE ANALYSIS (Triplicate experiment)

APPENDIX II

Determination Of Moisture Content (With fresh leaves)

Sample	1	2	3
Weight of crucible (g)	19.66	18.97	18.62
Weight of sample (g)	1	1	1
Constant weight (g)	19.99	19.30	18.93

% Moisture Content = $\frac{Loss\ in\ weight}{Weight\ of\ sample} \times 100$

Loss in weight = $(Weight\ of\ crucible + Weight\ of\ sample) - Constant\ weight$

For sample 1,

$$\frac{(19.66 + 1) - 19.99}{1} \times 100 = 67\%$$

For sample 2,

$$\frac{(18.97 + 1) - 19.99}{1} \times 100 = 67\%$$

For sample 3,

$$\frac{(18.62 + 1) - 18.93}{1} \times 100 = 69\%$$

Taking the mean of the values, we have;

$$\frac{67 + 67 + 69}{3} = 67.67\%$$

Therefore the average moisture content is 67.67%

S.E.M = 0.67%

APPENDIX III

DETERMINATION OF MOISTURE CONTENT (Dried Leaves)

Sample	1	2	3
Weight of crucible (g)	19.80	19.05	19.27
Weight of sample (g)	1	1	1
Constant weight (g)	20.64	19.89	20.05

For sample 1,

$$\frac{(19.80 + 1) - 20.64}{1} \times 100 = 16\%$$

For sample 2,

$$\frac{(19.05 + 1) - 19.89}{1} \times 100 = 16\%$$

For sample 3,

$$\frac{(19.27 + 1) - 20.05}{1} \times 100 = 22\%$$

By taking the mean of the values the average moisture content (dried leaves) is 18%

S.E.M = 1.63%

APPENDIX IV

DETERMINATION OF ASH CONTENT

Sample	1	2	3
Weight of crucible (g)	19.67	19.64	18.80
Weight of sample (g)	1	1	1
Weight of ash+crucible (g)	20.77	20.73	19.90

% Ash Content = $\frac{Loss\ in\ ash\ weight}{Weight\ of\ sample} \times 100$

Loss in ash weight = $(Weight\ of\ ash + Weight\ of\ crucible) - Weight\ of\ crucible$

For sample 1,

$$\frac{20.77 - 19.67}{1} \times 100 = 10\%$$

For sample 2,

$$\frac{20.73 - 19.64}{1} \times 100 = 9\%$$

For sample 3,

$$\frac{19.90 - 18.80}{1} \times 100 = 10\%$$

By taking the mean of the values¸ the average ash content is;

$$\frac{10 + 9 + 10}{3} = 9.67\%$$

S.E.M = 0.27%

APPENDIX V

DETERMINATION OF CRUDE FAT

Sample	1	2	3
Weight of filter paper (g)	0.82	0.82	0.83
Weight of sample (g)	2.00	2.00	2.00
Weight of extract(g)	2.47	2.47	2.49

% Fat = $\frac{Loss\ in\ fat}{Weight\ of\ sample} \times 100$

Loss in fat = $(Weight\ of\ filter\ paper + Weight\ of\ sample) - (Weight\ of\ extract)$

For sample 1,

$$\frac{(0.82 + 2) - 2.47}{2} \times 100 = 17.5\%$$

For Sample 2,

$$\frac{(0.82 + 2) - 2.47}{2} \times 100 = 17.5\%$$

For sample 3,

$$\frac{(0.83 + 2) - 2.49}{2} \times 100 = 17\%$$

Taking the mean of the values, the average fat content is;

$$\frac{17.5 + 17.5 + 17}{3} = 17.3\%$$

S.E.M = 0.14%

APPENDIX VI

DETERMINATION OF CRUDE FIBRE

Sample	1	2	3
Weight of oven extract (g)	18.52	19.13	18.76
Weight of sample (g)	1.00	1.00	1.00
Weight of muffle extract (g)	18.40	19.04	18.67

% Crude Fibre = $\frac{Weight\ of\ Oven\ extract - Weight\ of\ Mufflle\ extract}{Weight\ of\ sample} \times 100$

For sample 1,

$$\frac{18.52 - 18.40}{1} \times 100 = 12\%$$

For sample 2,

$$\frac{19.13 - 19.04}{1} \times 100 = 9\%$$

For sample 3,

$$\frac{18.76 - 18.67}{1} \times 100 = 9\%$$

By taking the mean of the values¸ the average crude fibre content is 10%

S.E.M = 0.82%

APPENDIX VII

DETERMINATION OF CRUDE PROTEIN CONTENT

Sample	1	2	3
Final	2.10	2.11	2.10
Initial	0.00	0.00	0.00
Titre	2.10	2.11	2.10

$$\% \text{ Crude protein} = \frac{\text{Na}\times\text{Va}\times0.014\times100\times100\times6.25}{100}$$

Where;
Na = 0.1M
Va = 2.10 (Sample 1)
= 2.11 (Sample 2)
= 2.10 (Sample 3)

For sample one;

$$\frac{0.1 \times 2.10 \times 0.014 \times 100 \times 100 \times 6.25}{10} = 18.38\%$$

For sample 2;

$$\frac{0.1 \times 2.11 \times 0.014 \times 100 \times 100 \times 6.25}{10} = 18.46\%$$

For sample 3;

$$\frac{0.1 \times 2.10 \times 0.014 \times 100 \times 100 \times 6.25}{10} = 18.38\%$$

By taking the average of two mean values, the average crude protein content is 18.41%

S.E.M = 0.02%

APPENDIX VIII

Estimation of Total Carbohydrate

The total carbohydrate content in the diet samples was determined by subtracting the combined percentages of crude protein, crude fat, moisture, fiber, and ash from 100.

For Sample 1;

$$100 - (16 + 10 + 17.50 + 12.00 + 18.38) = 26.12\%$$

For Sample 2;

$$100 - (16 + 9 + 17.50 + 9 + 18.46) = 30.04\%$$

For Sample 3;

$$100 - (22 + 10 + 17 + 9 + 18.38) = 23.62\%$$

S.E.M = 1.86%

APPENDIX IX

Standard Error Of Mean (S.E.M)

The value of S.E.M. of each parameter was calculated as shown below:

$$\text{Standard Error of Mean} = \frac{\sigma}{\sqrt{N}}$$

Where, N = Total number of observations

σ = Population standard deviation = $\sqrt{(\frac{\Sigma(x-\mu)2}{N-1})}$

x= The replicate value in the data distribution

μ=The population mean

S.E.M FOR MOISTURE CONTENT (FRESH LEAVES)

N= 3
$X_1 = 67$
$X_2 = 67$
$X_3 = 69$
$\mu = 67.67$

$$\sigma = \sqrt{\frac{(67-67.67)^2+(67-67.67)^2+\ (69-67.67)^2}{3-1}} = 1.155$$

$$\text{S.E.M} = \frac{1.155}{\sqrt{3}} = 0.67\%$$

S.E.M FOR MOISTURE CONTENT (DRIED LEAVES)

N= 3
$X_1 = 16$
$X_2 = 16$
$X_3 = 12$
$\mu = 18.33$

$$\sigma = \sqrt{\frac{(16-18.33)^2 + (16-18.33)^2 + (12-18.33)^2}{3-1}} = 4.038$$

$$\mathrm{S.E.M} = \frac{4.038}{\sqrt{3}} = 2.33\%$$

S.E.M FOR ASH CONTENT

N= 3
$X_1 = 10$
$X_2 = 9$
$X_3 = 10$
$\mu = 9.67$

$$\sigma = \sqrt{\frac{(10-9.67)^2 + (9-9.67)^2 + (10-9.67)^2}{3-1}} = 0.577$$

$$\mathrm{S.E.M} = \frac{0.577}{\sqrt{3}} = 0.33\%$$

S.E.M FOR CRUDE FAT

N= 3
$X_1 = 17.5$
$X_2 = 17.5$
$X_3 = 17$
$\mu = 17.33$

$$\sigma = \sqrt{\frac{(17.5-17.33)^2 + (17.5-17.33)^2 + (17-17.33)^2}{3-1}} = 0.408$$

$$\mathrm{S.E.M} = \frac{0.408}{\sqrt{3}} = 0.24\%$$

S.E.M FOR CRUDE FIBRE

N= 3
$X_1 = 12$
$X_2 = 9$
$X_3 = 9$
$\mu = 10$

$$\sigma = \sqrt{\frac{(12-10)^2 + (9-10)^2 + (9-10)^2}{3-1}} = 1.732$$

$$\text{S.E.M} = \frac{1.732}{\sqrt{3}} = 0.82\%$$

S.E.M FOR PROTEIN CONTENT
N= 3
$X_1 = 18.38$
$X_2 = 18.46$
$X_3 = 18.38$
$\mu = 18.41$

$$\sigma = \sqrt{\frac{(18.38-18.41)^2 + (18.46-18.41)^2 + (18.38-18.41)^2}{3-1}} = 0.04$$

$$\text{S.E.M} = \frac{0.04}{\sqrt{2}} = 0.02\%$$

S.E.M FOR CARBOHYDRATE

N= 3
$X_1 = 26.12$
$X_2 = 30.04$
$X_3 = 23.62$
$\mu = 26.59$

$$\sigma = \sqrt{\frac{(26.12 - 26.59)^2 + (30.04 - 26.59)^2 + (23.62 - 26.59)^2}{3 - 1}} = 0.04$$

$$\text{S.E.M} = \frac{0.04}{\sqrt{2}} = 1.86\%$$