PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *Alstonia boonei* LEAF

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CERTIFICATION

We the undersigned, certify that **AZOBU ELOGHOSA BLESSING** with matriculation number **LSC1906456** 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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DEDICATION

I dedicate this Project research work to my Parents, Mr. and Mrs. Moses Azobu**,** for their immense contributions and support in carrying out this project work, the love, assistances, understanding and advices.

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ABSTRACT

Alstonia boonei De wild is a plant belonging to the Aponcyanacea family. Its leaves, root bark and stem bark parts have various traditional uses in parts of West Africa for the management of some ailments such as: Malaria, hypertension and cancer. The nutritional composition and phytochemical content of the leaf of *Alstonia boonei* De wild were explored under standard analytical methods in order to ingress the numerous potential of the plant. The qualitative phytochemical screening of aqueous extract of *Alstonia boonei* De wild, leaves showed the presence of saponins, tannins, alkaloids, phenols, steroids, cardiac glycosides, coumerins, phlobatannins and protein; with saponins, tannins, steroids, and phenol highly present. Flavanoids, terpenoids, emotins, anthraquinones and anthocyanins were seen to be absent. Variation of this composition was observed in the ethanol extract which showed that saponins, phenols, terpenoids, steroids, alkaloids, protein and anthocyanins were seen to be present in moderate proportion, whereas flavonoids, phlobatannins,coumerin, emotins and anthraquinones were seen to be absent, while cardiac glycosides was highly present. The medicinal value of *Alstonia boonei* De wild is influenced by the presence and levels of these secondary metabolites. The proximate analysis revealed that *Alstonia boonei* leaves are rich in carbohydrates (57.45 ± 1.38%), have a moderate content of Ash (3.67± 0.16%), crude protein (8.05 ± 00.05%), crude fats (9.50 ± 0.24%), and crude fibre (11.00 ± 0.47%); but a moderate content of moisture (10.30 ± 0.27%). The presence of high carbohydrates, protein, crude fats and fibre contents of the leaves may be responsible for their nutritive values

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

1.1: BACKGROUND INTO HERBAL MEDICINE

In this 21st century, advances in science and technology have greatly improved and witnessed, drugs unveiling on the other hand is not left behind. This is witnessed in the massive on-going search and discovery for novel molecules in the pharmacological industry of several biotopes. Notwithstanding, in advancing and developing communities, some parts of Asia, this coordinated search has not diminished the popularity of traditional herbal medicine, but rather, it has helped in the health-care of the common man. In Nigeria, and some other sub-Saharan African countries, there is a huge reliance on herbal medicine for the treatment of some common ailments like malaria, typhoid fever, ulcers, diabetes, etc. and in the maternal health-care (Malan and Neuba, 2011). This herbal medicinal treatment is commonly referred to as "Root and Herbs" in most parts of Nigeria.

The prevalent uses of herbal medicines in African, seems to be conflicted, its high usage has been reported (James *et al.,* 2018). According to WHO, 'there is need, to harness the rich natural resources available in Africa which can contribute immensely in the fight against diseases, particularly with the global concern of antimicrobial drug resistance' (WHO, 2021).

The plant *Alstonia boonei* De Wild is among the numerous plants reported and classified to have remarkable pharmacological properties. *Alstonia boonei* is a common, large deciduous plant species that occupies a prominent healing effect in the medicinal landscape, found in African

Republic, Ghana, Democratic Republic of Congo, Cote d'Ivoire (Adotey *et al.,* 2012). Taxonomically, *Alstonia boonei* belongs to the family of Apocynaceae, it is habitually referred to as 'Cheese wood, pattern wood or stool wood' as its English name, In Ghana, it is known as 'Onyame-dua in Ashanti and Emain' in Cote d'ivore (Malan and Nebua, 2011) and as 'Ekouk' in Frang (Obame-Engonga *et al.,* 2019). In the trade market, it is known as Emien as its French trade name. In Ghana it is grown as a spice crop because of its ethnobotanical values.

Botanically, *Alstonia boonei* is a tall deciduous tree that can grow as tall as 40-45 m, and 3 m in girth, with cylindrical boles, narrowed and deep-fluted butteresses. In Congo, these trees have swollen bases like those of the Bald Cypress and Water Tupelo. The leaves are borne in whorls at the nodes, the leaf shape is oblanceolate, with the apex rounded to acuminate the lateral veins at the right angles to the midrib. The flowers are yellowish-white and borne in lax terminal cymes. The fruit are pendulous, paired, with slender follicles up to 16 cm long, containing seeds bearing a tuft of silky, brown floss at either end to allow dispersal by the wind. The latex is white in colour and abundant in nature.

Tropically, *Alstonia boonei* is an evergreen tree and a native to tropical West Africa, with a range extending into Ethiopia and Tanzania.

Ethnobotanically, the leaves of *Alstonia boonei* are use to reduce swellings, for the treatment of sores, rheumatic pains, muscular pains, hypertension, and also for the treatment of resistant malaria (Omoya and Oyebola, 2019). The stem bark is also importantly used for the management of dizziness, impotence, breast pain, tooth ache, asthma (Osadebe, 2003; Akinmoladun *et al*., 2007), malaria, as snake bite anti-venom (Olanlokun and Olorunsogo, 2018; Osuntokun and Ajiga, 2020), and as arrow poison (Akinloye *et al*., 2013), use to treat painful micturition and

rheumatic conditions (Ojewole, 1984; Asuzu and Anaga, 1991). The stem bark extract of *Alstonia boonei* has also been reported to be used to induce labour, remove plancenta and also for the management of post-partum hemorrhage (Uzor *et al*., 2017). The root bark is also of great medicinal value used also for the treatment of rheumatic and breast pain (Osadebe, 2003). The latex from the stem acts as a stimulant for lactation and taken as a laxative, for children the latex is mostly boiled in water and drunk for the remedy of fever. Herbal mixtures having some parts of *Alstonia boonei*, or in conjunction with other parts of other plants (locally called 'concoction' medicine) has been evidently used for the management of various ailments such as malaria and typhoid fever (Etame *et al*., 2019), hypertension (Turkson *et al*., 2019), gastric pain, pelvic and chest pains, skin infections and cancers (Languon *et al*., 2018). The wood is used for light construction, carpentry, open boat, furniture, interior joining, boxes, pencils, sculptures and plywood. It is use for household production because of its working durable properties and stability. In Ghana, it is used to make the popular Asante stools, while in Nigeria, it is used for musical instruments to make sound boxes for the Western people. In most African countries, where *Alstonia boonie* is a sacred tree and worshipped in the forest, Humans in those countries do not cut down or eats any part of the tree (Surya H. *et al*., 2001).

Tropically, *Alstonia boonei* is an evergreen tree and a native to tropical West Africa, with a range extending into Ethiopia and Tanzania. It grows in an evergreen and deciduous forest in damp situations, as well as secondary moist evergreen to dry semi-deciduous forest at elevation up to 1,200 meters. *Alstonia boonie* is sometimes seen in swampy areas and stream sides.

1.1.2 STATEMENT OF PROBLEM

Having a good medicinal purpose, *Alstonia boonei* plant contains some chemical compositions, and nutritional elements that helps it to achieve these stated purposes. This study will help to determine the proximate analysis and phytochemical constituents of the leaves and extracts of the plant.

1.1.3 AIM AND OBJECTIVE OF STUDY

The aim of this work was to valuate the proximate and phytochemical compositions of *Alstonia boonei*.

1.1.4 SPECIFIC OBJECTIVES OF THE STUDY

The following specified aims, are compelled to this plant study:

Proximate analysis of the leaf of *Alstonia boonei*

Phytochemical screening of the leaf of *Alstonia boonei*

1.2. LITATURE REVIEW

1.2.1: Taxonomical and Systematic Classification of *Alstonia boonie* De Wild

Super-kingdom----------Eukaryota

Kingdom----------Plantae

Sub-kingdom----------Virdiplantae

Infra-kingdom----------Streptophyata

Super-division----------Embryophyata

Division----------Tracheophyata

Subdivision----------Streptophytina; Spermatophyta

Class----------Magnoliopsida

Order----------Gentianales

Family----------Apocynaceae

Subfamily----------Rauvolfioideae

Tribe----------Alstonieae

Genus----------*Alstonia*

Species----------*Alstonia boonei*

1.2.2 Nomenclature

Scientific Name: *Alstonia boonei*

Common Name(s): Stoolwood, Devil Tree, Pattern wood.

Nigeria Name(s): Uhu, Ukhu, Ogiegbukhun (Edo); Egbu, Egun, Egbe, Egbwu-ora, Akpo (Igbo); Ahun, Awun (Yoruba); Ndodo (Efik); Egbu (Ijaw).

1.2.3 Botanic Description

A tall tropical rain-forest tree, *Alstonia boonei* is 45 m in height, it is a very large deciduous plant and a tropical rain-forest tree. It is 1 .2 m in diameter, bole is around 7 m, with buttresses, greyish-green or grey bark, and a milky latex. The leaves borne whorls at the node, oblanceolate in shape, with a rounded apex to acuminate and lateral veins prominent at the right angle of the midrib. The flowers are whitish-yellow in colour with borne in lax terminal cymes. Fruits are 16 cm long, pendulous, paired with slender follicles, containing seeds bearing a tuft of silky, brown floss at ends to allow dispersal by wind. The latex is white, copious and abundant (Abbiw, 1990).

1.2.3 Habitat Ecology

As an evergreen and deciduous forest plant in damp conditions. Primary, and also as well as a secondary moist evergreen and semi-dry deciduous plant with an elevation of 1,200 m (Palla *et al*., 2005). This plant thrives and survives greatly in swampy and damp locations or riverbanks. *Alstonia boonei* grows into a giant tree in most tropical rain-forests of West Africa, extending into Ethiopia and Tanzania. It is also seen in deciduous and fringing forest of Ghana (Abbiw, 1990).

‘Alstonia’ is named after Dr C. Alston (1685-1760), a professor of botany at Edinburgh University.

1.2.4 Cultivation

The records of flowering and fruiting are millificent, even in places of its natural habitat. In a place like Sierra Leone, tree shreds its leaves at the end of the rainy season and flowering occurs immediately in October and November, with the growth of new leaves. Fruits, matures in January and February. The seeds are likely to have hairs on both ends, which aids dispersal by wind (Abbiw, 1990).

Plate 1: *Alstonia boonei* plant

Source: Brunken *et al*., (2008).

Plate 2: Leaves of *Alstonia boonie*

Source: Brunken *et al*., (2008).

1.2.5 Phytochemistry

The phytochemical consistuents isolated from the leaves, bark, stem and root bark of *Alstonia boonei* De wild is enriched in tannins, alkaloids, saponins, steroids, triterpenes, cardiac glycosides, cyanogenetic glycosides, carbohydrates and reducing sugars in various amounts (Osadebe, 2003; Ojo *et al*., 2014; Opoku and Akoto, 2014; Akinnawo *et al*., 2017; Omoya and Oyebola, 2019; Ajose *et al*., 2019; Arogbodo, 2019). An amount of calcium, phosphorus, iron, sodium, potassium and magnesium have documented to be present in some parts of the plant (Akinmoladun *et al*., 2007). Some bioactive secondary metabolites have been ascertained/discovered from the leave part of the plant, such include; Flavonoid and Phenolic acid.

1.2.6 Traditional and Pharmacological Uses

Traditional uses

The various parts of *Alstonia boonei* have been used locally as medicine for the treatment of sores, rheumatic pain, resistant malaria, breast pain, hypertension, muscular pain, anti-venom against snake bite, to induce labour, as stimulant for lactation, etc. Traditionally, the stem bark, leaves, stem latex and root bark all has medicinal effects for the treatments of one or more ailment (Majekodunmi et al., 2008; Ojewole, 1984).

Pharmacological uses

The claim to attest or authenticate the usefulness of *Alstonia booonei* ethanomedicines led to the pharmacological activities of the various parts of the plant (Patwardhan, 2005).

Anti-Cancer Activity

Methanol extract obtained from the stem bark of *Alstonia boonei* has been seen to be cytotoxic to human colon carcinoma (Ohiagu *et al*., 2020). Eugenol in leaf, also 1,2-benzenedicarboxylic acid present in the root bark of extracts (Ohiagu *et al*., 2020). *Alstonia boonei* present as a Ghanaian herb product Kantinka Herbaltics which is popularly used for the management of cancer, though the actual part of the plant used has not been told. This herbal product, was investigated to be cytotoxic against some human cancer cell lines (Languon *et al*., 2018).

Anti-Inflammatory Activity

Methanol extract of leaves was analysed to have a profound anti-inflammatory dosage activity on Wister rats induced with rat paw odema (Iniaghe *et al*., 2012). Same observation was made also from the methanolic extracts of the stem and root barks respectively. The aqueous and ethyl acetate of leaves (Akinnawo *et al*., 2017) as well as the solvent fraction such as n-hexane fraction (Olanlokun *et al*., 2021) and compounds (Okoye *et al*., 2014) has shown a tremendous anti-inflammatory activity. These observations have reported to the use of *Alstonia boonei* De Wild for the treatment of tooth ache, breast, muscular and rheumatic pains, justifying the traditional use as a pain reliever.

Anti-Malaria Activity

The leaves of *Alstonia boonei* revealed anti-malaria activity by the aqueous and methanol extracts, which showed a dependent dose chemo-suppression and cure of parasitaemia of infected rodents (Dibua *et al*., 2013; Omoya and Oyebola, 2019) which was compared with the antiplasmodial effect of Chloroquine (Imam *et al*., 2017). Similar reports were recorded with

ethanol, methanol and aqueous extraction of the stem bark, which justifies the use of *Alstonia boonei* parts in the treatment of malaria.

Anti-Microbial Activity

The leaves and stem barks methanol and ethanol extracts show mild to moderate antimicrobial activity (Irulandi *et al*., 2017; Arogbodo, 2019; Ajose *et al*., 2019; Obame-Engonga *et al*., 2019). The antimicrobial activity of aqueous and ethanol extracts *Alstonia boonei* has significant inhibition to common strains of bacteria and and fungi such as *Escherichia coli, Bacillus subtilis, Psuedomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*. This antimicrobial activity has served pharmacologically for the basic use of *Alstonia boonei* in the treatment of typhoid fever, sores, tooth ache and diarrhea (Melogmo *et al*., 2020).

Antioxidant Activity

The leaf extracts and fractions of *Alstonia boonei* have showed a dose dependent antioxidant activity by the use of various models (Omoregie *et al*., 2014). The antioxidant activity of leaves was traced to have the presence of two caffeic acid derivatives, 5- caffeoylquinic acid and 4,5-dicaffeoylquinic acid and other flavonoid glycosides, all detected from the leaves (Okoye and Okoye, 2016). According to Nkono *et al* (2014) the stem bark has antioxidant activity. The consistent antioxidant activity of the plant extracts of various parts of *Alstonia boonei* has been useful for many medicinal purposes and properties, justifying its use in the management of various ailment.

Anti-Diabetic Activity

The anti-diabetic studies on the extraction of the leaves, stem bark (Akinloye *et al*., 2013; Nkono *et al*., 2014) and roots of the plant (*Alstonia boonie*) showed a good hypoglycemic effect on a rat model, with the stem bark having the highest anti-diabetic activity (Osadolor *et al*., 2015; Owolabi *et al*., 2014).

Anti-Ulcer Activity

The stem bark by extraction of the aqueous (Christopher *et al*., 2016) and methanol (Adjouzem *et al*., 2020) of *Alstonia boonei* has anti-ulcer activity. The stem bark extracts have great inhibition and healing effects on test rats.

Central Nervous System Depressant Effect

Alstonia boonei has been linked as a central depressant effect which may explain the traditional impact in the management of mental health conditions. This is because of a study, both aqueous and methanol extract of stem bark and leaves resulted in drowsiness among test mice (Omoya and Oyebola, 2019; Dibua *et al*., 2013; Idowu *et al*., 2010).

Oxytocic Effect

The validation of *Alstonia boonei* stem bark has proved to have oxytocic activity of various fractions thereby justifying its folkloric use in the inducement of labour and as a childbirth for the removal of placenta (Uzor et al., 2017). However, by the observed oxytocic effect, the administration of herbal mixtures/remedies containing *Alstonia boonei* leaves, stem barks and root bark extracts should be disallowed during pregnancy as it could lead to termination of pregnancy.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 PLANT SAMPLE

Fresh leaves of *Alstonia boonei* De Wild was plucked in January, 2024 from a compound located at Street 18, Off Palace Road, Uteh community, Ikpoba-okha L.G.A, Benin City, Edo State. Fresh leaves of the plant 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. The plant was given a voucher number of UBH-A343.

2.1.2 REAGENTS

The following reagents were used to carried out this experimental study:

Hydrochloric acid (Sigma-Aldrich, United States)

Hager's reagent (Hager & Werken GmbH & Co. KG, Germany)

Chloroform (Sigma-Aldrich, United States)

Ammonium (Sigma-Aldrich, United States)

Sodium chloride (Sigma-Aldrich, United States)

Ferric chloride (Sigma-Aldrich, United States)

Sodium hydroxide (Sigma-Aldrich, United States)

Olive oil (Goya company, America)

Sulphuric acid (Sigma-Aldrich, United States)

Chloroform (Sigma-Aldrich, United States)

Oxalic acid (Sigma-Aldrich, United States)

Acetic anhydride (Sigma-Aldrich, United States)

Petroleum ether (Sigma-Aldrich, United States)

Ethanol (Sigma-Aldrich, United States)

Ammonium hydroxide (Sigma-Aldrich, United States)

2.1.3 EQUIPMENT/ APPARATUS

The apparatus used in this study include:

Weighing balance (Atom-A110C, China)

Soxhlet apparatus (Everest., China)

Heating mantle (Witeg Labortechnik, Korea)

Micro-Kjeldahl digestion flask (Pyrex, Nigeria)

Digester (Hanon Lab., China)

Burette (Pyrex, Nigeria)

Restort stand (Atico export, India)

Muffle furnace (Kejia furnace, China)

Beakers (Pyrex, Nigeria)

Conical flasks (Technico, India)

Standard flask (Technico, India)

Thermosetting Oven (Ele International., England)

Filter paper (Whatman, United Kingdom)

2.2 METHODS

2.2.1 PREPARATION OF SAMPLES

The freshly plucked leaves of *Alstonia boonei* were dried in an open air under the sun for 7 days, the twigs were separated by hand. Thereafter, the plant sample was grinded and ready for assayed.

2.2.2 EXTRACTION OF SAMPLES FOR PHYTOCHEMICAL SCREENING.

This 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 ethanol 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. This method was repeated again for water as a second solvent.

2.2.3 PROXIMATE ANALYSIS

Moisture Content Determination

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

Principle: These methods and procedures were dependent on the measurement of the content which was determined by measuring the mass of leaf sample before and after water content evaporation.

Procedure: An empty washed and clean beaker was oven dried and weighed. Exactly 2g of the sample was measured and added into the beaker and labeled, the beaker with the sample content was transferred into a thermosetting oven at a temperature of 105^0C for 3hours. After 3hours, the beaker content was taken off the oven and allowed to cool down in a desiccator for 30minutes and weighted. This step was repeated as the constant weight was re-weighed at 10mins interval until a constant weight was achieved. This experiment was done in triplicate.

Calculation

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

Weight loss= $[(W_2+W_1)$ — C] (g)

Where; W_1 = Weight of crucible (g)

W_2= Weight of sample (g)

C= Constant weight (g)

Ash Content Determination

This ash content was carried out using the method of AOAC (1990).

Principle: This is based on complete incineration at a high temperature in a muffle furnance. At the high temperature, all hydrocarbon constituent of the sample were decomposed, left with only the inorganic matter in the beaker. Thus, the end product of the ash content is the inorganic matter of the sample whose weight is thus recorded.

Procedure: An oven dried beaker was weighed and labeled, exactly 1g of the leaf sample was weighed and added to the beaker. The beaker was then transferred into the furnance at a temperature of 550^0C for 3 hours. The beaker with the ash sample was removed from the furnace and allowed to cool for 30mins. The weight was taken after cooling and recorded.

Calculations

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

Loss of Ash weight (g) = $(Weight\ of\ crucible\ (g) + Weight\ of\ sample(g)) -$

$Constant\ weight\ (g)$

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 weighed 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: Exactly 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 (1984), was employed.

Principle: This method is based on the Kjelhal method, which determines the total nitrogen content of a sample multiplied by a conversion factor. The sample is digested using $CuSO_4$ and $FeSO_4$ as catalysts, this digestion then converts nitrogen (N) to ammonia (NH_3). This method is used to determine protein content in wide variety of organic matters.

Procedure for Digestion: Three (3) 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.

Procedure: Exactly 0.5g of the sample was weighed and then transferred into a conical flask. About 25ml of H_2SO_4 with the addition of a mixed catalyst was added to the conical flask, it was heated on a heater on a low heat for 15mins, it was then increased to a medium heat for 30mins. Finally, it was gradually increased until digestion, the flask was rotated at interval until digestion cleared off. The sample was allowed to cool, the digest was made up to 100ml mark, about 5ml of 2% boric acid was placed into 50ml conical flask to trap down the ammonia vapour from the digest. Thereafter, about 3 drops of indicator was added, which was can prepare together 10ml of 40% NaOH, and 30ml of distilled water was then added.

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

Where 6.25 = Conversion factor of 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.

Calculations

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

2.2.4 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 $P < 0.05$.

2.3 PHYTOCHEMICAL SCREENING

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

5 Detection of Alkaloids

Principle of Hager's test: This test is a chemical test that is primarily used to detect the presence of alkaloids in a solution. It involves the formation of a yellow precipitate when

Hager's test, which is picric acid (a yellowish crystalline solid that is a powerful explosive), is added to a solution containing alkaloid substances. If alkaloid is present, it will react with the picric acid (Hager., 1879).

Procedure: About 2.0ml of plant extract was filtered into a test tube, few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to the filtrate, the formation of a crystalline yellow precipitate indicate the presence of alkaloid.

6 Detection of Tannins

Principle: Tannins are a group of polyphenolic compounds that have the ability to bind to and precipitate protein. The major principle behind tannins test is its ability to form complexes with proteins or metals, leading to precipitation or color change. It involves adding a protein solution (commonly gelatin or casein) to a tannins-containing extract, if tannins are present, it binds to the protein causing it to precipitate. Also, chemical indicators such as ferric chloride ($FeCl_2$), which reacts with tannins to produce a color change (Barnett., 1964).

Procedure: About 1ml extract + 2ml H_2O was measured into a test tube, few drops of 1% ferric chloride was added. A colouration of brownish green to a blue green color indicate the presence of tannins.

7 Detection of Phenols

Principle: The presence of phenols is based on the formation of a colored complex using Iron (iii) chloride ($FeCl_3$), this formation is based of a colored complex formed $FeCl_3$ and the phenolic hydroxyl group. Phenols react with $FeCl_3$ to form a colored complex, typically purple, due to the formation of an Iron (iii)-phenolate complex. Phenol compound reacts with Iron (iii) chloride, the hydroxyl group (-OH) present in the phenol molecule donates a pair of electrons to the Iron(iii) ion (Fe^3+), this forms a coordination complex between the phenolate ion and iron ion, the resulting complex is deep purple color, indicating the positive test for the presence of phenols. The typical color observed with the reaction between phenols and iron (iii) chloride is purple, but is can also appear as a dark green color under certain phenolic compounds or variations in concentration, therefore, the dark green color is also indicate the presence of phenols in a phenol-containing sample (Tiwari *et al*., 2011).

Procedure: This was done by treating 2ml of the plant extract with about 4 drops of 10% $FeCl_3$ solution. Formation of a dark green color indicated the presence of phenols.

8 Detection of Saponins

Principle: Saponins are natural glycosides present in plants, are amphipathic molecules, having both hydrophilic and hydrophobic regions. The test of saponins is based on agitation, the surfactant properties of Saponins tends to reduce the surface tension of water, allowing air to become entrapped within the solution. The air is then stabilized by the hydrophobic portions of the saponin molecules, forming a stable froth or emulsion on the surface of the solution. This froth persist tends to give the continuous presence of Saponins in the solution, which maintains

its surface characteristics, therefore the persistent formation of froth or foam indicates the presence of Saponins in a test sample (Vicken *et al*., 2007)

Procedure: The froth test methods were used in the detection of saponins. About 1ml of the extract, 5ml of distilled H_2O was added and heat, the mixture in the test tube was shaken, 3 drops of olive oil was added and shaken vigorously. The formation of emulsion which persisted for 10 minutes indicating the presence of saponins.

9 Detection of Flavonoids

Principle: This test is based on the used of specific chemical reagents to detect the presence of flavonoids in a sample. Shinoda test is a common test used commonly for the detection of flavonoids, where the reaction between flavonoids and concentrated acids such as Hydrochloric acid (HCl) and Sulphuric acid (H_2SO_4) which produces a characteristics color change. Another test for the detection of Flavonoids is the Lead acetate test, which forms a yellow precipitate with the Lead acetate solution. These tests depend on the chemical properties of flavonoids to produce observable reactions (Tiwari *et al*., 2011).

Procedure: In the lead acetate test, 5ml of dilute ammonia was added to 1ml of extract, 1ml of concentration H_2SO_4. The presence of a yellow color indicate the flavonoid in the sample.

10 Detection of Steroids

Principle: Steroids, having conjugated double bonds in their structure has the ability to undergo a chemical reaction known as Unsaturated test or Liebemann test. Burchard test, when submerged to a combination of acetic acid and sulfuric acid. The degree of color alteration observed during this test reaction is dependent on the specific steroids being examined and its

concentration. The appearance of a reddish-brown ring or hue at the junction of the test-tube, indicates the presence of steroids in the sample (Nath *et al*., 1946).

Procedure: About 1m of extract was added to 2ml acetic anhydride with 2 ml of H_2SO_4 test tube. The formation of reddish brown at the junction signified the presence of steroids.

11 Detection of Terpenoids

Principle: This test is based on the Salkowski test using chloroform (Siddiqui *et al.,*2009). Chloroform selectively extracts terpenoids from plant material, leaving behind other components such as sugars, proteins and salts which are often soluble in chloroform. This extract causes a portioning whereby the Chloroform partitions the terpenoids from the solid plant material between terpenoids and other component present in the plant material.

Procedure: Exactly 2ml of the extract of the plant sample was mixed with 2 ml of chloroform ($CHCl_3$), few drops of concentrated H_2SO_4 (about 2-3 drops) were carefully added to form a layer. A reddish-brown coloration in the interface indicated positive results for the presence of terpenoids.

12 Detection of Glycosides

Principle: The detection of cardiac glycosides through the use of glacial acetic is based on specific chemical reactions that result in the development of color changes. In this method of detection, cardiac glycosides are exposed to glacial acetic acid and then heated, they undergo a particular chemical reaction that give rise to the formation of colored solution or precipitate. The test used here is the Keller-Killiani Test (Singh *et al*., 2017). This test relies on the specific interaction between certain cardiac glycosides and ferric ions (Fe^{3+}) in an acidic environment.

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 1ml of concentrated H_2SO_4. A blue-colored solution obtained at the interface indicated the presence of glycoside.

13 Detection of Emodins

Principle: This test involves the use of Ammonium hydroxide (NH_4OH) as a reagent, which reacts with the emodin sample solution to form a coloured complex (Rauf, A, *et al*., 2013).

Procedure: 2ml of NH_4OH was added to 2ml of extract, then after 3ml of benzene was added. A red colouration indicate the presence of emodins.

14 Detection of Anthocyanins

Principle: This involves the use of a pH indicator to detect changes in acidity, which affects color of anthocyanin pigment. Anthocyanins are basically red or pulple pigments, that changes color depending on the pH of the environment, which involves altering the pH and observing color change. Hydrochloric (HCl) acid and Ammonia (NH_3), are chemical reagents used for antocyanins testing. The acidic and alkaline nature of these solutions can change the pH of the environment, thereby affecting the anthocyanin pigment.

Procedure: 2ml of extract was added to 2ml HCL (2N) and NH_3. The presence of a pinkish red and bluish-violet colouration indicate the presence of anthocyanide.

15 Detection of Coumarins

Principle: This involves the use of Sodium Hydroxide (NaOH) as a reagent. Coumarin reacts with NaOH to undergo hydrolysis, leading to the formation of various products. This reaction is exploited in certain analytical methods for the detection of Coumarin particularly in alkaline

hydrolysis procedure used for the release aglycones from glycosidic coumarin derivatives. NaOH is an important reagent in the qualitative analysis of Coumarin test (Kumar *et al*., 2018).

Procedure: 2ml of extract was added to 3ml NaOH (10%). A yellow colour indicate the presence of coumarins.

16 Detection of Phlobatannins

Principle: Phlobatannins as a class of polyphenolic compounds, testing for them involves the addition of hydrochloric acid (HCl) to the plant's sample. If present, a reddish precipitate will form, this reaction is used to identify the presence of phlobatannins in various botanical extracts (Smith., 2020).

Procedure: 2ml of 1% HCL was added to 2ml extract. A red color precipitate proves the presence of this phytochemical in the plant

17 Detection of Protein

Principle of Protein test: The underlying principle of this detection technique is the creation of a yellow precipitate following the treatment of proteins with concentrated nitric acid. The presence of proteins in the solution is indicated by the formation a yellow color solution (Tiwari *et al*., 2011).

Procedures: The presence of protein in the plant extract is confirmed by observing the formation of a yellow color precipitate upon adding 1 ml of concentrated nitric acid to 1 ml of the extract.

18 Detection of Anthraquinones

Principle: This involves the characteristics color reaction with specific reagent. Anthraquinone derivation reacts with alkaline solution, to form colored products which exhibits hues in their identification (Njoku *et al*., 2009).

Procedure: About 2ml of chloroform was added to 1ml of plant extract, 2.5ml of NH_3 was also added. The observation of a pink, violet or red coloration proves the presence of this phytochemical test in the sample.

CHAPTER THREE

3.1 RESULTS

3.1.1 Proximate Composition

The proximate analysis of the fresh leaves of *Alstonia boonei* de wild, revealed it contained an average moisture content of 67.67%. However, the moisture content contained in the dried leaf samples had an average value of only 10.30% and an ash content that was miminum at 3.67%. The leaf turned out to have fat that cumulated to only 9.50% of its total proximate composition and had a gross 11.00% in fibre content. Showing a total protein content that amounted to 8.05%, and finally, from these results, the carbohydrate content was also estimated to be 57.45% of its proximate composition. Table 3.1 explicitly shows the proximate composition values of the *Alstonia boonei* de wild

Table 3.1: Proximate Composition of *Alstonia boonei* leaf.

S/N	PARAMETER	VALUES
		Mean ± S.E.M (%)
1	Moisture Content	
	Dried sample	10.30 ± 0.27
	Wet sample	67.67 ± 2.65
2	Ash	3.67 ± 0.16

3	Crude Fat	9.50 ± 0.29
4	Crude Fibre	11.00 ± 0.47
5	Crude Protein	8.05 ± 0.05
6	Carbohydrate	57.45 ± 1.38

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

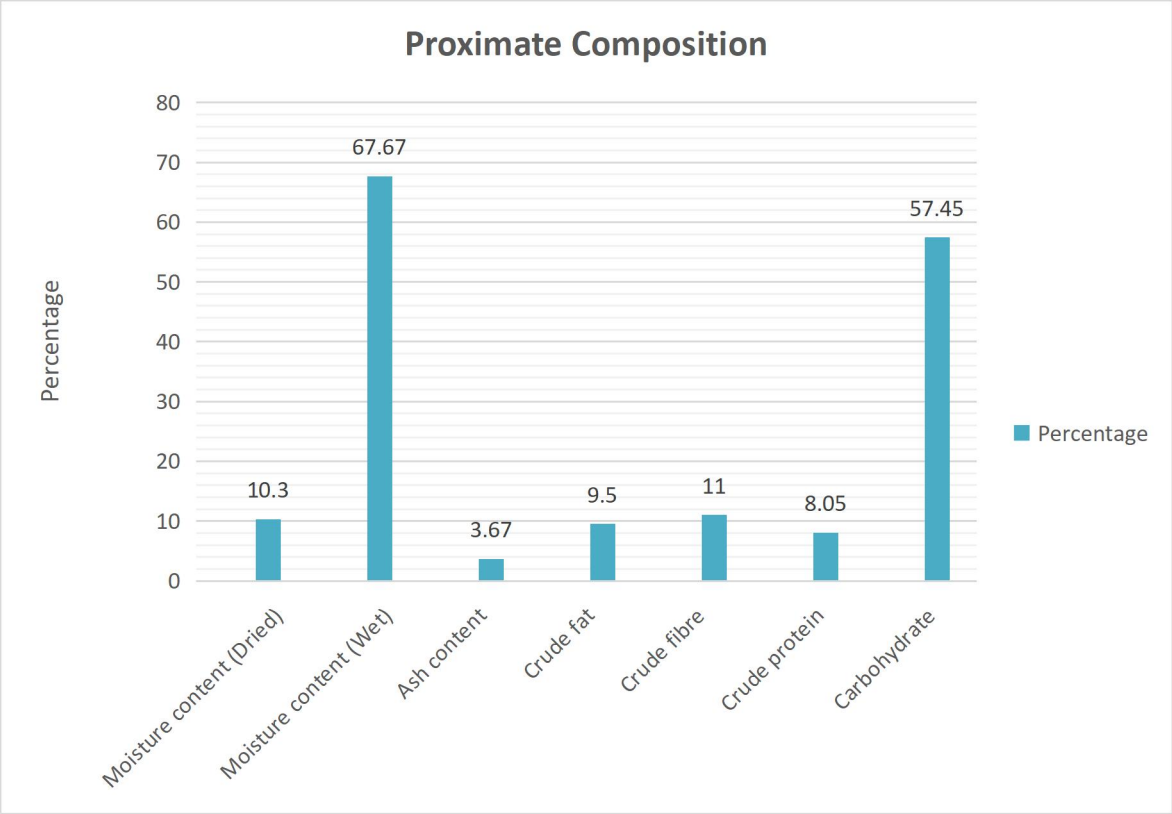

Figure 1: A graphical representation showing the proximate composition of *Alstonia boonei* leave

3.2 Phytochemical Content of *Alstonia boonei* leaf

The phytochemical content of the *Alstonia boonei*. leaf extract (extracted with water) revealed that tannins, saponins, steroids, and phenols are in abundance; alkaloids, cardiac glycosides, phlobatanins, coumarins, and proteins are moderately present; while flavonoids, terpeniods, anthraquinones, emodins and anthocyanins are absent. The results are presented in Table 3.2 below.

The phytochemical content of the *Alstonia boonei* de wild. leaf extract (extracted with ethanol) showed that cardiac glycosides, is highly present; tannins, saponins, phenols, terpeniods, steroids, alkaliods and proteins are moderately present; while emodins, anthraquinones, phlobatannins, coumarins, and anthocyanins are absent. The results are presented in Table 3.3 below.

Little variation is 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 *Alstonia boonei* ***Leaf Aqueous Extract.***

S/N	PARAMETERS	OBSERVATION	INFERENCE
1	Flavonoids	No yellow coloration was observed	-
2	Tannins	A brownish green to blue green color was highly salient	++
3	Cardiac glycosides	A brown interface was present	+

4	Saponins	Formation of frothing and emulsion was highly salient	++
5	Steriods	A salient reddish-brown precipitate was at the junction of test-tube	++
6	Phenols	A dark green color was highly salient	++
7	Terpenoids (Salkowski test)	Absence of a deep red/brown pink to violet	-
8	Phlobatannins	A red precipitate was formed	+
9	Coumarins	A yellow coloration was observed	+
10	Emodins	Absence of a red coloration	-
11	Protein	A white precipitate was observed	+
12	Anthraquinones	Absence of a pink, violet or a red coloration	-
13	Alkaloids	A yellow precipitate was observed	+

14	Anthocyanins	Absence of a pinkish red to bluish violet coloration	-

Table 3.3: Qualitative Phytochemical Analysis of *Alstonia boonei* ***Ethanol Extract.***

S/N	PARAMETERS	OBSERVATION	INFERENCE
1	Flavonoids	No yellow color was observed	-
2	Tannins	A brownish green to blue black color was present	+
3	Cardiac glycosides	A brown interface was highly salient	++
4	Saponins	Formation of frothing and emulsion was present	+
5	Steriods	A reddish brown at the junction of test-tube was observed	+
6	Phenols	A dark green color was present	+
7	Terpenoids	The present of a deep red/brown pink to violet colouration	+
8	Phlobatannins	A red precipitate was not observed	-

9	Coumerins	No yellow coloration was not observed	-
10	Emotins	No red coloration was observed	-
11	Protein	A white precipitate was present	+
12	Anthraquinones	No pink, violet or a red coloration was observed	-
13	Alkaloids	A yellow precipitate was present	+
14	Anthocyanins	No pinkish red to bluish violet coloration was observed	-

- = Absent

+ = Moderately Present

++ = Highly Present

CHAPTER FOUR

DISCUSSION

4.1 DISCUSSION

This study was carried out in order to have a comprehensive understanding of the nutritional, traditional and medicinal potentials of *Alstonia boonei* de wild. By the proximate analysis carried out, *Alstonia boonei* de wild, leaves proved to be a good source of carbohydrates (57.45%), crude protein (8.05%) and crude fibre (11.00%). Crude fat made up 9.50% of the total proximate content while the remainder was cumulated by both the moisture and ash content (10.30 and 3.67% respectively). There was a considerably high moisture content in the fresh (wet) samples (67.67%). Comparing these results to the experiment carried out by Abu *et al*., 2016 on the Southern research for the proximate composition of *Alstonia boonei* leave, this research yielded the following results; Crude Fat:0.844, crude protein: 2.106%, moisture content: 67.254%, crude fibre: 7.153%, ash content: 2.155%, and carbohydrate: 20.488%, respectively. The comparison of these experimental results and value difference explains that the ecological factors, such as soil, climate change, and temperature, tends to have a great retarded influence on nutritional prowess, plant's genetics, environmental factors or maturity of *Alstonia boonei* in various locations. The nutritional composition of the leaves of *Alstonia boonie*, showed moderate quantities of the proximate compounds, such as carbohydrate, crude protein, crude fat, crude fibre, ash content, and high moisture content. The crude fibre of this plant sample is quite high. Fibre helps in the absorption of excess water in the colon, helps retain a good amount of moisture in the fecal matter and can also offer protection against conditions like hemorrhoids, colon cancer, chronic constipation and rectal fissures (Jimaima *et al*., 2003). The low fats composition

of *Alstonia boonei* leaves proves the fact that vegetables are relatively low in calories and fats (Jayaraj *et al*., 2008). The energy balance and metabolism are the two most important processes regulated by carbohydrates (Maughan, 2009). Hence, high carbohydrate content of this leave reveals a good source of carbohydrate. Proteins gives strength to the immune system and facilitate cell development and division, the gauge of water activity and a predictor of stability and susceptibility to microbial contamination, plant material's moisture content is measured (Lang and Steinberg, 1980; Uriah and Izuagbe, 1990). The amount of ash determines the inorganic matter plant sample present (AOAC, 1990).

The presence of non-nutritive plant chemicals, which is also known as phytochemicals, has vary degrees of disease prevention. Plant phytochemicals have the; antibiotic, antiviral, anti-plasmodial and anti-parasitic properties which has been reported in several studies (Oliver-Bever, 1986; Adomi, 2008; Ene *et al*., 2008; Alshawsh *et al*., 2009). These phytochemicals are known and responsible for the medicinal importance of the plant. This work corroborated the works of Abu *et al*., (2014), by confirming the presence of phenol steroids, saponins, tannins, glycosides, alkaloids and so on, in either the aqueous or ethanolic extracts. In addition, this study confirms the absence of coumarin and flavonoids in both the aqueous and ethanol extract respectively, and the presence terpenoids in the ethanol extract.

These phytochemicals serve a variety of purposes and are sources of immeasurable fundamental components for both conventional and complementary therapies. Flavonoids, are effective as free radial scavengers, and have been observed in several studies (Del-Rio *et al*., 1994; Selah *et al*., 1995; Okwu, 2004). Flavonoids have antiallergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic properties (Middleton and Kandaswami,1993), hence, its absence in this work, is in disagreements with the report of Abu *et al.,* (2014). *Alstonia boonei*

leave is rich in phenolic compounds, which are known for their antioxidant properties. Phenols have been reported as one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). They possess several biological properties, which include, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). Shi *et al*., (2004) provided an overview of the effects of saponins, stating that they decrease cholesterol levels and have an impact on the immune system, which helps shield the body from cancer. Saponins yields the formation of foams in aqueous solutions, range from haemolytic activity to cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004). Plant parts have saponins, thus increasing their efficacy as drugs making them capable of boosting immune system (Okwu, 2004), known to have anti-diabetic properties. However, the presence of steroids in this plant makes it useful against cerebral malaria, thus, confirming its effectiveness as anti-plasmodial agents as reported by David *et al*., (2004). Alkaloids, the most essential of all phytochemicals. They have a wide range of activities, which anti-parasitic properties are one of them (Louw *et al*., 2002; Abu *et al*., 2014). Cardiac glycosides are predominantly used for the treatment of heart problems that may result from severe malaria attack (Fatoba *et al*., 2003). As reported by Alshawsh *et al.,* (2007), tannins also have anti-plasmodial activity.

4.2 CONCLUSION

Alstonia boonei complete proximate analysis and phytochemical screening as carried out in this study, have yielded important insights into the composition and possibly bioactive components of this plant. The plant is a potential nutritional resource because the results show that it contains considerable amounts of proteins, lipids, carbohydrates and necessary macronutrients.

Additionally, a wide variety of secondary metabolites; including phenols, alkaloids, steriods, and saponins, all of which have been linked to a variety of pharmacological activities, were found using phytochemical screening. These results provide credence to the nutritional and ethno-medical applications of *Alstonia boonei*, as well as its possible usage in the pharmaceutical sectors.

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APPENDIXES

APPENDIX I

PARAMETER	REPLICATES			Mean ± S. E. M.
	1	2	3	%
	%	%	%	
Moisture Content				
Dried Sample	10.00	10.00	11.00	10.30 ± 0.27
Wet Sample	74.00	65.00	64.00	67.67 ± 2.65
Ash Content	4.00	3.00	4.00	3.67 ± 0.16
Crude Fat	10.00	9.50	9.00	9.50 ± 0.29
Crude Fibre	12.00	10.00	11.00	11.00 ± 0.47
Crude Protein	8.14	7.96	8.05	8.05 ± 0.05
Carbohydrate	55.86	59.54	56.95	57.45 ± 1.38

DETAILED RESULTS OF THE PROXIMATE ANALYSIS of *Alstonia boonei*

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)	18.82	18.20	18.96
Weight of sample (g)	1	1	1
Constant weight (g)	19.08	18.55	19.32

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

Loss in weight(g) =

$(Weight\ of\ crucible(g) + Weight\ of\ sample(g)) - Constant\ weight$(g)

For sample 1,

$$\frac{(18.82 + 1) - 19.08}{1} \times 100 = 74.00\%$$

For sample 2,

$$\frac{(18.20 + 1) - 18.55}{1} \times 100 = 65.00\%$$

For sample 3,

$$\frac{(18.96 + 1) - 19.32}{1} \times 100 = 64.00\%$$

Taking the mean of the values, we have;

$$\frac{74 + 65 + 64}{3} = 67.67\%$$

Similar steps are followed for the other calculations.

APPENDIX II

DETERMINATION OF MOISTURE CONTENT (Dried Leaves)

Sample	1	2	3
Weight of crucible (g)	19.66	19.36	20.22
Weight of sample (g)	1	1	1
Constant weight (g)	20.56	20.26	21.11

For sample 1,

$$\frac{(19.66 + 1) - 20.56}{1} \times 100 = 10\%$$

For sample 2,

$$\frac{(19.36 + 1) - 20.26}{1} \times 100 = 10\%$$

For sample 3,

$$\frac{(20.22 + 1) - 21.11}{1} \times 100 = 11\%$$

By taking the mean of values¸ the average moisture content (dried leaves) is 10.30%

S.E.M = 0.27%

APPENDIX III

DETERMINATION OF ASH CONTENT

Sample	1	2	3
Weight of crucible (g)	18.82	19.05	19.85
Weight of sample (g)	1	1	1
Weight of ash (g)	18.86	19.08	19.89

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

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

For sample 1,

$$\frac{18.86 - 18.82}{1} \times 100 = 4\%$$

For sample 2,

$$\frac{19.08 - 19.05}{1} \times 100 = 3\%$$

For sample 3,

$$\frac{19.89 - 19.85}{1} \times 100 = 4\%$$

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

$$\frac{4 + 3 + 4}{3} = 3.67\%$$

S.E.M = 0.27%

APPENDIX IV

DETERMINATION OF CRUDE FAT

Sample	1	2	3
Weight of filter paper (g)	0.82	0.82	0.81
Weight of sample (g)	2	2	2
Weight of extract(g)	2.62	2.63	2.63

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

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

For sample 1,

$$\frac{(0.82 + 2) - 2.62}{2} \times 100 = 10\%$$

For Sample 2,

$$\frac{(0.82 + 2) - 2.63}{2} \times 100 = 9.5\%$$

For sample 3,

$$\frac{(0.81 + 2) - 2.63}{2} \times 100 = 9\%$$

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

$$\frac{10 + 9.5 + 9}{2} = 9.5\%$$

S.E.M = 0.29%

APPENDIX V

DETERMINATION OF CRUDE FIBRE

Sample	1	2	3
Weight of oven extract (g)	23.18	22.70	24.85
Weight of sample (g)	1	1	1
Weight of muffle extract (g)	23.06	22.60	24.74

$$\% \text{ Crude Fibre} = \frac{Weight\ of\ Oven\ extract\ (g) - Weight\ of\ Muffle\ extract(g)}{Weight\ of\ sample\ (g)} \times 100$$

For sample 1,

$$\frac{23.18 - 23.06}{1} \times 100 = 12\%$$

For sample 2,

$$\frac{22.70 - 22.60}{1} \times 100 = 10\%$$

For sample 3,

$$\frac{24.85 - 24.74}{1} \times 100 = 11\%$$

By taking the mean values, the average crude fibre content is 11%

S.E.M = 0.47%

APPENDIX VI

DETERMINATION OF CRUDE PROTEIN CONTENT

Sample	1	2	3
Final	0.93	0.91	0.92
Initial	0.000	0.000	0.000
Titre	0.93	0.91	0.92

% Crude protein = $Na \times Va \times 0.014 \times 100 \times 100 \times 6.25$

Where;

Na = 0.1M

Va = 0.93 (Sample 1)

= 0.91 (Sample 2)

= 0.92 (Sample 3)

For sample one;

$$0.1 \times 0.93 \times 0.014 \times 100 \times 100 \times 6.25 = 8.14\%$$

For sample 2;

$$0.1 \times 0.91 \times 0.014 \times 100 \times 100 \times 6.25 = 7.96\%$$

For sample 3;

$$0.1 \times 0.92 \times 0.014 \times 100 \times 100 \times 6.25 = 8.05\%$$

By taking the mean of the values, the average crude protein content is 0.85%

S.E.M = 0.05%

APPENDIX VII

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.

Sample 1

$$100 - (10 + 4 + 10 + 12 + 8.14) = 55.86\%$$

Sample 2

$$100 - (10 + 3 + 9.5 + 10 + 7.96) = 59.54\%$$

Sample 3

$$100 - (11 + 4 + 9 + 11 + 8.05) = 56.95\%$$

S.E.M = 1.38%

APPENDIX VIII

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 = 74

X_2 = 65

X_3= 64

μ= 67.67

$$\sigma = \sqrt{\frac{(74 - 67.67)^2 + (65 - 67.67)^2 + (64 - 67.67)^2}{3}} = 4.49$$

$$\text{S. E. M} = \frac{4.49}{\sqrt{3}} = 2.65\%$$

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

N= 3

$X_1 = 10$

$X_2 = 10$

$X_3 = 11$

$\mu = 10.30$

$$\sigma = \sqrt{\frac{(10 - 10.30)^2 + (10 - 10.30)^2 + (11 - 10.30)^2}{3}} = 0.47$$

$$\text{S. E. M} = \frac{0.47}{\sqrt{3}} = 0.27\%$$

S.E.M FOR ASH CONTENT

N = 3

$X_1 = 4$

$X_2 = 3$

$X_3 = 4$

$\mu = 3.67$

$$\sigma = \sqrt{\frac{(4-3.67)^2 + (3-3.67)^2 + (4-3.67)^2}{3}} = 0.707$$

$$\text{S. E. M} = \frac{0.707}{\sqrt{2}} = 0.5\%$$

S.E.M FOR FAT CONTENT

N = 3

$X_1 = 10$

$X_2 = 9.5$

$X_3 = 9$

$\mu = 9.50$

$$\sigma = \sqrt{\frac{(10 - 9.50)^2 + (9.5 - 9.50)^2 + (9 - 9.50)^2}{3}} = 0.41$$

$$\text{S. E. M} = \frac{0.41}{\sqrt{2}} = 0.29\%$$

S.E.M FOR FIBRE CONTENT

N= 3

$X_1 = 12$

$X_2 = 10$

$X_3 = 11$

$\mu = 11.00$

$$\sigma = \sqrt{\frac{(12 - 11.00)^2 + (10 - 11.00)^2 + (11 - 11.00)^2}{3}} = \frac{\sqrt{2}}{\sqrt{3}}$$

$$\text{S. E. M} = \frac{\frac{\sqrt{2}}{\sqrt{3}}}{\sqrt{2}} = 0.47\%$$

S.E.M FOR PROTEIN CONTENT

N= 3

X_1 = 8.14

X_2 = 7.96

X_3= 8.05

μ= 8.05

$$\sigma = \sqrt{\frac{(8.14 - 8.05)^2 + (7.96 - 8.05)^2 + (7.96 - 8.05)^2}{3}} = 0.07$$

$$\text{S. E. M} = \frac{0.07}{\sqrt{2}} = 0.05\%$$