PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *Azadirachta indica* AND *Pennisetum purpureum* LEAVES

BY

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A A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES,

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DECLARATION

I hereby declare that this project has been written by me, under the supervision of Dr. Elizabeth Olawumi Oyebanji and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university, All citations and sources of information are clearly acknowledged by means of reference provided.

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DATE

CERTIFICATION

This is to certify that the content of this project titled '**Proximate and phytochemical analysis of *Azadirachta indica* and *Pennisetum Purpureum*** was prepared and submitted by **ADEOYE SIMILOLUWA TEMITOPE** in partial fulfillment of the requirements for the degree of BACHELOR OF SCIENCE IN MICROBIOLOGY.

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DEDICATION

I dedicate this project to the God Almighty, who has been my strength, provider and my sustainer, Also, to my family and my supervisor for unending support.

ACKNOWLEGMENT

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ABSTRACT

Medicinal plants have an essential role in society because almost 80% of low-income earners in developing nations rely on traditional medicine for their primary health treatment. *Pennisetum purpureum* is an excellent choice for treating eye problems, Headache, Skin disorders and Wound. Leprosy, eye diseases, bloody nose, intestinal worms, stomach distress, skin ulcers, and heart and blood vessel illness have all been treated using *Azadirachta indica*. Furthermore, many Indian cultures rely on these plants for their therapeutic properties. Many of the investigations on these plants have concentrated on phytochemical isolation and characterization. As a result, the focus of the study was on the qualitative determination of phytochemicals and proximate constituents. The analyses were carried out utilizing industry-standard methodologies. Flavonoids, Coumarins, Polyphenols, and Saponins were found in the plants, according to research. The plants studied had varying concentrations of moisture, ash, crude protein, crude fiber, and Ether Extract, according to proximate analysis. The highest percentage by mass were; moisture content 9.0% in *Azadirachtaindica*, crude protein 23.72% in *Pennisetum purpureum,* crude fibre 25.22% in *Azadirachtaindica*, Ether extract 13.91% in *Pennisetum purpureum* and ash 9.1% in *Pennisetumpuroureum* . The dried plant parts were used for the study. The findings suggested that the medicinal plants studied have a promising potential for curing diseases and maintaining a healthy lifestyle, and that they should be explored further to uncover the active components.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

1.1.1 *Azadirachta indica*

Azadirachta indica, also known as neem, it is a mahogany tree in the Meliaceacee family. It is one of two species in the Azadiractha genus. It is native to India and has naturalized in most tropical and subtropical countries. It has a high medicinal value and is widely distributed across the world. The plants part contains compounds with proven antiviral, antiseptic, antipyretic, anti-fungi and anti-inflammatory uses. It has great potential in the field of pest management, environment protection and medicine. Neem has the potential to be a natural source of environmental friendly insecticides, pesticides, and agrochemicals. (Brahmachari, 2004).Neem is taken into account to be a part of India's genetic diversity (Sateesh, 1998). A lot of biologically active compounds are often derived from neem's chemical constituents, including flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones, with azadirachtin being the foremost active. Salannin, volatile oils, nimbin and meliantriol are samples of biologically active compounds. The drought tolerance of the arishth is well established. It thrives in areas with sub-humid conditions and annual rainfall of 400 to 1200mm, but it's heavily reliant on well water in these areas. Neem can grow in a very style of soils, but it prefers rich, sandy soils that are well drained. it is a typical/ tropical/ subtropical tree that thrives in temperatures starting from 21 to 32 degrees Celsius. It can withstand temperatures of less than 4 degrees Celsius. Temperature is one of the most important factors influencing seed growth; higher temperatures speed up respiration, gas diffusion, and metabolic processes. Germination is dependent on all these processes and thus is strongly affected by temperature. Neem can be used for a variety of things, including the processing of wood and heat, the providing of shade, and a variety of medicinal uses, as well as a biological insecticide. The Middle East, Southeast Asia, Australia, the Pacific Islands, the Caribbean, Central and South America, and the southern United States have all effectively incorporated indica (Tewari, 1992; Vietmeyer, 1992). The largest global almost-pure *Azadirachta indica* stand is most likely in Saudi Arabia's Arafat Plains near Makkah, where 50,000 trees have been planted (Vietmeyer, 1992). The majority of these introductions took place all across the nineteenth century. During this century, emigrants from

Southeast Asia took it to Fiji, Mauritius, and Guyana, and the British introduced it to Egypt, where it has since become one of the fastest-growing trees with a wide range in the tropics and subtropics (World Agroforestry Centre, 2002). In many locations where it has been introduced, A. indica has been proven to be invasive. In the Dominican Republic (IABIN, 2003) and other Caribbean countries, it has been connected to dry soils, and Puerto Rico has labeled it invasive. Personal communication, 2002, Hamilton A, World Wide Fund for Nature, Godalming, UK) and Ghana where a program to track its invasiveness was established (Chamberlain, 2000). It's a plant that's devouring natural ecosystems and displacing native plants in Gambia (WRM, 1999). It has also been reported to cause issues in Senegal (Chamberlain, 2000), particularly in the southern Casamance region, as well as Guinea Bissau, where it was declared a noxious weed in 1995. (Passiecznik N, CAB International, personal communication, 2004). Prior to (Reilly D, Department of Business, Industry and Resource Development, Government of Australia, personal communication, 2002). It was initially brought to Brazil in 1986, and since the 1990s, it has been cultivated commercially in the Southeast, Midwest, North, and Northeast of the country (Freire, 2013).

1.1.2 Pennisetum purpureum

Pennisetum purpureum (purple*pennisetum*) Elephant grass is additionally referred to as merker grass, napiergrass, Uganda grass, and a range of other names round the world. It was given the name napiergrass in honor of Colonel Napier of Rhodesia, who was the first to write to the Rhodesian Agricultural Department to alert them to the value of this plant. Elephant grass (*Pennisetum purpureum*) could be a monocot belonging to the Poaceae (grass familyand*Pennisetum* (*Pennisetum* Rich. ex Pers.; fountain grass).The Pennisetum genus is highly varied, with about 10,000 species with basic chromosomal numbers of 5, 7, 8, or 9 and ploidy ranging from diploid to octoploid, and a life cycle of annual, biennial, or perennial. (Martel, 1997). Remarkably, the species stay genetically closely related, and therefore the gene content of distinct species doesn't differ significantly (Bennetzen, 1997), variations in ploidy levels often accounting for differentiation. Elephant grass is an allopolyploid with chromosome constitution of 2n = 4x = 48 (Hanna, 1981). The allopolyploid carries two genomes A'A' BB within which chromosomes of A' genome are larger in size than the genome (Jauhar, 1981). Elephant grass is especially utilized in cut and carry systems (Zero grazing) and fed in stalls or

made into silage or hay. Elephant grass could also be grazed if it's left in an exceedingly lush vegetative state; Cattle value more highly to eat the younger leaves (FAO, 2015).

Elephant grass, as its name suggests, is a vital source of forage for African elephants (Tchamba et al., 1993; Francis, 2004). *Pennisetum purpureum* may be a plant that may be used for a spread of purposes. Humans may eat the young leaves and shoots, which may be prepared into soups and stews (Burkill, 1985). (1985, Burkill). Fences will be built using the culms, and also the entire plant may be utilized to construct thatch. within the US, it's thought to be a possible second-generation energy seed (EPA, 2013). Diuretic properties are identified for leaf and culm infusions (Duke, 1983).Elephant grass has many environmental uses. It may be accustomed make mulch and to supply eroding protection. it is a weed killer, and it has been documented in Africa that it has been used as a trap plant in push-pull management techniques to combat stemborers in maize crops (Khan, 2007).Elephant grass cultivars are grown everywhere the planet to accommodate local requirements, and that they are available a range of behaviors, yield capacity, and nutritional value. "Merker" varieties have lots of thin stems, short, often glabrous leaves, high yields, and are Helminthosporium tolerant. Dwarf cultivars ("Merkeron" and "Mott," grown at Tifton Station in 1955 and 1988, respectively) are leafy and high-value feed plants (Cook , 2005).Elephant grass can share alleles with other Pennisetum plants, and various hybrids are created as a result. bulrush millet (*Pennisetum glaucum*) and elephant grass hybrids ("King grass," "Pusa Giant," "Bana grass," "Florida," and others) have the benefit of pearl millet's desirable characteristics of vigour, drought resistance, disease tolerance, forage consistency, and seed size, while elephant grass offers rusticity, aggressiveness, perennity, palatability, and high DM yields (Timbo *et al.*, 2010).

1.2 STATEMENT OF PROBLEM

Because of the lack of knowledge about the chemicals present, the plant's protective nature for human health, and the nutritious properties of the plant's leaves, it is vital to investigate the proximate and bioactive components of the leaves.

1.3 AIM AND OBJECTIVES

AIM

To explore the proximate and bioactive component of *Azadirachta indica* and *Pennisetum purpureum.*

OBJECTIVE OF STUDY

- To determine the proximate analysis of *Azadirachta indica* and *Pennisetum purpureum*
- To determine of bioactive compounds from *Azadirachta indica* and *Pennisetum purpureum.*

1.4 SCOPE OF STUDY

This study is done to know the percentage of moisture content, crude fiber, crude protein, ash content and Ether extract present in the neem leaf. The phytochemical research method is thought to be useful in determining the bioactive characteristics of medicinal plants. Alkaloids, Tannin, Saponins, Phenol, Flavonoids, and Terpenoids are among the phytocompounds found in the plants studied in varying proportions.

1.5 SIGNIFICANCE OF STUDY

This study is considered effective in discovering the amount of macromolecules and also the bioactive profile of both *Azadirachta indica* and *Pennisetum purpureum*. Phytochemicals are observed to be present in the investigated plants in variable proportion.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BOTANY OF *Azadirachta indica*

It's a tough, quick-growing evergreen with a straight trunk, large leaves, and dense, rough, longitudinally fissured bark. Trees grow to be 7-15 meters (23-50 feet) tall when fully mature (Ogbuewu, 2008).The tree produces yellowish ellipsoidal drupes (fruits) in about four years, becomes completely viable in ten years, and can live for over 200 years. The leaves are complex and imparinnate, with up to 15 leaflets placed in alternating pairs at the end of the leaf (Ogbuewu, 2008).With a maximum length of 6 cm, the leaves are tiny and lanceolate. White panicles with a sweet scent bloom in the leaf axils. The approved method for producing plantation stands is seed prolongation in nurseries followed with direct planting in the region (Ogbuewu, 2008). The one-seed neem fruit is yellow and roughly an inch long when ripe (Ogbuewu, 2008). Neem blossoms grow in India from May to August (Koul, 2006).

2.1.1. TAXONOMIC HIERARCHY OF*Azadirachta indica*

The neem tree has been described as *A. indica* as early as 1830 by De jussieu and its taxonomic position is as follow:

Table 2.1 Scientific classification of *Azadirachta indica*

Rank	**Scientific and Common Names**
Kingdom	Plantae
Order	Rutales
Suborder	Rutinae
Family	Meliaceace (Mahogany family)
Tribe	Melieae
Genus	*Azadirachta*
Species	*India*
Latin	*Azadirachta indica*

2.1.2 ORIGIN AND HABITAT OF *Azadirachta indica*

Azadirachta indica grows in dry deciduous and thorny forests in India and Pakistan. It grows in mixed woodlands dominated by Acacia and Dalbergia sissoo in India (Champion, 1965). The

World Agroforestry Centre is a non-profit organization committed to promoting agroforestry (World Agroforestry Centre, 2002). Invasive in a variety of habitats, including fallow agricultural land, savannah, and dry and arid forests (Hamilton A, WorldWide Fund for Nature, Godalming, UK, personal communication, 2002), coastal forest in Ghana (Chamberlain 2000), lowland monsoon forest in Indonesia, and evergreen forest and dry deciduous forest in Africa (Hamilton A, WorldWide Fund for Nature, Godalming, UK, personal communication, (World Agroforestry Centre, 2002).

Table2. 2. Habitat List of *A. indica*

Category	Sub-category	Habitat	Presence	Status
Terrestrial	Managed	Cultivated/ Agricultural	Present, no further details	Harmful(Pest/ Invasive)
Terrestrial	Managed	Disturbed areas	Present,no further details	Harmful(Pest? Invasive)
Terrestrial	Natural/ Semi-natural	Natural forests	Present,no further details	Harmful(Pest/ Invasive)
Terrestrial	Natural/ Semi-natural	Natural grasslands	Present, nofurther details	Harmful (Pest/Invasive)

2.1.3. DISTRIBUTION OF *Azadirachta indica*

Neem is a plant that grows wild in India, Myanmar, and China's arid regions (Rojas- S andoval, 2014). It was first discovered in Thailand, Malaysia, and Indonesia, but has since expanded throughout tropical and subtropical regions. It has spread invasively in the Caribbean (Puerto Rico, Dominican Republic), Sub-Saharan Africa (Kenya, Gambia, Senegal, Guinea Bissau, Ghana, Tanzania), and the Pacific (Austalia, Fiji, Marshall Islands) (Rojas-Sandoval, 2014). Neem may be found in acacia woods, as well as dry deciduous and thorny trees. On its exotic range, it has grown invasive in fallow agricultural land, savannah, and dry desert woods (coastal forest in Ghana, lowland monsoon forest in Indonesia, and evergreen and dry deciduous forest in Africa) (Orwa, 2009). Neem may be found in regions with average annual rainfall of 400 to 1200mm and average annual maximum temperatures of 40 degrees, from sea level to 1500 meters above sea level. Adult trees can withstand any snow, but seedlings are more vulnerable. Neem can grow in a wide variety of soils, from acidic to alkaline in pH, but it thrives in shallow,

stony, sandy, poor soils, on rocky crevices, and in marginal sloping areas (Puri, 1999). Neem is a full-sun plant, but it can tolerate some shade in its early years (Orwa, 2009). Neem can derive nutrients from heavily leached sandy soils and can withstand PH ranges of 3 to 9. (Rojas-Sandoval, 2014). In well- drained soils, neem withstands up to 2500mm rainfall. Neemcan tolerate up to 2500mm of rainfall in well-drained soils. Neem has some salinity resistance and has been used in sugarcane plantations with high salinity soils (Orwa, 2009; Ahmed, 1997).

2.1.4 ECOLOGY AND CULTIVATION OF*Azadirachta indica*

Neem thrives in sub arid and sub humid climates with tropical and subtropical climates at elevations ranging from sea level to 700 meters. The average yearly temperature in its natural range is 21–32°C (recommended temperature range is 9.5–37°C) (Stoney, 1997). Neem can withstand high summer temperatures (up to 50 degrees Celsius), but not frost or cold below 4 degrees Celsius (leaf fall and death may result). Neem thrives best in places with annual rainfall ranging from 450 to 1200 mm (with optimum growth around 1100 mm), although it can endure annual rainfall as low as 150 mm provided its roots have access to ground water within 9–12 m of the ground surface (Stoney, 1997). Once established it is very drought tolerant and can survive 7–8 month dry seasons. Neem is known for growing in hard, dry, infertile soils, although it may thrive in a variety of soil types, including sandy, rocky, and extremely dry conditions. It may, however, be best suited to deep, porous, sandy soils. Its wide, deep root system appears to be a seasonal adaptation to arid conditions. Neem does not survive seasonally or chronically wet (poorly drained) soils, such as low-lying silty clays and clays, saline soils, or places with sub-surface hard-pan or laterite outcrops (NRC, 1992). It likes a pH range of 6.2–7.0 in the soil, but may thrive in a pH range of 5.0–8.0. (Stoney, 1997).It appears to have a preference for sandy river banks in North Queensland's Gulf region. Neem's original habitat is "seasonally dry, deciduous, mixed woods, occurring in association with Acacia spp." and Dalbergia sissoo," according to (Lemmenset, 1995). Neem grows natively in dry deciduous and thorn forests in India and Pakistan, according to (Champion, 1995). (CAB International 2000).

2.1.5. Uses of *Azadirachta indica*

Neem trees are grown commercially in plantations to produce azadirachtin, a chemical extracted from the seeds and leaves. Azadirachtin has been promoted as a new insecticide that is considered more 'environmentally friendly' than synthetic insecticides. Plantations have been established in tropical to subtropical regions of the world, including semi-arid and wet tropical regions, from sea level to about 700 m elevation (NRC, 1992).After the oil has been pressed from the seeds, the residue ('neem cake') can be used in cattle and poultry feed. Neem is also used in silviculture in India and for reforestation in Asia, Central America and the sub-Saharan region. It was grown as an ornamental plant and sold by commercial nurseries i.n Queensland (Lawson, 1996). It has been promoted as a street tree in some towns and cities (Hearne, 1975).Those advocating the planting of neem as a commercial crop cite a wide range of potential benefits (Table 3). Some go as far as saying neem is 'a tree for solving global problems' (NRC, 1992).While neem has a wide range of possible applications, the pest control characteristics of neem extracts are of particular economic relevance. While insecticidal effects of azadirachtin and other neem extracts have been demonstrated, the commercial feasibility of generating these pesticides looks to be in doubt. According to a 1988 economic evaluation of neem, "neem has limited contemporary demand, with no local manufacturing and just limited amounts of imports.

Table2.3. Potential uses of neem (Benge, 1986).

Potential Use	**Details**
Pesticide	The biologically active compound is azadirachtin (Extract of neem seed), repellent for a broad spectrum of agricultural and household insects.
Garden ornamental	An attractive tree with perfumed flowers
Shade	Planted as a shade tree for livestock in arid areas- well adapted to hot, Seasonally dry regions in tropical Queensland
Stock feed	Protein- rich stock feed is obtained by chemical processing of neem cake.
Timber	Used for timber and a fuel source.

Veterinary	The biologically active fraction separated from neem kernels shows antiviral activity against certain viruses and has blood-sugar lowering and antimicrobial properties. Leaves can be used as a poultice to treat cattle wounds and sores and to repel worms in livestock.
Medicinal uses	Compounds derived from various parts of the neem tree are used to treat fevers, thirst, nausea, vomiting, some skin diseases, heat rash and boils.
Contraception	Components of neem oil are reported to have contraceptive properties.
Soil improvement	Neem leaves and twigs can be used as mulch and fertilizer. Neem seed cake is organic manure with insecticidal properties and relatively high nitrogen content.
Soap	Neem oil replaces edible vegetable oil used in soap making. Soap has medicinal properties.

2.1.6. COMMON NAMES OF *Azadirachta indica*

Nim, Nimgachh, Danujhada, Limbado, Limbra, Limdo, Nimb, Nimba, Arista, Nimbah, Picumarda, Indian Lilac, Margosa tree, Neem tree.

2.1.7 PHARMALOGICAL ACTIVITY OF *Azadirachta indica*

2.1.7.1 ANTIMICROBIAL ACTIVITY

Neem extracts have been reported to have high antimicrobial activity, and studies have indicated that they may be useful in controlling some foodborne pathogens and other spoilage organisms (Mahfuzul, 2007). Zones of inhibition have been discovered in NLEs, confirming that they have antibacterial characteristics, and the extract had much more zones of inhibition than 3 percent sodium hypochlorite (Ghonmode, 2013). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration for extracts of the leaves and seeds against several dermatophytes were determined in another investigation (Arshad, 2018). Theminimum inhibitory concentration of seed extracts was found to be 31 g/mL for all of the dermatophytes tested. Seed extract at a concentration of 15 g/mL was also shown to be sufficient for altering the growth pattern of the organisms tested (Natarajan, 2003). On *Anopheles stephensi*, the effects of neem limonoids such as azadirachtin, salannin, deacetylgedunin, gedunin,

17hydroxyazadiradione, and deacetylnimbin were researched. The bioactivity of azadirachtin, salannin, and deacetylgeduninwas high at all doses, whereas the other neem limonoids were less active. Additionally, azadirachtin was the most powerful in all studies, causing almost 100% larval mortality at a dose of 1 ppm (Nathan, 2015). Antiviral activity of neem bark extract indicated that at 50–100 g/ml concentrations, bark extract effectively prevented HSV1 entry into cells (Tiwari, 2012). The antifungal efficacy of seed extracts on *Candida spp.* was also tested, and the study found that neem seed extract looks to be a promising anticandidal agent (Lloyd, 2005).

2.1.7.2 ANTI-INFLAMMATORY EFFECT

Various studies have discovered that neem plants have an anti-inflammatory effect (Arshad,2018). Nimbidin from neem trees was utilized orally in an experimental investigation based on rat models to evaluate its anti-inflammatory response (Arshad,2018). Phagocytosis was confirmed to be suppressed, and macrophage migration to their peritoneal cavities in response to inflammatory stimuli was also dramatically reduced. Furthermore, nimbidin prevented phagocytosis and phorbol myristate acetate stimulated respiratory burst in rat peritoneal macrophages when exposed to it in vitro (Arshad, 2018). In lipopolysaccharide stimulated macrophages, nitric oxide and prostaglandin E2 production were reduced by nimbidin after in vitro exposure(Kaur, 2004).The anti-inflammatory properties of neem fruit skin, as well as its active component, azadiradione, have been studied (Kaur, 2004). The mice given a 100 mg/kg dose of this fruit skin extract and azadiradione showed strong anti-inflammatory activity, according to the findings ofIlango (2013).Furthermore, rats with carrageenan-induced hind paw edema were used to test the anti-inflammatory activity of neem seed (Arshad, 2018).

2.1.7.3 WOUND HEALING EFFECT

The wound-healing abilities of neem leaves have been known as traditional medicine since ancient times. The effects of neem oil in the treatment of chronic, nonhealing wounds were studied in one study, and the results showed that after 8 weeks of treatment, 50 percent of patients had healed their wounds (Singh, 2014). Another study used an aqueous extract of neem leaves to test wound healing activities, and a substantial reduction in the wounds with the largest diameter was reported (Chundran, 2015). The wound-healing effects of aqueous extracts of neem leaves are thought to function biochemically through inflammatory response and neovascularization, according to research (Osunwoke, 2013).

2.1.7.4 IMMUNOMODULATORY EFFECT

Neem oil is also applied as a nonspecific immunostimulant since it helps to activate cell-mediated immunological processes, allowing for a better response to following mitogens (Upadhyay, 1992). Furthermore, when utilized at a concentration of 50 ml/l of fresh drinking water, neem infusion has been shown to boost antibody titer growth performance (Durrani,2008).

2.2 BOTANY OF Pennisetum purpureum

Elephant grass is vegetatively propagated and has a perpetual life cycle. It has a large root system that reaches deep into the soil, as well as a large number of fibrous roots that extend into the top soil horizons. The rhizomes (underground stems) are small and creeping, with fine roots and culms developing at nodes.There are two sorts of plants: giant (tall) and dwarf (little). The plant grows in clumps and upwards, with thick cane-like stems that are densely branched.Theculms are erect and tall, varying in height from 2 to 6 m.It has long, tapering leaves that are rigid. Bristle-like ridges run along the leaf margins. The upper leaf surface is coated with stiff hairs, and the leaf sheaths are likewise hairy. (Nyambati, 2010) offered the following morphological parameters based on an average of 12 cultivars cultivated in Kenya: tillers number 60; tiller diameter 5.4 cm; tiller angle (stem to ground angle) 79.2°; leaf length 61.2 cm; and leaf width 2.0 cm. Extracts or rudimentary pieces of the tree are frequently combined with stored seeds like maize, rice, and beans to protect them from insects. In India, neem-based pesticides have been created. With neem extracts, plants can be protected from insects that eat their leaves while pollinators like honeybees are not affected. Other limonoid chemicals in neem have a diverse set of properties. Melantriol and salannin are insect repellents. Antiviral activity has been demonstrated for nimbin and nimbindin (the latter a bitter chemical found at 2% in the seed). It produces no or very few flowers when grown in sub-temperate climates because the temperature drops too low to allow for further growth by the time the days shorten for blossoming. The panicles are the plant's reproductive organs, and they have a tawny or reddish hue, sessile fascicles, and sparsely plumose bristles. Cross-pollination is possible because elephantgrass flowers have a receptive stigma before anther exertion. The stigma exerts itself for 3–4 days at the apex of the inflorescence, followed by anthers releasing pollen for a similar amount of time. Self-fertilization is further hampered by a substantial percentage of self-incompatibility. In crosses with other genotypes, certain genotypes, like as 'Merkeron,' have been discovered to self or produce seed on maternal parents (Hanna, 2004). The seed set is flimsy, and

the seeds break readily (Cheng, 1991). The seeds are quite little, weighing about 3.8 million seeds per kilogram (Skerman and Riveros, 1990), and they are dispersed by the wind. Seeds have a poor germination rate, and seedlings are sluggish to develop.

2.2.1 TAXONOMY OF *Pennisetum purpureum*

Table 2.4. Taxonomic position of *Pennisetum purpureum* according to Cronquist (1981)

RANK	**SCIENTIFIC AND COMMON NAMES**
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	*Pennisetum*
Species	*Purpureum*

2.2.2 ORIGIN AND HABITAT OF *PENNISETUM PURPUREUM*

Pennisetum purpureum is an invasive plant that grows in farm fields, pastures, and along roadsides. It thrives in marshes, floodplains, riverbanks, swamps, forest margins, disturbed areas, and waste ground, particularly in mesic to moist environments (Francis, 1992). *P. purpureum* is drought tolerant and may be seen populating dry lowlands, such as on the Galapagos Islands. (McMullen, 1999).

Table 2.5. Habitat List *Pennisetum purpureum*

CATEGORY	SUB-CATEGORY	HABITAT	Presence	Status
Terrestrial	Managed	Cultivated/ agricultural land	Present,no further details	Harmful(pest or invasive)
Terrestrial	Managed	Cultivated/ agricultural land	Present,no further details	Natural
Terrestrial	Managed	Manage forests, plantations and orchards	Present,no further details	Harmful(pest or invasive)
Terrestrial	Managed	Manage forests, plantations and orchards	Present,no further details	Natural
Terrestrial	Managed	Manage forests, plantations and orchards	Present,no further details	Productive /non-natural
Terrestrial	Managed	Managed grasslands (grazing systems)	Present,no further details	Harmful (pest or invasive)
Terrestrial	Managed	Managed grasslands (grazing systems)	Present,no further details	Natural

Terrestrial	Managed	Managed grasslands (grazing systems)	Present,no further details	Productive /non natural
Terrestrial	Managed	Disturbed areas	Present,no further details	Harmful (Pest or invasive
Terrestrial	Managed	Disturbed areas	Present,no further details	Natural
Terrestrial	Managed	Rail / roadside	Present,no further details	Harmful (Pest or invasive
Terrestrial	Managed	Rail / roadside	Present,no further details	Natural
Terrestrial	Natural / semi natural	Natural grassland	Present,no further details	Harmful(pest or invasive)
Terrestrial	Natural / semi natural	Natural grassland	Present,no further details	Natural
Terrestrial	Natural / semi natural	Riverbanks	Present,no further details	Harmful(pest or invasive)
Terrestrial	Natural / semi natural	Riverbanks	Present,no further details	Natural
Terrestrial	Natural / semi natural	Wetlands	Present,no further details	Harmful (Pest or invasive)
Terrestrial	Natural / semi natural	Wetlands	Present,no further details	Natural
Terrestrial	Natural / semi natural	Scrub/ shrublands	Present,no further details	Harmful (Pest or invasive)

Terrestrial	Natural / semi natural	Scrub/ shrublands	Present,no further details	Natural
Terrestrial	Natural / semi natural	Arid regions	Present,no further details	Harmful (Pest or invasive)
Terrestrial	Natural / semi natural	Arid regions	Present,no further details	Natural

2.2.3 DISTRIBUTION OF *PENNISETUM PURPUREUM*

Elephant grass is native to tropical Africa's sub-Saharan region (Clayton, 2013). As a fodder plant, it has been introduced to most tropical and subtropical locations across the world. It was initially launched in 1913 in the United States, and later in the 1950s in Australia. It can become invasive if it becomes naturalized. Elephant grass is usually found between the latitudes of 10oN and 20oS. It's a pioneer species in dry areas like the Galapagos Islands, and it can withstand drought (CABI, 2014). Elephant grass is a summer-growing grass that may be found from sea level to 2000 meters in height. Temperatures ranging from 2 to 40 degrees Celsius are ideal for it (FAO, 2015). and when the annual rainfall total exceeds 1500 mm. It is frost-sensitive and stops growing below 15 degrees Celsius, although it can regenerate from its adventitious roots if the soil is not frozen (Duke, 1983). Elephant grass is drought tolerant and thrives in areas with 200 to 400 mm of rainfall. Elephant grass prefers well-drained soils and cannot tolerate floods. Because of the inadequate drainage, it thrives in high beds .Elephant grass grows best in rich, deep soils like friable loams, but it can also grow in poorly drained clays with a heavy texture or excessively drained sandy soils with a pH ranging from 4.5 to 8.2 (FAO, 2015).Elephant grass is a full-sun species that can produce in partial shade but not total shade under a dense canopy of trees.

2.2.4 ECOLOGY AND CULTIVATION OF *PENNISETUM PURPUREUM*

2.2.4.1 ECOLOGY

The best environment for Elephant grass development is one that is comparable to its natural region, with light rains interspersed with bright sunlight.The optimum daytime temperature for growth is 30–35°C (Ferrais, 1978), while no growth occurs below 10°C (Bogdan, 1977). Elephantgrass thrives in all tropical and subtropical climates. It can tolerate annual rainfall ranging from 750 to 2500 mm and elevations ranging from sea level to 2100 m, however it is prone to frost damage (Skerman, 1990). Elephantgrass grows all year in the tropical climate. The aboveground section of the plant is destroyed by cold, but the soil ensures its survival by preserving the subterranean rhizomes, which sprout and produce new tillers when the weather improves. Rhizomes of elephantgrass cultivar 'Merkeron' was able to endure winter temperatures as low as -18°C and resurface for growth the following spring. Although elephantgrass prefers warm temperatures for optimal development, its capacity to recover quickly allows it to thrive in moderate temperate regions (35°N).Early development is severely hampered in temperate locations due to low spring temperatures, but the plant quickly recovers with summer increasing temperatures. There have been reports of biomass yields of around 80 dry t ha1 year1 in the tropics and reaching 45 dry t ha1 year1 (Prine, 1988) in 30°N latitude (Gainesville, Florida, USA). Elephantgrass has been documented to overwinter as far north as 36°N in Oak Ridge, Tennessee (USA) . Yield appears to have declined as a result of the abbreviated summer (30 dry t ha1 for 'N-51' and 22 dry t ha1 for 'PI 300086'). (Nagasuga, 2005: Kubota, 2006) Elephantgrass was well suited to temporary shadow circumstances similar to tropical rainy season gloomy weather.

2.2.4.2 CULTIVATION SELECTION

According to (Kretschemer, 2001), there are around 25 cultivars of elephantgrass and 16 hybrids with millet under cultivation. There are cultivars that have been adapted to different locations of the world. Farmers favour dwarf cultivars for fodder because they adapt better to hand-harvesting practices used in underdeveloped nations, and they are also superior to regular height cultivars for grazing (Williams, 1995). (Mukhta,2003) discovered that dwarf cultivars had greater tiller number, LAI, and dry blade % of leaf blade but lower plant height, mean tiller dry weight, and total dry weight than normal cultivars, however the difference in total plant dry weight narrowed as plant densities increased. 'Mott' is a well-known dwarf elephantgrass variety.

In East Africa, cultivars such as ‘Bana,' ‘French Cameroon,' ‘Clone 13', and ‘Pakistan hybrid' are prevalent, with ‘Bana' being the most popular because to its short stems and large leaves, as well as reduced stem herbage at maturity (Nyambati*e,* 2010).Cultivars with thick stems should be more suited as biofuel feedstock since the stem acts as a cellulose sink. ‘Wrunkwona' is touted as a superior-yielding elephantgrass cultivar with thick stems and great in vitro dry matter digestibility (Ishii, 2005). Another cultivar with favorable features for biofuel feedstock is ‘Taishigrass No.2'. It grows quickly, adapts well to a short growth season, and hence is broadly adaptable, yielding large volumes of biomass (Tsai, 2009).

2.2.4.3 *PENNISETUM PURPUREUM* PLANT

Elephant grass does not reproduce by seed for a variety of reasons. First, because of cross-pollination, the seeds are a mixed bunch, thus the plants generated from them will be uneven and unpredictable in performance. Seeds frequently germinate poorly, and seedlings are feeble. Furthermore, seed supply is hampered by poor seed-setting and shattering. As a result, vegetative growth of elephant grass is a common procedure. The plant is easily propagated, and the procedure ensures germplasm stability and quality preservation. Elephant grass is propagated by stem cuttings (setts), rooted stems, or rhizome splitting. Setts are the most often used method of planting. Mature stems from the basal 2/3 zone with well-developed leaf buds are cut into three-node portions and planted upright by burying two basal nodes in the soil. Another, more popular way is to lay chopped portions horizontally into shallow trenches 5–10 cm and cover the trenches. (Knoll, 2012) Cuttings obtained from the plant's basal region were more suitable for planting than those obtained from the plant's younger top half. Furthermore, five- and ten-node cuttings exhibited a greater incidence of after-winter emergence in sub temperate temperatures than one- or two-node cuttings.(Woodward,1985) Cutting stems into short pieces because apical dominance kept the bud at the central node quiescent while buds at the two ends began to develop normally. Depending on soil quality, cultivar, and harvesting purpose, different planting densities have been reported from different areas of the world. For forage production, intra-row and inter-row spacing of 0.5 m × 1 m in Ghana (Ansah, 2010), 1 m × 1 m in Kenya (Nyambati, 2010) and 50 cm × 50 cm in Japan (Ishii, 2005) have been suggested. (Mukhtar, 2003) where plants have to go through overwintering, high population density impacted adversely the number of stubble tillers emerging from underground stems.

2.2.4.4 *PENNISETUM PURPUREUM* FERTILIZATION

The rate of fertilization varies with the soil and is dependent on the existing fertility level. The reported range of optimum fertilization rate especially of N is wide. (Mohammad,1988) AN rate of 80 kg N ha1 was adequate, (Miyagi, 1983) Forage yield increases of up to 600 kg N ha1. (Sunusi, 1999) There is a continuous increase in dry matter yield by increasing N supply to 1200 kg N ha1. (Walsmsley,1978), There is no advantage to yielding more than 170 kg N ha1.There have been numerous reports of elephantgrass being planted in conjunction with legumes to increase fodder yield and nutrient value (Mureithi, 1995; Njoka-Njiru, 2006). Trailing or shrub legumes are recommended for companion cropping in the tropics because low growing types may be shaded out by the elephantgrass canopy. (Tiley, 1989) A combination of desmodium (Desmodiumintortum Mill) and 120 kg ha−1 fertilizer N produced maximum yield of elephantgrass. The cover crop is allowed to develop in the field, die, and become mulch in this trial. It has been shown to be beneficial in providing ground cover to reduce weed issues in the spring. The cover crop reseeds itself after first year planting. (Quesada, 2005) Rich soil is needed to generate high protein elephantgrass feed, this plant has the potential to digest equivalent amounts of dry matter but with lower N composition in restricted N soils. Because low N concentration is a desirable attribute for biofuel feedstock, this presents a once-in-a-lifetime chance for infertile soil to be utilized and availability may become a concern since elephantgrass is commonly farmed on acidic soils in Africa, South America, and even in the south-eastern United States in the future. Inoculation with mycorrhizae can help plants absorb more P and (Hung,1990) inoculated plants grew faster at all P levels.

2.2.5 USES OF *Pennisetum purpureum*

Farmers in Africa, Asia, and other tropical and subtropical parts of the world employ a variety of grass types as fodder crops. Napier grass is one of the most important fodder crops in Eastern and Central Africa, especially among smallholder farmers. (Kabirizi, 2015; Lukuyu, 2012). It's mainly used for animal cut-and-carry feeding systems (+Farrell, 2002). It is a flexible fodder crop that may be grazed directly or turned into silage or hay (Orodho, 2011). In Nepal (Pandit, 2004) and Bangladesh (Shaha, 2015), for example, grass carp and tilapia have been fed Napier grass. In a recent report from Nigeria, young stems of Napier grass were also found to be

consumed as a cooked vegetable (Akah, 2014). These many implementations demonstrate how Napier grass may help alleviate poverty and nutritional insecurity in a variety of ways.Napier grass may be used to create fences, as a windbreak, and to demarcate borders between neighbouring farms, in addition to its usefulness as a feed crop. The dried material can also be utilized as a fuel source (Orodho, 2011). It's utilized as a mulch to minimize weed invasion and soil erosion in agricultural land management approaches (Kabirizi, 2015), as well as a trap plant in the push–pull method, which uses repellent intercrop "push" plants and attractant trap "pull" plants. Control of insect pests in Africa, particularly the maize stem borer (Khan, 2017; Khan, 2007).Napier grass has been used in phytoremediation techniques such as cleaning up cadmium-affected soil and decreasing cadmium levels in soil to a depth of 15 cm (Ishii., 2013). Large biomass plants have been promoted as second- or next-generation biofuel crops in the hunt for alternative biofuel sources, as worldwide interest in decreasing fossil fuel usage and worries about climate change has grown. Because of its perennial nature and capacity to expand fast, Napier grass has been estimated to give a dry matter (DM) output of up to 78 tons per hectare per year (on average 35–41 tons per hectare) (Oliveira, 2014From Napier grass varieties grown in Thailand, (Rengsirikul, 2013) predicted a maximum ethanol yield of 329 L/ton DM and a maximum ethanol output of 350–460 L/ton DM. This potential was shown to be 6% and 15% greater than that of the tropical forages Brachiariabrizantha and Panicum maximum, respectively, and around 15% and 17% higher than Eucalyptus bark and sugarcane, respectively (Lima, 2014). As a result, Napier grass may be used for phytoremediation, with the enormous crop being transported to biofuel production plants.

2.2.6 COMMON NAMES OF *PENNISETUM PURPUREUM*

Elephant grass, merker grass, Napier grass, napier, uganda grass, bana grass, barner grass (English).

2.3 PROXIMATE ANALYSIS

Proximate analysis refers to the quantitative analysis of macromolecules in food. It comprises the mass percentage of macromolecules which are obtained from a series of standardized tests. The total ash, moisture, crude fat, protein and fiber content given as the percentage composition of the sample is calculated by proximate analysis (Self, 2005).The proximate analysis system is both predictive and comparative. We may perform legitimate comparisons of plant specific

nutritional value against micro-organisms using proximate analysis. Moisture, ash, volatile matter, and fixed carbon are parameters been tested in proximate analysis.

2.3.1 MOISTURE CONTENT

A sample's moisture content is measured as the difference in mass following dehydration (Self, 2005).The value of dry matter (DM) or total solids is the mass after dehydration. The most popular drying methods are oven drying and freeze drying. Moisture, also known as water, is a universal fluid that can dissolve other substances and transport nutrients within the body, allowing organs like the kidney, liver, and stomach to work properly and efficiently. A high moisture content (matric potential >-0.01 MPa) decreases rates of organic matter decomposition, due to low oxygen supply, while low soil moisture decreases microbial activity by reducing diffusion of soluble substrates, microbial mobility and intracellular water potential (Csonka, 1989 and Killham et al., 1993). According to the research done by Yi (2011), on theinfluence of moisture content on microbial activity and silage quality during ensilage of food processing residues,moisture content is one essential factor affecting *microbial activity* and silage quality. The optimum Moisture content for silage production varies depending on the feedstock and microbial inoculation. The Moisture content range studied has varied from 40 to 90% (Richard, 2001). In addition, this study reported that the presence of lactic acid bacteria can improve the lactic acid fermentation and inhibits undesirable secondary fermentation which decomposes the biomass and deteriorates silage quality. In this study, stabilization and storage of seasonal and highly putrescent food processing wastes such as TomatoePomace andSugar beet Pulpwere examined using ensilage technology. Results from the study identify moisture content and basic inocula for ensiling TomatoePomace andSugar beet Pulp, and elucidate the long-term stability of ensiled materials with adequate moisture to reduce water addition in the downstream processes.

2.3.2 CRUDE PROTEIN

Proteins are immune boosters that aid in the growth and division of cells in the body and blood plasma are also immune boosters which help in the growth and division of cells in the body (Okeke and Elekwe, 2006). In the crude protein determination, the total nitrogen content of the sample is measured as total nitrogen by applying a conversion factor of 6.25 after digestion, salt neutralization, and titration of the ammonia formed against standard acid (Kjeldahl method). A

conversion factor is used to calculate total protein. Some functional classes, such as -NO2 and -N=N, rarely react**.**

2.3.3 CRUDE FIBRE

The crude fibre material is used to determine the structural carbohydrate content of a sample. Fibers are insoluble in the human digestive system and are found in fruits, grains, and vegetables. Furthermore, dietary fibers slow the release of glucose into the bloodstream in the human body, lowering the risk of hyperglycemia (Bouttwell, 1998). They can also function in reducing levels of plasma cholesterol, colon cancer and cardiovascular diseases. (Davidson et al., 1975). A sample is heated sequentially with dilute acid and then with dilute alkali, then washed with ethanol and diethyl ether, with the residue being separated from the ash and the product being identified as crude fiber.

A 2017 study found that the importance of fiber is intimately tied with the importance of our gut microbes(Sarah, 2018). A proper fiber diet feeds and makes these bacteria thrive. In turn, they increase in number and kind. The more microbes we have in our intestines, the thicker the mucus wall and the better the barrier between our body and our busy bacteria population. While the mucus barriers lower inflammation throughout the body, the bacteria aids in digestion, creating a dual benefit.

2.3.4 ASH CONTENT

The ash content of a plant sample is a characteristic of the mineral elements present. Dietary ash has long been used to build and maintain a balanced acid-alkaline blood system, as well as to detect hyperglycemia. In a research carried out by(Pichtel,1990), on the influence of fly ash on soil microbial activity and populations, it was observed that the means of microbial numbers were markedlyaffected by the ash and sludge amendment.When 5% ash was applied to the soil, bacterial numbersincreased, though not significantly. Lowlevels of the ash may have increased bacterial numbers due tothe addition of nutrients .Actinomycetes and fungi declined with 5% ash and allpopulations declined at the 10 and 20% rate. With 20%ash bacteria, actinomycetes and fungi decreased by 57%,80%, and 86%, respectively.

2.4 PHYTOCHEMICAL

Phytochemicals are plant-derived compounds (Molyneuexet, 2007). Phytochemicals, derived from the Greek word phyto, which means "plant," are substances generated by plants as a result of their main or secondary metabolism (Harborne, 1999). They usually have biological activity in the plant host and help the plant develop or defend itself against predators, diseases, or rivals. Examples of phytochemicals; Tannins, Saponins, Alkaloids, Flavonoids, Glycosides, , Steroid, Anthraquinones, Protein, Fat and Oil, Phenol, coumarin, Cardiac glycosides, Phytosterol, Carbohydrates, Phlobotannins.

Table 2.6 Microbial activities of Phytochemicals

PHYTOCHEMICALS	**MICROBIAL ACTIVITIES**
Flavonoids	Anti-viral and anti- inflammatory Okwu, 2001
Phenols	antimicrobial (Alves et al., 2013)
Glycoside	Antibacterial activity (Lorian, 1991)
Terpenoids	Anti-microbial (Park, 2016) Anti-bacterial (Pattnaik, 1997)
Steroids	Anti-fungal (Sung-Kee, 1998)
Tannins	Antimicrobial (Akiyama, 2001), Antiviral (Orlowski et al., 2014).
Saponins	Anti-viral activity(Hayashi,1997)
Anthraquinones	Antibacterial and antifungal (Marco Masi, 2020)
Coumarin	Anti-bacterial (Goth, 1945)
Cardiac glycoside	Antibacterial activity (Lorian, 1991)
Fat and Oil	Anti-microbial, Anti-fungi (Marianne, 2015)

Protein	Anti-microbial (Alejandro, 20201)
Phytosterol	Anti-inflammatory
Carbohydrate	anti-inflammatory (Garcia-Vaquero, 2017)
Phlobotanin	Antiviral, Anti-bacterial (Jalpa, 2015**)**
Alkaloid	(Antibacterial, 1984)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION AND IDENTIFICATION

The leaves were collected as part of the plant for both proximate analysis and Phytochemical screening. The fresh plant sample of *Azadirachta indica*and *Pennisetum purpureum* was collected around the Lake Close to the college of Basic and Applied Science, Mountain Top University, Prayer City, Ogun state. They were found growing freely as wild plant collected on Monday 8th February 2021 and identified by a taxonomist Dr. George from the Department of Botany, University of Lagos, Nigeria.

3.2 PREPARATION AND STORAGE OF PLANT MATERIALS

The leaves of the identified plants were washed thoroughly and carefully with distilled water and dried in an oven with circulating air at 50 degree C for 48 hours, it was pulverized to powder using a grinder in the laboratory and kept in an air-tight container and labeled to prevent mix up until needed for further extraction and various processes.

3.3 AQUEOUS EXTRACT

100 grams of pulverized plant leave was weighed and mixed with 400 ml of distilled water in a volumetric flask. This was left for 3 days in sterile environment and then filtered using a what-man paper no. 40

3.4 SOLVENT EXTRACT

100 gram of pulverized leave was weighed and mixed with 200 ml of ethanol in a volumetric flask. This was left for 3 days in a sterile environment and then filtered using a Whatman filtered paper no.40.

3.5 METHODS

3.5.1 PROXIMATE ANALYSIS

Standard analytical procedures were used to determine the moisture content, crude protein, ash content, and crude fiber content of powdered Elephant grass and Neem leaf samples (AOAC, 1990; Kirk and Sawyer, 1980; James, 1995).

3.5.2 MOISTURE CONTENT DETERMINATION

Moisture was determined by oven drying (Gravimetric method), where 2.0 g of the ground crude sample was accurately weighed in a clean and dry crucible. Then it was placed in an oven at 105^{o}C for 4hours, this procedure was done repeatedly until a constant weight was achieved. The sample was placed in a desiccator to cool for some time. The weight of moisture lost was calculated with the equation (a) below and expressed as percentage of weight of sample analysed.

$$\% \text{ Moisture} = \frac{\text{Weight of sample + dish before drying + dish after drying}}{\text{Weight of sample taken}}$$

3.5.3 ETHER EXTRACT DETERMINATION

The fat and oil in a feed represents the ether extract. The Soxhlet device is used to determine the concentration of ether extract. It is made up of three primary components.

1. An extractor, which consists of the thimble that retains the sample.
2. Condenser: To cool and condense the ether vapour.
3. A flask of 250 mL

In the flask, pour around 150ml of anhydrous diethyl ether (petroleum ether) with a boiling point of 40-600C. 2-5g of sample is weighed into a thimble and cotton wool is used to plug the thimble. The extractor is filled with the contents of the thimble, and the ether in the flask is heated. The ether soluble substances are dissolved and carried into solution through the siphon tube back into the solvent is distilled from the flask into the extractor as the ether vapour reaches the condenser through the side arm of the extractor and condenses to liquid from the drop back into sample in the thimble. The flask is then disconnected and placed in an oven at 65^{0}C for 4hrs, cool in desiccator and weighted.

$$\%\text{Ether extract} = \frac{\text{Weight of flask + Extract – Tare weight of flask}}{\text{Weight of sample}} \times 100$$

3.5.4 Crude Fibre

The organic residue left after sequential extraction of feed with ether can be used to determine the crude fibre, The residue is washed several times with boiling water (Until residue is neutral to litmus paper) and transferred back into the beaker. Then 200mls of pre-heated 1.25% Na_2SO_4 is added and boiled for another 30minutes. Filter under suction and wash thoroughly with hot

water and twice with ethanol. The residue is dried at 65^{o}C for about 24hrs and weighed. The residue is transferred into a crucible and placed in muffle furnace (400-600) and ash for 4hrs, then cool in desiccator and weigh.

$$\%\text{Crude fibre} = \frac{\text{Dry weight of residue before ashing} - \text{weight of residue after ashing}}{\text{Weight of sample}} \times 100$$

3.5.5 Crude protein

Crude protein is determined by measuring the nitrogen content of feed and multiplying it by a factor of 6.25. this factor is based on the fact that most protein contains 16% nitrogen. Crude protein is determined by kjeldahl method**.** The method involves: Digestion, Distillation and Titration.

Digestion: weigh about 2g of the sample into kjeldahl flask and add 25mls of concentrated sulpuric acid, 0.5g of copper sulphate, 5g of sodium sulphate and a speck of selenium tablet. Slowly heat in a fume cupboard to avoid excessive foaming, then continue to digest for 45 minutes until the digesta is clear pale green. Allow it cool fully before adding 100mL of distilled water. Rinse the digesting flask 2-3 times before adding it to the bulk.

Distillation: Distillation is done with a Markham distillation equipment. Allow around 10mls of the digest to be poured into the distillation equipment via a funnel and allowed to boil. To prevent ammonia loss, add 10mL sodium hydroxide from the measuring cylinder. Distil into 50mL of 2 percent boric acid containing methyl red indicator that has been tested.

Titration: the alkaline ammonium borate formed is titrated directly with 0.1*N* HCI. The titre value which is the volume of acid used is recorded. The volume of acid used if fitted into the formula which becomes

$$\%N = \left\{ \frac{14 \times VA \times 0.1 \times w}{1000 \times 100} \times \frac{x}{100} \right\} 100$$

VA = volume of acid used

W= weight of sample

% crude protein = %N x 6.25

Ash

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600^{0}C in muffle furnace for 4hrs. 2g of the sample is weighed into pre- heated crucible. The crucible is placed into muffle furnace at 400-600^{0}C for 4hrs or until whitish- grey ash is obtained. The crucible is then placed in the desiccator and weighed.

$$\%Ash = \frac{\text{wt of crucible + ash – wt of crucible}}{\text{wt of sample}}$$

3.6 PHYTOCHEMICAL ANALYSIS

The Aquoeus and Solvent extract of the plants were subjected to phytochemical test to determine their chemical constituents using standard methods.

3.6.1 QUALITATIVE ANALYSIS

3.6.1.1 TEST FOR TANNINS

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

3.6.1.2 TEST FOR SAPONINS

2ml of extract, 2ml of distilled water were added and shaken in a graduated cylinder for 15mins lengthwise. It resulted in the formation of 1cm layer of foam that indicated the presence of saponins .

3.6.1.3 TEST FOR ANTHRAQUINONES

To 1ml of extract few drops of 10% ammonia solution was added, appearance of pink colour precipitate indicates the presence of Anthraquinones.

3.6.1.4 TEST FOR CARBOHYDRATES TEST

Presence of carbohydrate was confirmed when 2ml of extract was treated with 1ml of molioch's reagent and few drops of concentrated sulphuric acid, which resulted in the formation of purple or reddish color.

3.6.1.5 TEST FOR CARDIAC GLYCOSIDE TEST

0.5ml of extract, 2m of glacial acetic acid, few drops of ferric chloride, 1ml of conc sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycoside.

3.6.1.6 TEST FOR NINHYDRIN TEST

2ml of extract, 0.2% ninhydrin reagent was added and heated for 5minutes. Formation of blue colour indicates presence of ninhydrin (Amino acid).

3.6.1.7 TEST FORFAT AND OIL TEST

Small quantity of the extract was pressed between two filter papers. The appearance of oil stain on the paper indicated the presence of fat and oil.

3.6.1.8 TEST FOR PHLOBOTANNIN TEST

If aqueous extract is boiled with1% aqueous hydrochloric acid and red precipitate is deposited. This indicates presence of phlobotannin.

3.6.1.9 TEST FOR TERPENOIDS TEST

5ml of aqueous extract is mixed with 2ml of $CHCL_3$ in a test tube, 3ml of conc H_2SO_4 is carefully added to the mixture to form a layer. An interface with reddish brown colouration is formed, terpenoids is present.

3.6.1.10 TEST FOR PHYTOSTEROL (LIBERMAN BURCHARD'S)

1ml of extract was dissolved in 5ml of distilled water. 2drops of conc H_2SO_4 was added slowly alongside of the test tube. Change in colour`(violet blue) indicated the presences of steroid.

3.6.1.11 TEST FOR FLAVONOID

1ml of extract was dissolve in 5ml of distilled water and few drops of hydroxide (N_a OH) solution were added. The formation of intense yellow was presence of flavonoids.

3.6.1.12 TEST FOR ALKALOID

1ml of the crude extract was dissolve in 5ml of distilled water. 2ml of (HCL) was added and heated gently. 3ml of mayer's reagent was added. Presence of turbidity of the precipitate indicates the presence of Alkaloids.

3.6.1.13 TEST FOR PHENOL (FERRIC CHLORIDE)

1ml of extract was dissolved in 5ml of distilled water and few drops of ferric chloride was added. The formation of blush black color indicates the presence of phenol.

3.6.1.14 TEST FOR GLYCOSIDES

To 2ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates presence of glycosides.

3.6.1.15 TEST FOR STEROID

To 1ml of extract equal volume of chloroform was added and a few drops of concentrated sulphuric acid added. Appearance of bluish brown ring indicates the presence of phytosteroids.

3.6.1.16 TEST FOR COUMARIN

1ml of 10% sodium hydroxide was added to 1ml of extract. Formation of yellow colour indicates the presence of coumarins.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 PROXIMATE ANALYSIS

Proximate analyses of crude extracts of selected medicinal plants namely *A. Indica*and*P.purpureum* leaves were carried out using the standard methods. The determination of the proximate constituents is necessary in assessing its nutritional levels and consequently, its microbial impact. These analyses revealed important findings and results obtained as presented in (Figures and Tables below).

The moisture content of the plants ranged from 9.20 to 7.0%, *Azadirachtaindica*has a higher moisture content.The high moisture content improves the activity of water soluble enzymes and co-enzymes essential for these plants' metabolic activities (Iheanacho, 2009). Fibre aids in the prevention of constipation, bowel issues, and piles. Dietary fibers are required for digestion and proper waste disposal, and they can decrease blood cholesterol, as well as the risk of coronary heart disease, hypertension, constipation, diabetes, colon, and breast cancer (Sodipo, 2000).The ash content of *A. indica* varies from 3.50 percent to 9.1 percent for *P. purpureum*. The presence of ash suggests that the medicinal plants are mineral-rich.The medicinal plants' nutritional benefit was shown by their increased protein content of 13.48 and 23.72.

Table 4.1 Percentage proximate composition in *Azadirachtaindica*and *Pennisetum purpureum*

Parameters	***Azadirachta indica* (%)**	***Pennisetum purpureum* (%)**
Moisture	9.20	7.0
Ether Extract	7.4	13.91
Crude Fibre	25.22	12.12
Crude Protein	13.48	23.72
Ash	3.50	9.1

PROXIMATE ANALYSIS

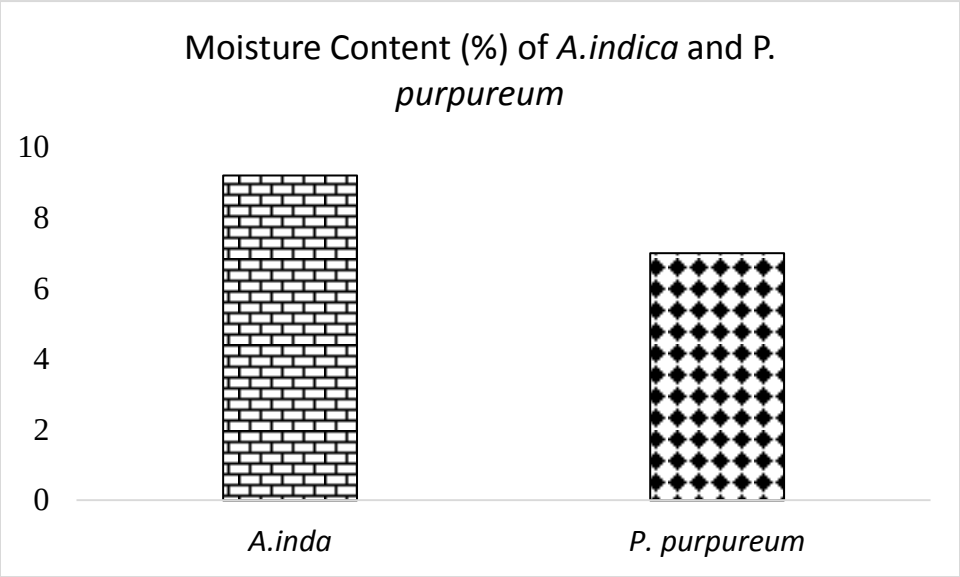

Figure4.1: Graphical Representation of Moisture Content of *Azdairachta indica* and *Pennisetum purpureum.*

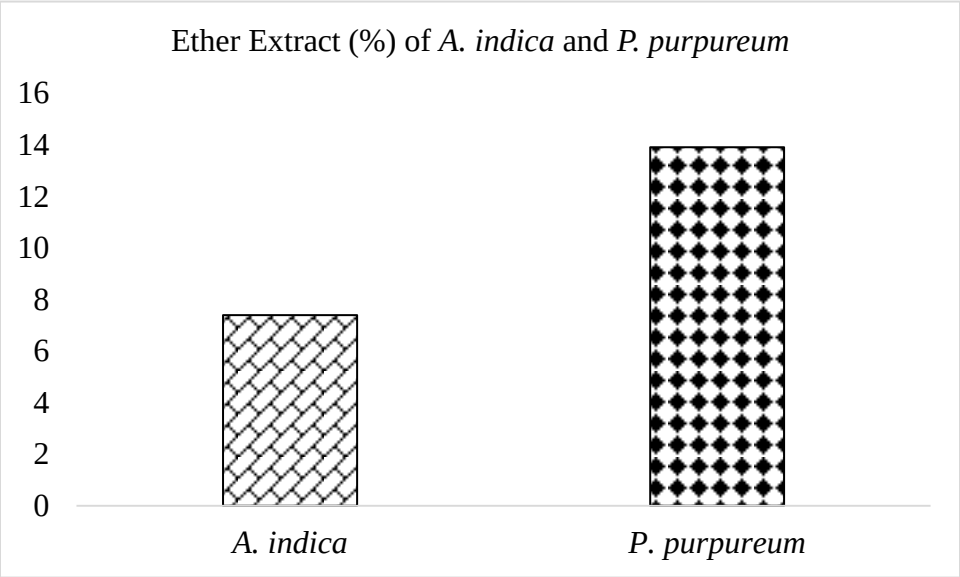

Figure 4.2: Graphical Representation of the Ether extract*Azdairachta indica* and *Pennisetum purpureum.*

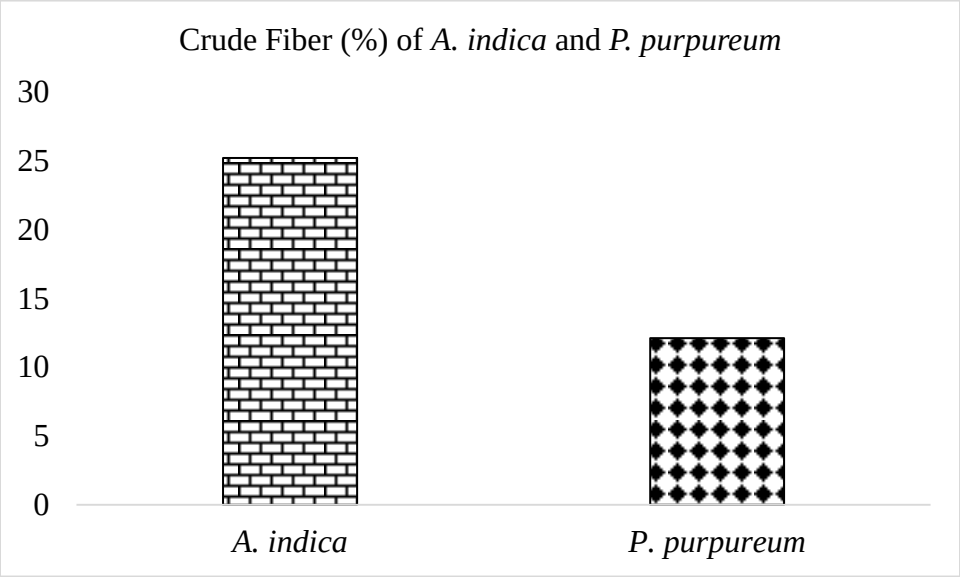

Figure 4.3: Graphical Representation of Crude Fiber content of *Azdairachta indica* and *Pennisetum purpureum.*

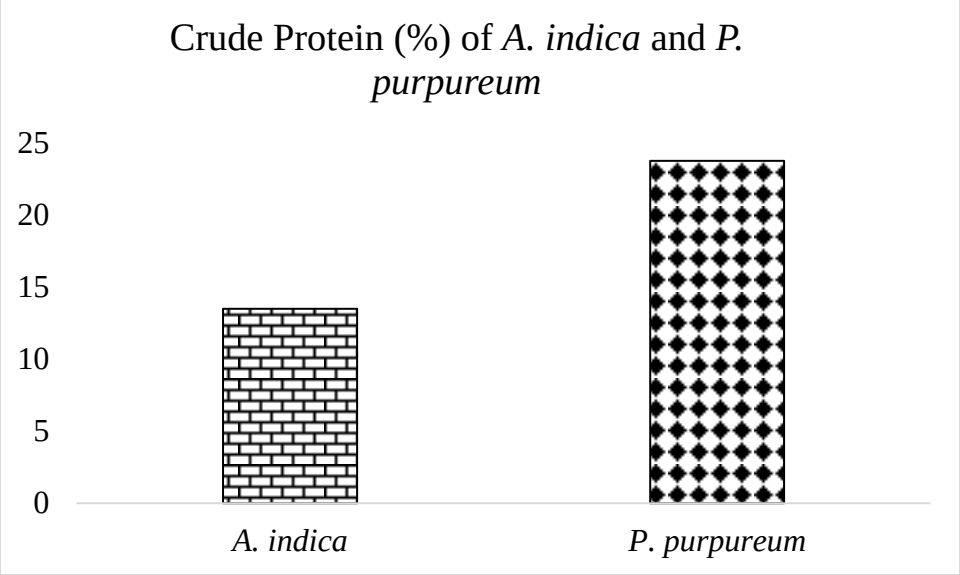

Figure 4.4: Graphical Representation of Crude Proteinof *Azadirachta indica* and *Pennisetum purpureum.*

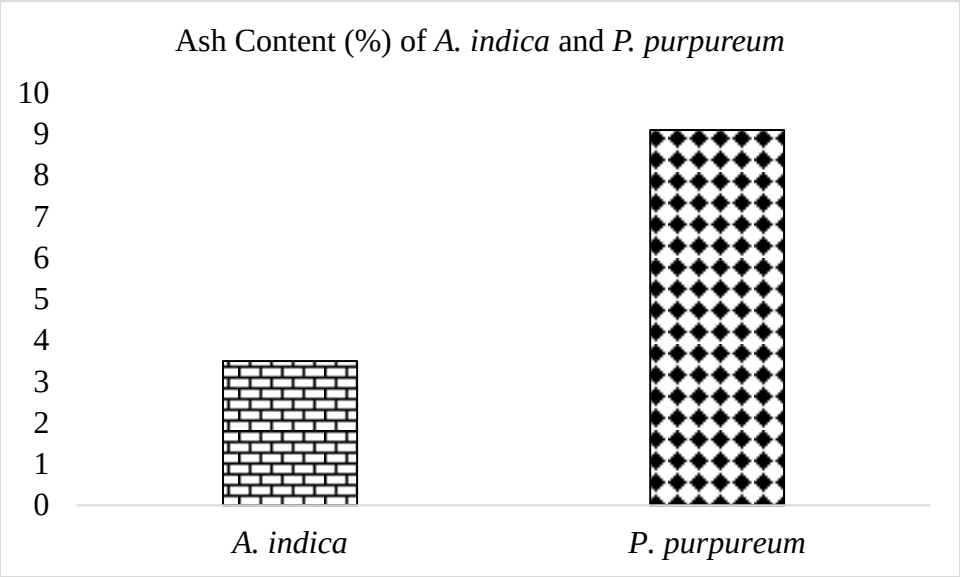

Figure 4.5 : Graphical Representation of Ash Content*Azdairachta indica* and *Pennisetum purpureum.*

4.2 QUALITATIVE DETERMINATION OF PHYTOCHREMICALS

Qualitative tests were used to assess the presence or absence of phytochemicals, and the results are reported in table 4.3 and 4.4 below.The phytochemicals Terpenoids, Phenols, Tannins, Fat and Oil, Flavonoids, Steroids, Saponins, Glycosides, Alkaloids, and Coumarin were found in the *Azadirachtaindica* studied, but Cardiac glycoside, Phlobotannis, Carbohydrate, Phytosterol and Anthraquinones were not. Protein was found in Ethanol extract but not in Aqueous extract. Flavonoids, Coumarins, Polyphenols, Saponins, and Fat and Oil were discovered in the Aqueous extract of *P. purpureum*, however Phenol, Terpenoids, Steroids, Tannis, and Cardiac glycosides were not.

Flavonoids are water-soluble compounds that play a significant role in phenolic protection. They contain antioxidant properties and can protect cells from oxidative damage and tumorigenesis (Naser et al., 2013). They have anti-inflammatory properties and have a significant impact on illnesses of the lower intestine and the heart (Okwu, 2001).This is the reasonwhy these plants are being used for skin diseases. The presence of flavonoid is effective against wide array of microorganisms. Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides (Okwu,2001).

Phenolic chemicals are one of the most common and widely distributed groups of plant metabolites (Singh, 2007). They have biological features such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis, cardiovascular protection, and endothelial function enhancement, as well as suppression of angiogenesis and cell proliferation (Han,2007). The antioxidant effects of medicinal plants that are high in phenolic compounds have been studied in several research (Brown , 1998; Krings, 2001).The historic usage of these medicinal plants in illness treatment may be due to the presence of phytochemicals.

Table 4.2 Percentage phytochemical constituent of *Azadirachtaindica*

Phytochemical	**Aqueous Extract**	**Ethanol Extract**
Flavonoids	+	+
Phenols	+	+
Glycoside	-	+
Terpenoids	+	+
Steroids	+	+
Tannins	+	+
Saponins	+	+
Anthraquinones	+	_
Coumarin	+	+
Cardiac glycoside	-	_
Fat and Oil	+	+
Protein	+	_
Phytosterol	_	_
Carbohydrate	_	_
Phlobotanin	_	_
Alkaloid	+	+

Key : Absent = -, Present = +

Table 4.3. Percentage phytochemical constituent of *Pennisetum purpureum*

Phytochemicals	**Result**
Flavonoids	+
Phenols	-
Coumarins	+
Terpenoids	-
Polyphenol	+
Steroids	-
Tannins	-
Saponins	+
Fat and oil	+
Cardiac glycoside	-

Key : Absent = -, Present = +

CHAPTER FIVE

5.0 CONCLUSION

This study was carried out to evaluate the proximate and phytochemicals to ascertain their compositions in the selected plants namely *Azadirachta indica* and *Pennisetum purpureum*. The presence of proximate constituents and phytochemicals revealed in this study shows that medical plants investigated, if consumed in sufficient amounts and doses, could significantly contribute to meeting human nutritional requirements for adequate protection/treatment against various diseases subject to favourable toxicity test. The presence of these phytochemicals in *Azadirachta indica* suggests that it includes components that enable it to provide therapeutic effects.

5.1 RECOMMENDATION

From the study, Clinical studies is recommended to determine at what level the nutrients and method of plant extraction become toxic to human and farm animals and also ascertain the side effects.

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