PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *Plumbago zeylanica* Linn. LEAF

BY

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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 **MOMOH OSHOMAH PRAISE** with matriculation number **LSC1806348** 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

TO MY FATHER, MR. JOHN A. MOMOH.

I experience your guidance every day.

ACKNOWLEDGMENTS

First and Foremost, heartfelt appreciation to God Almighty for unending grace to complete this exercise successfully.

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God bless you all.

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ABSTRACT

The nutritional composition and phytochemical content of the leaf of *Plumbago zeylanica* Linn. were investigated using standard analytical methods in order to access the numerous potential of the plant. The qualitative phytochemical screening of aqueous extract of *Plumbago zeylanica* Linn. leaves revealed the presence of saponins, flavonoids, alkaloids, eugenols, phenols, and reducing sugars; with saponins being highly present. Glycosides, terpenoids, steroids and tannins were seen to be absent. Variation in this composition was observed in the ethanol extract which showed that glycosides, saponins, phenols, eugenols, terpenoids, steroids, alkaloids, flavonoids and reducing sugars were present in moderation, whereas tannins were seen to be absent. The medicinal value of *Plumbago zeylanica* Linn. is influenced by the presence and levels of these secondary metabolites. The proximate analysis revealed that *Plumbago zeylanica* Linn. leaves are rich in carbohydrates (34.990 ± 1.088%), have high content of Ash (8.606 ± 0.73%), crude protein (19.942 ± 0.154%), crude lipids (9.194 ± 0.258%), and crude fibre (27.267 ± 1.62%); but low content of moisture (4.993 ± 0.035%). Vitamin C content was also determined to be 4.724 ± 0.095%. The presence of high carbohydrates, protein, crude lipids and fiber contents of the leaves may be responsible for their nutritive value.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Plumbago zeylanica Linnaeus, a perennial angiosperm, is a plant species that occupies a prominent position in various ecological, cultural, and medicinal landscapes. Belonging taxonomically to the Plumbaginaceae family, this plant is colloquially referred to as the Ceylon leadwort because of its historical connection to the Indian subcontinent, specifically Sri Lanka. It is widely distributed in Africa and Asia. *Plumbago zeylanica* **L**inn. is used to treat unspecified medicinal disorders and has environmental uses as a poison, medicine, and food (Chetty, 2006; Azad *et al*., 1982; Mandavkar Y. D. and Jalalpure S. S., 2011) The botanical characteristics, cultural significance, and multifaceted applications of *Plumbago zeylanica* Linn. collectively contribute to its intriguing profile, warranting a comprehensive exploration.

Botanically*, Plumbago zeylanica* Linn. exhibits distinctive features that have attracted the attention of researchers and botanists. It is characterized by slender stems, lanceolate leaves, and delicate funnel-shaped flowers that come in shades of blue and white. The ability of plants to thrive under diverse climatic conditions, ranging from tropical to subtropical, has facilitated their distribution across regions. Additionally, the role of *Plumbago zeylanica* Linn. as a potential soil stabilizer owing to its robust root system adds to its ecological importance (Bhattacharjee, 1998; Chen *et al*., 2011).

In addition to its botanical attributes, *Plumbago zeylanica* Linn. boasts of a rich cultural heritage that is deeply intertwined with traditional practices across its native range. This plant has been used in rituals, ceremonies, and cultural festivals for centuries. Its vibrant blue and

white flowers symbolize purity, serenity, and auspiciousness in various cultural contexts, leading to their incorporation into ornamental gardening and landscape design (Ignacimuthu *et al*., 1998).

Plumbago zeylanica Linn. is extensively utilized in traditional and folk medicine systems. Ayurveda, Siddha, and other indigenous healing traditions have harnessed the pharmacological properties of plants for various medicinal purposes. The presence of bioactive compounds such as plumbagin in plants has attracted the attention of modern scientific research. Plumbagin is known for its anti-inflammatory, antimicrobial, and antioxidant properties, opening avenues for further pharmacological investigation (Mandavkar Y. D. and Jalalpure S. S., 2011; Aleem, 2020).

In essence, *Plumbago zeylanica* Linn. stands for a botanical, cultural, and medicinal marvel, weaving a tapestry that spans the realms of ecology, tradition, and modern science. Their botanical characteristics make them an intriguing subject for taxonomists, whereas their cultural significance enriches our understanding of the interplay between plants and human society. Furthermore, its potential medicinal value underscores the relevance of traditional knowledge systems. This exploration of the multifaceted dimensions of *Plumbago zeylanica* sets the stage for an in-depth study that bridges the gap between traditional wisdom and contemporary scientific understanding (Pant *et al*., 2012).

1.1.1 STATEMENT OF PROBLEM

The leaves of *Plumbago zeylanica* Linn. is believed to have several chemical composition including vitamins and minerals also having a good nutritional and medicinal purpose. This study will help to ascertain the proximate analysis and phytochemical constituents of the leaves and extracts of the plant.

1.1.2 AIM AND OBJECTIVE OF THE STUDY

The purpose of this work is to evaluate the proximate and phytochemical screening of *Plumbago zeylanica* Linn, as well as its vitamin C content.

1.1.3 SPECIFIC OBJECTIVES OF THE STUDY

The study has the following precise aims:

- Proximate analysis of the leaf of *Plumbago zeylanica* Linn.
- Vitamin C content determination of the leaf of *Plumbago zeylanica* Linn.
- Phytochemical screening (qualitative) of the leaf of *Plumbago zeylanica* Linn.

1.2 LITERATURE REVIEW

1.2.1 Scientific Classsification of Plant Species

Domain……Eukaryota

Kingdom……. Plantae

Phylum……Tracheophyta

Subphylum……..Angiospermae

Class……Magnoliopsida

Order…….. Caryophyllales

Family…… Plumbaginaceae

Genus……. *Plumbago*

Specie…….*zeylanica*

1.2.2 Nomenclature

Scientific Name: *Plumbago zeylanica* Linnaeus

Common Name(s): Ceylon leadwort, Doctor bush, Garden plumbago, Chitrak, Wild plumbago.

Nigerian Name(s): *Onayo ako* (Igbo), *Inabiri* (Yoruba), *Ebe atogbon* (Esan).

1.2.3 Description

There is no consensus in the literature regarding the classification of *Plumbago zeylanica* Linn. as either an herb or a shrub. Some authors have described it as a perennial dicot herb (Chetty *et al*., 2006; Kumar *et al*., 2009), while others have classified it as a shrub (Dhale *et al*., 2011). *Plumbago zeylanica* Linn. is a plant that grows to a height of about 0.5-2 m (1.6-6.6 ft). Its leaves are alternate, simple, ovate or ovate-lanceolate, elliptical or oblong, and can measure from 0.5-12 cm in length with a tapered base and often have a hairy margin. Stipules are absent, and petioles are narrow (0-5 mm long) with small auricles in young leaves. The inflorescence is a terminal raceme-type, measuring about 6-30 cm in length, and bears many flowers. The flowers are white in color and are borne in axillary and terminal elongated spikes. They are bisexual, regular, pentamerous, pedicellate, and have a sweet scent. The stamens are free and included. The style is filiform with five elongated stigma lobes, and the ovary is superior and single-celled. The flowers are also characterized by a tubular calyx (7-11 mm long and 5-ribbed) with glandular trichomes (hair) secreting sticky mucilage. The plant flowers throughout the year and is primarily pollinated by insects. Mucilaginous glands assist in capturing insects and facilitating the distribution of fruits through animal

interactions.The fruit of the plant is an oblong (7.5-8 mm long) five-furrowed capsule containing a single seed. Each seed is oblong in structure, 5-6 mm long, and reddish-brown to dark brown in color. Roots are straight, smooth, branched, or unbranched, with or without secondary roots, and can reach a length of 30 cm or more and a diameter of 6 cm. They are light yellow when fresh and reddish-brown when dried. The roots have a strong and characteristic odor, with an acrid and bitter taste (Lubaina *et al*., 2011; Chetty *et al*., 2006).

1.2.3 Habitat

The native range of *Plumbago zeylanica* Linn. is Tropics and Subtropics. It grows throughout Asia and Africa (Jain and Bashir, 2011).

1.2.4 Ecological Conditions

Plumbago zeylanica Linn. can grow in various soils, but prefers well-drained, deep sandy loam or clayey loam soil with high organic content (Sivanesan *et al*., 2009).

1.2.5 Propagation

Plumbago zeylanica Linn. can be propagated through seeds, rooted shoots from the base of the plant, or semi-ripe cuttings treated with a growth hormone. The seeds take 21–30 days to germinate, but prolonged storage (over 3 months) results in a significant decline in germination rate. Sowing seeds in a nursery and transplanting into the field at a density of 60 x 60 cm is a common method of propagation. In natural habitats, the plants thrive in moist soil with high organic content and partial shade. However, conventional propagation methods have been inadequate for meeting the growing demand for the plant in the market due to poor seed germination and the premature death of seedlings (Sivanesan *et al*., 2009). To address

this issue, the technique of *in vitro* propagation has been successfully utilized using various explants and callus cultures (Pant *et al*., 2012).

Plate 1: The leaves of *Plumbago zeylanica* Linn.

Source: Google

Plate 2: Flowers of *Plumbago zeylanica* Linn.

Source: Google

1.2.6 Phytochemistry.

Naphthaquinones, alkaloids, glycosides, steroids, triterpenoids, tannins, phenolic compounds, flavonoids, saponins, coumarins, carbohydrates, fixed oils, fats, and proteins are present in various parts of *Plumbago zeylanica* Linn. plant (Ming *et al.,* 2011). Previous chemical analyses of this plant revealed that its roots contain plumbagin, 3-chloroplumbagin, 2,3-biplumbagin, 6,6-biplumbagin, zeylinone, isozeylinone, chitranone (3, 3'-biplumbagin), droserone, plumbagic acid, plumbazeylanone, glucose, fructose, and enzymes such as protease and invertase. There was little to no plumbagin in the leaves or stems. The aerial parts also contained amino acids, hentriacontane, sitosterol, lupeol, and lupenyl acetate. In addition, the plant contains zeylinone, isozeylinone, plumbagic acid, plumba-zeylanone, naphthelenone, isonaphthelenone, and isoshinanolone (Anonymous, 1989; Chen *et al*., 2011; Kumar *et al*., 2009). Aspartic acid, tryptophan, tyrosine, threonine, alanine, histidine, glycine, methionine, and hydroxyproline were also isolated from the aerial parts (Williamson, 2002).

1.2.7 Beneficial Attributes and Medicinal Potential.

Locals in Southern Nigeria consume the leaves of the plant as a vegetable (personal observation), likely as a result of its nutritional value. However, additional research is needed to support this claim as study on proximate and nutritive analysis of *Plumbago zeylanica* Linn. is scarce. Instead, emphasis is placed on the medicinal potential.

Traditional Uses

Plumbago zeylanica Linn. play a significant role in Ayurvedic medicine. The use of leaves in the treatment of infections and digestive issues, such as dysentery. Leaf paste is applied to

treat chronic itchy skin conditions or painful rheumatoid joints (Chaudhari and Chaudhari, 2015). According to Sharma *et al*. (2000), the leaves are caustic, vesicant, and aphrodisiac.

Antibacterial Properties

The aqueous extract and its partition (petroleum ether, dichloromethane, methanol, and aqueous residue) were effective against *Salmonella gallinarum, Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeruginosa, and Staphylococcus aureus* in mice. Inoculated with antibiotic-resistant strains of *Salmonella paratyphi*, *Staphylococcus aureus*, *Escherichia coli,* and *Shigella dysenteriae* were tested against multidrug resistance of clinical origin using an alcoholic extract from the roots of *Plumbago zeylanica*. The extract exhibited potent antibacterial activity against all the tested bacteria. In BALB/C, plumbagin showed inhibitory activity at higher concentrations, while still enhancing macrophage bactericidal activity by potentiating oxyradical release at low concentrations. This results in delayed growth due to resistance. However, growth was completely prevented when the bacteria were grown in a medium containing antibiotics and Plumbagin (Durga *et al*., 1990).

Anticonvulsant Activity

A study on the pharmacological and clinical therapeutic uses of Ayurvedic medicinal plants, including *Plumbago zeylanica* Linn., was conducted by Vishnukanta and Rana in 2010. The leaf extract of *Plumbago zeylanica* Linn. was tested for its ability to prevent convulsions induced by maximum electroshock and PTZ. However, it was found that the extract did not possess anticonvulsant properties.

Anti-fertility Activity

The efficacy of *Plumbago zeylanica* Linn. leaf extract as an anti-fertility agent was examined by Edwin *et al*. in 2009. At 200 and 400 mg/kg, the effects of petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of *Plumbago zeylanica* Linn. leaves on the estrous cycle of rats were investigated. The estrous cycle of the rats was most effectively broken by acetone and ethanol extracts (p 0.05). The animals' prolonged diestrous stage of the estrous cycle, which corresponds to momentary inhibition of ovulation, was evident. Upon stopping the treatment, the anti-ovulatory activity could be reversed. Significant estrogenic and anti-estrogenic activities were observed for both extracts (p 0.05).

Antiviral Activity

A study on the antiviral properties of Ethiopian medicinal plants used to treat dermatological disorders was carried out by Neubert et al. in 2006. This study examined the antiviral activities of 80% methanolic extracts of *Acokanthera schimperi, Euphorbiae schimperi, Inula confertiflora, Mutinus elegans,* and *Plumbago zeylanica* Linn. against cox-sackievirus B3 (CVB3), influenza A virus, and herpes simplex virus type 1 (HSV-1) using cytopathic effect (CPE) inhibitory assays in HeLa, MDCK, and GMK cells, respectively. The results demonstrated that the *Plumbago zeylanica* Linn. extract inhibited CVB3, whereas the *I. confertiflora* extract inhibited HSV-1..

Hepatoprotective Activity

Rajesh *et al*. (2009) examined the hepatoprotective effects of a methanolic extract of the aerial parts on wistar rat liver toxicity in CCl4. By lowering serum total bilirubin, SGPT, SGOT, and ALP levels, the extract of the aerial parts of *Plumbago zeylanica* Linn.

demonstrated significant hepatoprotection against CCl4-induced hepatotoxicity in Wistar rats. Histopathological studies also supported the hepatoprotective properties of the extract.

Anti-Inflammatory Activity

Sheeja *et al.* (2010) used in vivo experimental models and two dose levels (200 and 400 mg/kg, p.o.) to test the anti-inflammatory and anti-nociceptive effects of different leaf extracts of *Plumbago zeylanica* Linn. (Petroleum ether, chloroform, acetone, ethanol, and aqueous). Compared with the control group, the acetone extract significantly ($p < 0.01$) reduced inflammation in carrageenan-induced rats. Regarding the analgesic effect, petroleum ether and acetone extracts only significantly ($p < 0.01$) reduced the pain stimulus during the later stage of the formalin test, indicating that the medication may act peripherally.

Hyperlipidaemic activity

Alpana (1996) investigated the impact of *Plumbago zeylanica* Linn. on hyperlipidemic rabbits and how vitamin E alters this effect. Triglyceride, LDL, and total serum cholesterol levels were significantly decreased. A significant reduction was observed with the *Plumbago zeylanica* Linn. and vitamin E formulations. The LDL-to-HDL and total-to-HDL cholesterol ratios were found to have significantly (p0.01) decreased.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 PLANT SAMPLE

Fresh leaves of *Plumbago zeylanica* Linn. collected in June, 2023 from a fallowed farm at Idelegbagbon Street, Off Pipeline, Eyaen, Uhunmwonde L.G.A., Edo State. Fresh parts of the plants were identified prior to analysis by Prof. Emmanuel I. Aigbokhan at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

Voucher Number: UBH-P636 issued by Dr. Henry A. Akinnibosun.

2.1.2 REAGENTS

The reagents used in this study were manufactured by Loba Chemie Pvt Ltd, India and they include:

Hydrochloric acid

Mayers reagent

Wagner's reagent

Benedict solution

Fehling's solution A and B

Gelatin

Sodium chloride

Ferric chloride

Sodium hydroxide

Lead acetate

Sulphuric acid

Chloroform

Oxalic acid

Potassium permanganate

Ascorbic acid

Petroleum ether

Ethanol

Phenol colorimetric kit

Sodium potassium tartarate

Sodium hypochlorate

Potassium nitrate

Sodium sulphate

Selenium

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 (Labconco, USA)

Digester (Hanon Lab., China)

UV/Visible spectrophotometer (Bruker Physik, Germany)

Muffle furnace (Kejia furnace, China)

Beakers (Pyrex, Nigeria)

Conical flasks (Technico, India)

Standard flask (Technico, India)

2.2 METHODS.

2.2.1 PREPARATION OF SAMPLES

The freshly collected leaves of *Plumbago zeylanica* 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 and assayed.

2.2.2 EXTRACTION OF SAMPLES FOR PHYTOCHEMICAL SCREENING.

Extraction was done using the method described by Jimoh *et al*., (2010). Exactly 10g of sample was weighed and transferred into an electric blender and 100ml of solvent 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.

2.2.3 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 Mayer's Test: Alkaloids consist of nitrogen atoms which have lone pair electrons. The lone pair electrons form covalent coordinate bonds with metal ions, specifically potassium ions (K^+) provided by potassium tetraiodomercurate (II) in the sample. This results in the precipitation of yellow potassium-alkaloid complex, indicating the presence of alkaloids (Altemimi *et al*., 2017).

Principle of Wagner's Test: In the Wagner test, the K^+ metal ion forms a covalent coordinate bond with nitrogen in the alkaloid producing a yellow precipitate of potassium-alkaloid complex. The positive results of alkaloids test in Wagner's test is indicated by the appearance of the brownish to yellowish precipitate (Altemimi *et al*., 2017).

Procedure: This was done by first evaporating to dryness, 2.0ml of the plant extract. Then the resultant residues were dissolved in 5ml of HCl (2mol/ dm^3) and filtered. The filtrate was separated into two separate test tubes. To the first test tube, few drops of Mayer's reagent were added, and formation of a yellow-coloured precipitate indicated the presence of alkaloids.

a. The second test tube was treated with few drops of Wagner's reagent, and the formation of brownish red precipitate indicated the presence of alkaloids.

2. **Detection of Reducing Sugars**

Principle: Reducing sugars are able to reduce copper ions (Cu^{2+}) in an alkaline solution, resulting in the formation of a coloured precipitate of copper (I) oxide (Cu_2O). The intensity of the color change corresponds directly to the concentration of reducing sugars in the sample (Singh *et al*., 1970).

Procedure: This was done by dissolving 2ml of the plant extract in 2ml of water. The resultant solution was divided into two test tubes. The first test tube was treated with Benedict's reagent and then heated gently. The appearance of orange-red precipitate signified the existence of reducing sugars.

a. The second test tube was treated with 20 drops of boiling Fehling's solution (A and B). The formation of a brick – red precipitate in the bottom of the tube indicated the presence of reducing sugars.

3. **Detection of Tannins**

Principle: Tannins have a high affinity for binding to proteins due to their ability to form hydrogen bonds and other non-covalent interactions. In the gelatin/ NaCl test for a sample containing tannins, the tannin molecules interact with the gelatin molecules. The tannin molecules bind to the gelatin molecules through hydrogen bonding and other intermolecular forces. The binding results in the formation of insoluble complexes or aggregates. This forms a white precipitate which indicates the presence of tannins in the sample (Sungur and Uzar, 2008).

Procedure: To 1.0ml of the extract, 1.0ml of 1% gelatin solution containing sodium chloride was added. No formation of white precipitate indicated the absence of tannins.

4. **Detection of Phenols**

Principle: Compounds with a phenol group, such as enols, hydroxamic acids, sulfinic acids, and oximes, will form a blue, violet, purple, green, or red-brown color upon addition of aqueous ferric chloride ($FeCl_3$). This is as a result of the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by phenols in the plant sample (Dai and Mumper, 2010).

Procedure: This was done by treating 1.0ml of the plant extract with 4 drops of ferric chloride solution. Formation of a bluish black colour indicated the presence of phenols.

5. **Detection of Saponins**

Principle: Saponins have surfactant properties. When a solution of a sample containing saponins is agitated, air is introduced into the solution. The surfactant property reduces the surface tension of water, allowing air to become trapped in the solution. This results in the formation of a stable froth or foam on the surface of the solution (Vincken *et al*., 2007).

Procedure: The foam test method and froth test methods were used in the detection of saponins. In the foam test method, 0.5g of the plant extract was shaken with 2.0ml of distilled water. The formation of foam which persisted for 10 minutes indicating the presence of saponins.
In the froth test method, 5.0ml of the extract was diluted with distilled water to 20.0ml and this was shaken in a 50ml graduated cylinder for 15 minutes. Formation of 1cm layer foam indicated the presence of saponins.

6. **Detection of Flavonoids**

Principle: Flavonoids, when subjected to an alkaline environment in sodium hydroxide test, undergo a chemical reaction known as alkaline hydrolysis. This reaction often leads

to a color change in the solution. Flavonoids can change from colorless or pale yellow to various colors, depending on their specific chemical structure. The observed color change is indicative of the presence of flavonoids (Litvinenko and Makarov, 1969). In the lead test, flavonoids chelate lead ions (Pb^{2+}) contributed by lead acetate resulting in the formation of insoluble lead-flavonoid complexes that are yellow in colour and signals the presence of flavonoids (Malesev and Kuntic, 2007).

Procedure: This was done using the sodium hydroxide test and the lead acetate test. In sodium hydroxide test, the extract was treated with few drops of 2mol/dm^3 solution of sodium hydroxide. The formation of an intense yellow colour which became colourless on addition of dilute hydrochloric acid (2mol/dm^3), indicated the presence of flavonoids. In the lead acetate test, the plant part extract was treated with few drops of lead acetate solution. The emergence of a yellow precipitate signified the prsence of flavonoids.

7. **Detection of Eugenols**

Principle: Eugenols are converted into water-soluble form (potassium eugenolate) through an alkaline reaction with KOH. Then, it's reverted back to eugenol in an acidic environment with HCl, allowing for precipitation of eugenol appearing as a pale yellow (Ntamila and Hassanali, 1976).

Procedure: About 2ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. Few drops of HCl were added to the filtrate. A pale yellow precipitate was indicative of positive test.

8. **Detection of Steroids**

Principle: Certain steroids, especially those with conjugated double bonds in their structure, can undergo a chemical reaction known as the "unsaturation test" or "Liebermann-Burchard test" when treated with acetic acid and sulfuric acid. Acetic acid is

a weak acid that is used to create an acidic environment. Sulfuric acid is a strong acid and is added gently into the solution. This reaction leads to a characteristic color change from violet to blue or green in the solution. The intensity of the color change can vary depending on the specific steroid present and its concentration. The color change, particularly the appearance of a blue or green color, serves as an indication of the presence of steroids in the sample (Nath *et al*., 1946).

Procedure: 2 ml of acetic anhydride was added to 0.5 g of the extract with 2 ml of H_2SO_4. The transition from violet to blue or green signified the presence of steroids.

9. **Detection of Terpenoids**

Principle: When chloroform ($CHCl_3$) is added to a sample containing terpenoids, it facilitates the extraction of lipophilic compounds including terpenoids from the sample. Vigorous shaking separates the chloroform phase from the aqueous phase, which contains other water-soluble compounds. Sulfuric acid is introduced to create an acidic environment within the mixture. If present in the sample, terpenoids undergo a chemical reaction with sulfuric acid. This reaction often leads to the appearance of various colors, which can range from reddish-brown to violet or blue, depending on the specific terpenoid compound present (Siddiqui *et al.,* 2009).

Procedure: Exactly 0.2 g of the extract of the plant sample was mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration in the interface indicated positive results for the presence of terpenoids.

10. **Detection of Glycosides.**

Principle: Upon reaction with ferric chloride ($FeCl_3$), certain glycosides undergo hydrolysis, which is the breaking of the glycosidic bond between sugar and non-sugar

component (aglycone) due to the acidic conditions created by acetic acid. Formation of colored complexes as well as the intensity of color change is characteristic of the type of glycoside present and the specific aglycone contained in the sample (Elderfield, 1935).

Procedure: About 1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. A lower layer was established by adding 1 milliliter of concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of glycoside.

2.2.4 Preparation of Sample for Vitamin C determination

The sample, *Plumbago zeylanica* Linn. leaves used in this study were prepared according to the method described by Elgailani *et al*., (2017) and, Aiyegoro and Okoh (2010) with little modification.

Procedure: The leaves were first washed with distilled water, sliced for easy blending and 100g of the sliced sample was weighed and transferred into the blender. About 200ml of distilled water was added and sample blended vigorously. This was followed by addition of 30 ml of oxalic acid (0.5% w/v) in order to prevent the oxidation of ascorbic acid (vitamin C). The mixture was filtered through a precleaned cloth and receiving the filtrate in a 250 ml Erlenmeyer flask. An aliquot (10ml) of sample was transferred to a 100 ml volumetric flask and then made up to the mark with oxalic acid solution (0.5%).

2.2.5 DETERMINATION OF VITAMIN C

Vitamin C concentration in the sample was determined using the method described by Pathy (2018).

Principle: Ascorbic acid reacts with potassium permanganate ($KMnO_4$) in an acidic solution. As ascorbic acid is oxidized, it reduces purple MnO^{4-} ions to colourless Mn^{2+} ions. The solution changes from purple to pale pink when the excess $KMnO_4$ is added allowing quantification of vitamin C based on the volume of $KMnO_4$ used.

Procedure: Exactly 10ml of the sample extract was accurately measured and transferred into a test tube and 1.0ml of $KMnO_4$ (100 µg/ml) was added and content mixed thoroughly. It was then allowed to stand for 5 minutes. The resultant solutions were read on a UV/Visible Spectrophotometer at 530nm against the reagent.

2.2.6 PROXIMATE ANALYSIS

Ash Content

Ash content was carried out using the method of AOAC (2000).

Principle: This is based on measuring the residue left behind when a sample is completely incinerated at high temperatures.

Procedure: Exactly 2g of the dried sample was placed into a porcelain crucible which initially was weighed and transformed into a preheated muffle furnace set at the temperature of 900^0C. The furnace was left on for one hour after which the crucible and it content was transferred to a dessicator and allowed to cool the crucible and it content was re-weigh and the weigh noted. The percentage ash content was then calculated from the relationship.

Calculations.

$$\% \text{ Ash} = \frac{\text{Wash}}{\text{Wo}} \times 100$$

W_{ash} = content weight after final incineration (g)

W_o = the dried weight of the sample (g)

Moisture Content

Moisture content was determined using the method of AOAC (2000).

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 W_1 (g). Exactly 2g of the sample was added to the crucible to obtain a weight recorded as W_2 (g). The crucible was then dried in an oven continuously. The dried sample was constantly re-weighed at 10minutes 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.

<u>Calculations</u>

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

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

Weight of sample= $W_2–W_1$ (g)

Crude Fibre Determination

This was conducted following the AOAC (1980) protocol.

Principle: This is based on the concept of sequentially removing different components of plant material to isolate the fibre fraction. Crude fibre represents the indigestible portion of a sample and consists mainly of cellulose, hemicellulose, and lignin.

Procedure: Briefly, 4 g of each moisture-free sample was weight into a 250 mL beaker and 50 mL of 4% H_2SO_4 was added followed by distilled water to a volume of 200 mL. Next, it was heated until it reached a boiling point and maintained at a boil for precisely 30 minutes over a Bunsen burner, while stirring continuously with a glass rod tipped with rubber to ensure all particles were dislodged from the sides of the beaker. The volume was maintained by adding hot distilled water as needed. After 30 min of boiling, the content was poured into a butchner funnel fitted with a Whatman no. 1 filter paper and connected to a vacuum pump. The beaker was rinsed multiple times with hot distilled water and then entirely transferred using a stream of hot water. The rinsing process continued through the funnel until the filtrate no longer showed acidity, as determined by litmus paper. The acid-free residue was transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200 mL. The mixture underwent a 30-minute boiling process with continuous stirring, following the previously mentioned method, while maintaining the volume with hot water. Subsequently, the mixture was filtered and subjected to the same washing process until it became free of alkalinity. Finally, the resultant residue was washed with two portions of 2 mL 95% alcohol. Residues on filter paper were transferred to a pre-weighed porcelain crucible. The content of the crucible was then dried in an oven maintained at 110°C to a constant weight after cooling in a desiccator. Crucible content was then ignited in a muffle furnace at 550°C for 8h, cooled and weighed. A triplicate determination was carried out on each sample.

Calculations

$$\% \text{ Crude fibre } = \frac{y - a}{x} \times 100$$

x = Weight of sample (g)

y = Weight of insoluble matter (g)

a = Weight of Ash (g)

Crude Fat Determination

The method of Pearson (1973) was employed.

Principle: This method was based on the principle that non-polar components of samples are easily extracted into organic solvents.

Procedure: Three grams, 3g (Moist-free) of each sample, was placed into fat free thimbles. These were then weighed, plugged with glass wool and introduced into soxhlet extractors containing 160 mL petroleum ether (b.p 60-80°C). Clean dry receiver flask weighed and fitted to the extractors. The extraction unit was then assembled and cold water was allowed to circulate, while the temperature of the water bath was maintained at 60°C. Extraction was carried out for 8 h. At the end of this time, the thimble containing the sample was removed and placed in an oven at 70°C for 3h and dried to constant weight. The weight of the Thimble and the content was then obtained using a standard analytical balance.

Calculations.

The crude fat was obtained as the difference in weight before and after the exhaustive extraction.

$$\% \text{ Crude fat} = \frac{X - Y}{Z} \times 100$$

where,

X = Weight sample and thimble and oil (g)

Y = Weight of empty thimble (g)

Z = Weight of sample (g)

Crude Protein Determination

For the determination of crude protein, a modified micro-Kjeldahl method, as outlined in AOAC (1990), was employed.

Principle: This method is based on the measurement of the nitrogen content in a sample, with the understanding that proteins contain a relatively consistent proportion of nitrogen.

Procedure for Digestion: Three grams each of the defatted samples were separately weighed on pre-weighed into micro-Kjeldahl digestion flask together with few anti bumping granules. In each flask, 2 grams of a catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:02 w/w) was introduced. Following this, 10 mL of concentrated H_2SO_4 free from nitrogen was also introduced into each flask. The flasks were positioned at an angle on a heating mantle within a fume hood. Digestion was started at temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30 min and finally at full heating (100°C) until a clear solution was obtained. The simmering process was extended below the boiling point for an additional 30 minutes to guarantee thorough digestion and the conversion of nitrogen into ammonium sulphate. After digestion was completed, samples were allowed to cool and then transferred quantitatively to 100 mL volumetric flasks with washing and cooling to room temperature. Distilled water was added to each container to reach the designated volume mark.

1) Exactly 5ml of the filtrate from the digest was transferred with the aid of a 10ml pipette into a 25ml standard flask. Exactly 2.5ml of the alkaline phenate was added and the solution shaken to mix properly. Then 1ml of sodium potassium tartarate was added, shaken properly followed by the addition 2.5ml of sodium hypochlorite. Following that step, the solution was adjusted to reach the 25 mL mark using distilled water, and the resulting solution's absorbance was measured using a UV/visible spectrophotometer at a wavelength of 630 nm. The nitrogen standards were subjected to the same procedure as the sample.

Calculations

$$\% \text{ Nitrogen} = \frac{\text{Instrument Reading x Slope Reciprocal x Color Vol. x Digest Vol.}}{Weight\ of\ Sample\ x\ Aliquot\ Taken \times 1000}$$

% Crude Protein = %Nitrogen×6.25

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.

Calculations

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

2.2.7 STATISTICAL ANALYSIS

All proximate assays were carried out in triplicates and the results were presented as Mean ± standard error of mean (S.E.M.). Statistical significance was determined through the use of Analysis of Variance (ANOVA).

CHAPTER THREE

RESULTS

3.1 RESULTS

3.1.1 Proximate Composition

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

Table 3.1: Proximate Composition of *Plumbago zeylanica* Linn.

S/N	**PARAMETER**	**VALUES** Mean ± S.E.M (%)
1	Moisture Content	4.993 ± 0.035
2	Ash Content	8.606 ± 0.73
3	Crude Fat Content	9.194 ± 0.258
4	Crude Fibre Content	27.267 ± 1.62
5	Crude Protein Content	19.942 ± 0.154
6	Carbohydrate Content	34.990 ± 1.088

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

3.1.2 Vitamin C Content

The analysis of *Plumbago zeylanica* Linn. included the determination of the vitamin C content, result of which is shown in Table 3.2. The results revealed that the plant sample contained poor quantity of vitamin C (4.724 ± 0.095).

***Table 3.2: Vitamin C content of the Plumbago zeylanica* Linn.**

PARAMETER	**VALUES** Mean ± S.E.M (mg/100g)
Vitamin C Content	4.724 ± 0.095

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

3.1.3 Phytochemical Content

The phytochemical content of the *Plumbago zeylanica* Linn. leaf extract (extracted with water) revealed that saponins are in abundance; flavonoids, alkaloids, eugenols, phenols, and reducing sugars are moderately present; while glycosides, steroids, terpenoids, and tannins are absent. The results are presented in Table 3.3 below.

The phytochemical content of the *Plumbago zeylanica* Linn. leaf extract (extracted with ethanol) showed that glycosides, saponins, flavonoids, alkaloids, eugenols, steroids, terpenoids, phenols, and reducing sugars are moderately present in the plant; while tannins are absent. The results are presented in Table 3.4 below.

Notable variation is seen in the results of the aqueous extract and ethanol extract. The aqueous extract showed lower content of phytochemicals and very high presence of saponins. Conversely, the ethanol extract revealed higher content of phytochemicals, present in moderation. Glycosides, steroids, terpenoids, and tannins are absent in the aqueous extract, whereas only tannins are absent in that of ethanol.

Table 3.3: Qualitative Phytochemical Analysis of *Plumbago zeylanica* Linn***. Leaf Aqueous Extract.***

S/N	PARAMETER	TEST METHOD	INFERENCE
1	Glycosides	Keller-Killiani Test	-
2	Saponins	Frothing Test Foam Test	++ +
3	Phenols	Ethanol/Ferric Chloride	+
4	Eugenols	KOH/HCl	+
5	Terpenoids	Salkowski Test	-
6	Steroids	Acetic Acid/H_2SO_4	-
7	Alkaloids	Mayer's Test Wagner's Test	+ +
8	Flavonoids	Sodium Hydroxide Test Lead Acetate Test	+ +
9	Tannins	Gelatin / NaCl Test	-
10	Reducing Sugars	Fehling's solution A and B Benedict reagent	+ +

- = Absent

+ = Moderately Present

++ = Highly Present

Table 3.4: Qualitative Phytochemical Analysis of *Plumbago zeylanica* Linn***. Ethanol Extract.***

S/N	PARAMETER	TEST METHOD	INFERENCE
1	Glycosides	General Test	+
2	Saponins	Frothing Test Foam Test	+ +
3	Phenols	Ethanol/Ferric Chloride	+
4	Eugenols	KOH/HCl	+
5	Terpenoids	Salkowski Test	+
6	Steroids	Acetic Acid/H_2SO_4	+
7	Alkaloids	Mayer's Test Wagner's Test	+ +
8	Flavonoids	Sodium Hydroxide Test Lead Acetate Test	+ +
9	Tannins	Ferric Chloride	-
10	Reducing Sugars	Fehling's solution A and B Benedict reagent Test	+ +

- = Absent

+ = Moderately Present

++ = Highly Present

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Plumbago zeylanica Linn. leaves were found to be a good source of carbohydrates, crude fibre, crude protein, and fat by its proximate analysis. Carbohydrates play a crucial role in regulating metabolism and energy balance (Maughan, 2009). Fibre is essential for promoting gut microbiota diversity, enhancing nutrient absorption, and reducing the risk of chronic diseases (Myhrstad *et al*., 2020). Proteins boost the immune system and contribute to cell growth and division (Okeke *et al*., 2009), while fats are necessary for hormone production, insulation, energy provision, and protection of vital organs (Duttta-Roy, 2000). The moisture content of plant materials is a measure of its water activity (Lang and Steinberg, 1980) and is used as an indicator of stability and susceptibility to microbial contamination (Uriah and Izuagbe, 1990). The ash content indicates the availability of mineral elements (AOAC, 2000).

The analysis of *Plumbago zeylanica* Linn. leaves revealed a vitamin C content of 4.724 ± 0.095 (mg/100g). Ascorbic acid plays a crucial role in various bodily functions such as, iron absorption, wound healing, collagen formation, immune system function, and the maintenance of connective tissues (Samtiya *et al*., 2021).

Non-nutritive plant chemicals, also known as phytochemicals, have varying degrees of disease prevention. They are priceless sources of basic ingredients in both conventional and alternative medicines. Numerous ways are known for phytochemicals to exert protective effects. For example, polyphenols and carotenoids can function as antioxidants and shield cells from the damage caused by free radicals (Omoregie and Osagie, 2012). By preventing tumor growth, they may also aid in lowering the risk of cancer (Devasagayam *et al*., 2004). Antibacterial activity and hormonal stimulation are the two additional modes of action (Mathew *et al.,* 2012). The results are in agreement with these assertions as a range of

phytochemicals were detected in both aqueous and ethanol extract of *Plumbago zeylanica* Linn. leaves, viz: saponins, flavonoids, alkaloids, eugenols, and phenols with varying amounts. The medicinal value of *Plumbago zeylanica* Linn. is influenced by the presence of these secondary metabolites. The presence of saponins justifies the cholesterol lowering properties of *Plumbago zeylanica* Linn. as reported in a study by Alpana, (1996). Saponins inhibit Na^+ efflux leading to higher Na+ concentration in cells, thereby activating a Na^+/Ca^{2+} antiport. According to Schneider and Wolfing (2004), this effect results in increased cytosolic Ca^{2+}, which strengthens the contraction of heart muscle and lowers congestive heart failure. The anti-inflammatory activity reported by Sheeja *et al*. (2010) was supported by the presence of flavonoids and eugenols. White blood cells use the alkaloids found in plants to eliminate harmful microorganisms and cell waste. Owing to their antioxidant properties, phenols can scavenge dangerous free radicals and potentially prevent oxidative damage (Tiwari and Rana, 2015).

4.2 CONCLUSION

The comprehensive proximate analysis and phytochemical screening of *Plumbago zeylanica* Linn. have provided valuable insights into the composition and potential bioactive constituents of this plant. The results indicate that the plant contains significant proportions of essential macronutrients, carbohydrates, proteins, and fats, making it a potential nutritional resource. Furthermore, the phytochemical screening revealed a diverse range of secondary metabolites, such as saponins, flavonoids, alkaloids, eugenols, and phenols, which have been associated with various pharmacological activities. These findings support the traditional use of *Plumbago zeylanica* Linn. in medicine and its potential application in the pharmaceutical and nutraceutical industries.

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APPENDICES

APPENDIX I

PREPARATION OF STOCK AND STANDARD SOLUTIONS OF ASCORBIC ACID.

Standard solution of ascorbic acid was prepared by dissolving an accurate weight of 0.01g of standard ascorbic acid in small amount of oxalic acid solution (0.5%.) and then completed to 100 ml with the same solution to obtain a concentration of 100 µg/ml. A series of dilutions 10, 8.0, 6.0, 4.0, and 2.0 µg/mL were prepared from the stock ascorbic acid solution.

PREPARATION OF POTASSIUM PERMANGANATE SOLUTION.

A solution of $KMnO_4$ of concentration of 100 µg/mL was prepared by dissolving an accurately 0.01 g of$KMnO_4$ in H_2SO_4 solution (5.0M), then transferred into a 100 mL volumetric flask and completed to the mark with distilled water and thoroughly mixed.

APPENDIX II

DETAILED RESULTS OF THE PROXIMATE ANALYSIS Of *Plumbago Zeylanica* Linn.

PARAMETER	REPLICATES			Mean ± S. E. M. %
	1 %	2 %	3 %	
Moisture Content	5.063	5.001	4.915	4.993 ± 0.035
Ash Content	10.076	6.989	8.753	8.606 ± 0.73
Crude Fat	9.781	9.105	8.697	9.194 ± 0.258
Crude Fibre	27.306	27.001	27.495	27.267 ± 1.62
Crude Protein	20.110	19.566	20.150	19.942 ± 0.154

Carbohydrate	32.727	37.339	34.905	34.990 ± 1.088

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

Calculations

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 = $\sqrt{(\frac{\Sigma(x-\mu)2}{N})}$

x= The replicate value in the data distribution

$\boldsymbol{\mu}$=The population mean

Using the moisture content values as an example:

$X_1 = 5.063\%$

$X_2 = 5.001\%$

$X_3 = 4.915\%$

Solving for population mean, $\boldsymbol{\mu} = \frac{5.063+5.001+4.915}{3} = 4.993\%$

Using the above formula,

Standard deviation, $\sigma = \sqrt{((5.063\text{-}4.993)^2/3+(5.001\text{-}4.993)^2/3+(4.915\text{-}4.993)^2/3)} = 0.06068497892\%$

Standard error of mean, S.E.M.= $\frac{0.06068497892}{\sqrt{3}} = 0.03503648892\%$

APPENDIX III

DETAILED RESULTS OF VITAMIN C CONTENT DETERMINATION

PARAMETER	REPLICATES			Mean ± S. E. M.
	1	2	3	Mg/100g
	Mg/100g	Mg/100g	Mg/100g	
Vitamin C	4.883	4.791	4.497	4.724 ± 0.095

The values of the mean and S.E.M. were obtained through the same procedure as in appendix III

APPENDIX IV

DETAILED RESULTS OF PHYTOCHEMICAL SCREENING OF THE *Plumbago zeylanica* LINN. LEAF AQUEOUS EXTRACT

PARAMETER	TEST METHOD	OBSERVATION	INFERENCE
Glycosides	General Test	No brown ring at interface	-
Saponins	Frothing Test Foam Test	Formation of foam on solution surface Moderate foaming up to 1.cm	++ +
Phenols	Ethanol/Ferric Chloride	Bluish-black colour formed	+
Eugenols	KOH/HCl Test	Pale yellow precipitate seen	+
Terpenoids	Salkowski Test	No reddish-brown colouration in the interface	-
Steroids	Acetic Acid/H_2SO_4	No colour change	-
Alkaloids	Mayer's Test Wagner's Test	Brownish precipitate formed Yellow-coloured precipitate	+ +
Flavonoids	Sodium Hydroxide Test Lead Acetate Test	Yellow precipitate formed Yellow precipitate formed	+ +
Tannins	Ferric Chloride	No colour change	-
Reducing Sugars	Fehling's solution A and B Benedict reagent Test	Brick-red precipitate in tube bottom Reddish precipitate seen	+ +

DETAILED RESULTS OF PHYTOCHEMICAL SCREENING OF THE *Plumbago zeylanica* LINN. LEAF ETHANOL EXTRACT

PARAMETER	TEST METHOD	OBSERVATION	INFERENCE
Glycosides	General Test	Brown ring at interface	+
Saponins	Frothing Test Foam Test	Formation of foam on solution surface Moderate foaming up to 1.cm	+ +
Phenols	Ethanol/Ferric Chloride	Bluish-black colour formed	+
Eugenols	KOH/HCl Test	Pale yellow precipitate seen	+
Terpenoids	Salkowski Test	Reddish-brown colouration in the interface	+
Steroids	Acetic Acid/H_2SO_4	Colour changed from violet to bluish green	+
Alkaloids	Mayer's Test Wagner's Test	Brownish precipitate formed Yellow-coloured precipitate	+ +
Flavonoids	Sodium Hydroxide Test Lead Acetate Test	Yellow precipitate formed Yellow precipitate formed	+ +
Tannins	Ferric Chloride	No colour change	-
Reducing Sugars	Fehling's solution A and B Benedict reagent Test	Brick-red precipitate in tube bottom Reddish precipitate seen	+ +

- = Absent

+ = Moderately Present

++ = Highly Present