

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Vigna unguiculata (L.) Walp is a member of the *Vigna* (peas and beans). It is commonly called cowpea. *Unguiculata* is Latin for "with a small claw", which reflects the small stalks on the flower petals (Ernest, 2009). All cultivated cowpeas are found within the universally accepted *V. unguiculata* subspecies *unguiculata* classification, which is then commonly divided into four cultivar groups: *Unguiculata*, *Biflora*, *Sesquipedalis*, and *Textilis* (Padulosi *et al.*, 1997 and Perrino *et al.*, 1993). Some well-known common names for cultivated cowpeas include black-eye pea, southern pea, yardlong bean, catjang, and crowder pea (Timko *et al.*, 2007). The first written reference of the word 'cowpea' appeared in 1798 in the United States (Ernest, 2009). The name was most likely acquired due to their use as a fodder crop for cows. Black-eyed pea, a common name used for the *unguiculata* cultivar group, describes the presence of a distinctive black spot at the hilum of the seed. Black-eyed peas were first introduced to the southern states in the United States and some early varieties had peas squashed closely together in their pods, leading to the other common names of southern pea and crowder-pea. *Vigna unguiculata* comes first ahead of other arable food legumes in the sub-Saharan Africa. Cowpeas can either be short and bushy (as short as 20 centimetres (7.9m)) or act like a vine by climbing supports or trailing along the ground (to a height of 2 metres (6.6ft)) (Sheahan, 2012 and NRC 2006). The tap root can penetrate to the depth of 2.4 metres after eight weeks (Davis, 1991). The size and shape of the leaves varies greatly making this an important feature for classifying and distinguishing cowpea varieties (Pottorff *et al.*, 2012) The recent annual global production of cowpea approximates to 3.3 million tons; Central and West African regions being the major areas of its cultivation (CGIAR, 2014). While it is chiefly a vegetable and grain crop for human who values it as a nutritional supplement to cereals and an extender of animal proteins, it provides a very safe fodder for livestock animals. In semiarid regions it is mainly grown by subsistence farmers, who sell the fresh or dried seeds, fresh pods and leaves as vegetables and the green or dried leftover parts of the plant. Leaves and stems (haulms) can be used as fodder for livestock (Inaizumi *et al.*, 1999). Cowpea has vast utility in the food culture of both man and animal (Tarawali *et al.*, 2002; Diouf and Hilu, 2005).

According to Faris (1964), *Vigna* spp belong to the genus Phaseoleae together with *Dolichos* spp and *Phaseolus* spp. The *Vigna* spp include subspecies *V.luteola*, *V.lutea*, *V.sinensis* and *V.vexillata*. *Vignasinensis* now commonly referred to as *V. unguiculata* include both the wild and the cultivated cowpea and *V.sesquipedalis* called yardlong bean and *V.cylindrica*, the *Cajan* bean are also known as subspecies of *Vigna*. Vavilov (1951) reported that cowpea must be originated from Asia. However, Faris (1964) provided evidence largely based on the abundance of many wild species to show that the centre of origin of this crop is in Africa. In India, which is one of the centres of diversities, selection is being made for the yardlong beans (*Vigna sesquipedalis*) which are small seeded and has long pods which the large grain small pod types were being positively selected in West Africa.

Considerable progress has been made during the past years on germplasm collection, characterization, evaluation, ecogeographical studies and taxonomy of cowpea and its wild relatives. The efforts have greatly contributed towards a better understanding of species diversity ecogeographical distribution and evolution of *V. unguiculata*. The genus *Vigna* family Fabaceae, formerly Leguminosae (peas or beans) is composed of more than 200 species that are native to the warm regions of both the old world and new world. It is one of the several species of the widely cultivated *Vigna*; belonging to the kingdom Plantae, family Fabaceae called Papilionaceae and sub-family, Fabioideae and order Fabales, tribe Phaseoleae, subtribe Phaseolinae, and section Catiang (Verdcourt, 1970; Marechal *et al.*, 1978). *Vigna* is a pantropical genus with several species, whose exact number varies according to authors: 184 (Philips, 1951), 170 (Faris, 1965 between 170 and 150) (Summerfield and Roberts, 1985) 150 Verdcourt, 1970) 154 (Steele, 1976), and about 84 (of which some 50 and about 84 (of which some 50 species are indigenous to Africa (Marechal *et al.* 1978,). In their revision of the genus *Vigna* Marechal *et al.*, (1978) subdivided the genus described earlier by Verdcourt (1970) into seven subgenera. In this classification *V. unguiculata* and *V. nervosa* Markotter constitute the section Catianj, one of the six sections of the subgenus *Vigna*. Species of the section *Catianj* are characterized by spurred Stipules below the attachment point of the leaf stalks and canoe shaped keel with beak. *Vigna* is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. *Vigna unguiculata* (L.) Walp (cowpea) one of the species of the genus *vigna* is an annual dicotyledonous legume and a warm weather crop, well adapted to drier regions of the tropics like Nigeria, where other food legumes do not thrive well (Sankie *et*

al., 2012). The planting area is more than 12.5million hectares worldwide, with an annual production of more than 3 million tons (Singh *et al.*, 1997). Nigeria is the largest producer and consumer, accounting for about 45 percent of its world's production (Ndonget *al.*, 2012, Lowenberg-Deboer and Ibro, 2008) while the whole of Africa accounts for about 75% (Brissibe *et al.*, 2011).Four subspecies are recognized; of which three are cultivated (more exist, including *textilis*,*pubescens*, and*sinensis*).Cowpea is also refered to as Southern pea, blackeye, crowder pea, lubia, niebe, caupi or frijole. All of these are the species of *Vigna unguiculata* (L.)Walp, which in older references may be identified as *Vigna sinensis*.

A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia and South America as its origin. It is believed that cowpea originated from West Africa by some workers, because both wild and cultivated species abound in the region. Others believed that it originated from Southern Africa. Some literatures indicate that cowpea was introduced from Africa to the Indian subcontinent, approximately 2000 to 3500 years ago. (Faris, 1964). This was the same time as the introduction of sorghum and millet, while others stated that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia. Speculations are that the Northern part of the Republic of South Africa (former Transvaal region) was the centre of speciation of *Vigna unguiculata*, owing to the presence of most primitive wild varieties. They further hypothesized that the species moved northwards from the Transvaal to Mozambique and Tanzania, where the subspecies *pubescens* evolved. (Singh *et al.*, 1997).Cowpea is now grown throughout the tropics and subtropics and has become a part of the diet of about 110 million people. Its production has spread to East and Central Africa, India, Asia, South and Central America. The drier savanna and the sahelian regions of west and centralAfrica produces about 70% of worldwide cowpea production with, Nigeria, Niger and Brazil being the largest producers. (Singhet *al.*, 2002). Cowpea fixes atmospheric nitrogen through symbiosis with nodule bacteria (Shiringani and Shimeles, 2011).When used with cereal crops, it can help restore soil fertility (Sanginga, 2001) therefore cowpea can play an important role in the development of agriculture. Cowpea is a popular leguminous crop in Africa which is known as beans in Nigeria and 'niebe' in the Francophone countries .The largest production is in the moist and dry savannah of sub-saharan Africa (SSA) where it is intensively grown as an intercrop with other cereal crops like millet, sorghum and maize as well as rice fallows (Ishiyaku *etal.*, 2010). Though it is grown in other parts of the world, Nigeria remains the largest producer and

consumer of cowpea in the world. According to FAO data (2001-2010), Nigeria produces an average of 2.58 ± 0.31 million metric tonnes. Demand deficit is met by Niger and Burkina Faso. Henshaw (2008) classified cowpea varieties into size categories based on their 100-seed weight. Varieties with seeds 10-15g are described as small, 15.1-20g as medium-sized seeds while large seeds have 20.1-25.0g. Seeds weighing over 25g are described as very large seeds. These different varieties of cowpea vary greatly in their growth habits, seed colour, from white, cream, yellow, and red, purple, brown to black and may be smooth or wrinkled according to Arthur (2009). These seeds differ in all physical properties which include hilum colour and seed coat texture. Seed shapes vary from the typical kidney shape for beans to globose, ovoid and rhomboid shapes (Henshaw, 2008). Cowpea (*Vigna unguiculata*) is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (FAO, 2002). This is why cowpea is called 'poor man's meat' (Diouf and Hilu, 2005) because the seed protein content ranges from 23% to 32% of seed weight rich in lysine and tryptophan and a substantial amount of mineral and vitamins (folic acid and vitamin B) necessary for preventing birth defect during pregnancy stage (Neilson *et al.*, 1993). Some varieties are rich in protein up to 30% and in addition contain micronutrients such as zinc, iron which are necessary for healthy living (Bouka *et al.*, 2010). It is for this reason that societies endowed with cowpea have evolved different ways of utilizing the grain for food. Perhaps the coinage of naman talaka (poor man's meat) by the Hausas of west and central Africa points to the perception about the nutritional attributes of cowpea grains. All parts of the cowpea are used for food. The leaves, green pods, green peas and the dry grains dishes are consumed differently. These parts are nutritious providing protein, vitamins and minerals especially micronutrients. The grains are rich in Amino acids, lysine and tryptophan making it better than cereals. In many parts of West Africa, cowpea hay is also critical in the feeding of animals during the dry season (West and Francois, 1982). There is a large morphological diversity found within the crop, and the growth habit ranges from indeterminate to fairly determinate with the non vining types tending to be more determinate. Plant types are often categorized as erect, semi-erect, prostrate (trailing), or climbing. Conditions and grower preferences for each variety vary from region to region. (Singh *et al.*, (2002). (Porter *et al.*, 1974) reported that morphological variability in cowpeas (*Vigna unguiculata* (L.) Walp) abounds in the tropics. Purple-hull pea, southern pea, sowpea, yard long bean, cream pea, pea bean, asparagus bean, cartjanga are seeds of cowpea. They are often called

'black eyed pea' due to its black or brown-ringed hilum. Poor concern for available but unutilized cowpea variants may promote the losses of diversity in the species. Morphological characters of plants are commonly used in the description of varietal differences in leaves, the photosynthetic organs of the plant growth and seed yield. The flat leaf blade is specialized for catching sunlight. Leaves vary greatly from plant to plant and are useful in classification and identification. Leaf appearance in crop plants is an important process involved in canopy development. There is wide range among the plant genotypes in shape, size and number of leaves produced by plants. In cowpea, the leaf shape is important for taxonomic classification and also for distinguishing cowpea varieties. However, there isn't a central naming convention for neither cowpea leaves nor detailed descriptions of the leaf shapes; thus, many researchers name the leaf shapes differently. The two largest cowpea germplasm agencies are the International Institute of Tropical Agriculture (IITA) and the United States Department of Agriculture (USDA). IITA, which houses 14,500 cowpea accessions from 65 different countries, classifies cowpea leaf shapes into four categories, sub-globose, sub-hastate, globose and hastate/lanceolate. The hastate leaf shape was reported to be dominant to the ovate leaf shape in several studies (Oluwatosin, 2002). This may indicate that the hastate shape is ancestral to the ovate leaf shape and the preponderance of the latter in most cultivated cowpea is due to direct or indirect selection by humans over time. The flowers of cowpea are zygomorphic (bilaterally symmetrical). The perianth is made up of a standard which is usually large and purple in colour especially in most of the wild varieties. There is a prominent pollen guide which is usually yellow in colour. There are two wings which are smaller and lighter in colour than the standard and there are two joined petals making up the keel. Flower colour varies (NRC, 2006), the purple colour is dominant over the white which is likely to be more recent on the evolutionary scale. However, there are several shades of purple colour and these may be due to multiple allelic series or to the effect of a modifier. Similar explanation may be given for the inheritance of flower with yellow petals recently noticed in one of the wild form. The flower is also found to be scented and possesses well developed nectaries which are added attractions for the insect pollinator. The surfaces of the pollen grains are reticulate with raised exine (De Leonardis *et al.*, 1993). Interspecific crosses made between the two species have not been successful (Mithen, 1987; Ng and Apeji, 1988; Ng, 1995). On the basis of a study on isoenzyme variation in generation *Phaseolus* and *Vigna*. Jaaska and Jaaska, (1988) proposed to raise the section *Catinj* to the rank of subgenus. Some varieties secrete a large

amount of nectar especially from the inflorescence of abscised flowers. Bees, which had been reported as the major insect pollinators (Leleji, 1974), are usually found in large numbers on cowpea fields. The stickiness of the pollen grains and the reticulate design on its exine are peculiar to insect-pollinated plants. Another distinguishing feature of cowpeas is the long 20-50 penduncles which hold the flowers and seed pods. One penduncle can support four or more seed pods. Wild cowpea are very small while cultivated varieties can have between 10 and 110 centimetres (3.9 and 43.3 in) (Davies *et al.*, 1991). Pods can have 6-13 seeds that are usually kidney shaped, although the seeds become more spherical the more restricted they are within the pod (Sheahan, 2012 and Davies *et al.*, 1991). Their texture and colour is very diverse. They can be smooth or rough coat and speckled, mottled or blotchy. Colours include white, cream, green, red, brown and black or various combinations (Davies *et al.*, 1991).

Some of the varieties as reported by the IITA include; TVX 3236 (Danknarda), IT82 E-60 (Ezorowo), Ife Brown L-25, Vita 4, ER-7, CA-I, II, III, IV (Henshaw, 2008). Others are Texas Cream 40, IFE BPC, Kanannado, Moola, L-80, and IT86D-1010.

Cowpeas have also been grouped into the following market classes based on seed type and colour: **Black eye and purple eye**—the immature pods shell easily because the hull (pod wall) is pliable and the seeds come out of the pod clean and free. The shelled peas are attractive, mild flavoured and suitable for processing. The white hilum is surrounded by black, pink, or light-red colour.

Brown eye—Pods vary in colour from green to lavender and have a wide range of lengths. The immature seeds, when cooked, are a medium to dark brown colour, very tender, and have a delicate flavour.

Crowder—Seeds are closely crowded in the pods and tend to be globular in shape.

Cream—Seeds of these types are generally cream coloured and have no noticeable "eye" (the hilum is inconspicuous).

Clay—these are generally older varieties that are medium to dark brown in colour and kidney shaped. They are no longer commonly grown.

White acre—the peas are kidney shaped with a blunt end. This type is a semi-crowder, generally tan in colour and somewhat small. Pods are quite stiff. (Marsh *et al.*, 1991).

Scientifically cowpea (*Vigna unguiculata*) is classified as follows:

Kingdom: Plantae
 (Unranked): Angiosperms
 (Unranked): Eudicots
 (Unranked): Rosids
 Order: Fabales
 Family: Fabaceae

Genus: *Vigna*

Species: *Vigna unguiculata*

Binomial name: *Vigna unguiculata*(L.) Walp.

Some of the common cultivars of cowpea (*Vigna unguiculata*) are;

Group Common name

Unguiculata Crowder –pea, Southern pea, Black eyed pea

Biflora Catjang, cowpea.

Sesquipedails Yardlong bean, asparagusbean Chinese long bean.

Textilis

Among the various cultivars of Cowpea. (*V. unguiculata*) cultivated in Nigeria include; large Kano white (iron beans), small Kano white (Potiskum beans), Ife Brown beans, Sokoto guzo, Oloka, Kafanji and Crowderpea (black beans).

1.2 Statement of the Problem

Various morphological variations are found in the genus *Vigna* and have resulted in the disorder in the identification and characterization of the species and this has led to inadequate information on the systematic studies of the plant.

1.3 Justification of the Study

This study could be justified from the economic importance of cowpea as food and drug, and the classification of *Vigna unguiculata* which has been an issue in plant taxonomy. This study therefore aimed at exploiting the various taxonomic parameters that will produce relevant and comprehensive information in the proper classification of the species thus reinforcing the accuracy of cowpea classification to advance the taxonomy of cowpea.

1.4 Significance of the Study

The various uses of cowpea, *Vigna unguiculata* (L.) Walp, and the potential they hold in agriculture as leguminous plants, the gross inadequacy of basic systematic information on the species and their ethnobotanical characteristics, were among the bases for the study of these Varieties namely; 'Kafanji', 'Ifebrown', 'Potiskum', Iron beans, 'Sokoto guzo', Crowderpea and 'Oloka'.

1.5 Aim and Specific Objectives of the Study

The aim of this research was to use the morphological, anatomical, cytological, phytochemical and proximate characters of seven varieties of *Vigna unguiculata* (L.) Walp Available in Anambra State to develop an acceptable taxonomic tool for the identification and characterization of seven varieties of *Vigna unguiculata* (L.) Walp.

The specific objectives include;

1. To determine various morphological parameters that will aid in the identification of the different varieties of *Vigna unguiculata* (L.) Walp.
2. To assess the anatomical features of different organs of the plants that will provide relevant information in the classification of these varieties.

3. To examine the cytological information on the mitotic studies of these varieties that will aid in solving taxonomic problems in the genus *Vigna*.
4. To evaluate the phytochemical and proximate constituents of the seven varieties for optimum utilization as food and in Ethnomedicine.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of Cowpea

The genus *Vigna* family Fabaceae is composed of more than 200 species that are native to the warm regions of both the old world and new world. *Vigna* is formerly closely related to *Phaseolus*, which is composed of more than 20 species also native to the warm or tropical regions of the New World. A number of species previously placed in *Phaseolus* are now placed in *Vigna*. *Vigna unguiculata* is a member of the *vigna* (peas or beans) genus and one of the widely cultivated *Vigna unguiculata*. It comes first ahead of other arable food legumes in the sub-Saharan Africa. All cultivated cowpeas are found within the universally accepted *Vigna unguiculata* subspecies *unguiculata* classification, which is then commonly divided into four cultivar groups; *unguiculata*, *biflora*, *sesquipedalis*, and *textilis* (Singh *et al.*, 1997). Some of the better known common names for cultivated cowpeas include blackeye pea, southern pea, yardlong beans, catjang and Crowder Pea. The classification of the wild relatives within *Vigna unguiculata* is more complicated, with over 20 different names having been used and between 3 and 10 subgroups described. (Singh, 1999). The original subgroups of *stenophylla*, *dekindtiana* and *tenuis* appear to be common in all taxonomic treatments, with the earlier described variations *pubescens* and *protractor* being raised to subspecies level by a 1993 characterization. (Singh *et al.*, 1997). Most taxonomists agree that cowpeas belong to the botanical species *Vigna unguiculata* (L.) Walp. However, classification and nomenclature of taxa at the intraspecific level are still debated. Verdcourt (1970), subdivided the species into 3 subspecies, Cowpea, (subspecies *unguiculata*, *catjang* (subspecies *catjang*), and yardlong bean (subspecies *sesquipedalis*). However, Marechal *et al.*, (1978), reclassified the subspecies *unguiculata*, *catjang*, and *sesquipedalis* as cultigroups *Unguiculata*, *Biflora* and *Sesquipedalis* respectively, and lumped these cultigroups under *Vigna unguiculata* subsp. *unguiculata*. Most cowpea breeders seem to have adopted (Marechal *et al.*, 1978) cultigroup scheme for classification of cultivated *Vigna unguiculata* taxa. The first written reference using cowpea appeared in 1798 in the United States. The name was most likely acquired due to their use as a fodder crop for cows. (Ehlers *et al.*, 1997). The common name of black-eyed pea, used for the *unguiculata* cultivar group, describes the presence of a distinctive black spot at the hilum of the seed. Black-eyed peas were first introduced to the southern states

in the United States and some early varieties had peas squashed closely together in their pods, leading to the other common names of southern pea and crowderpea. *Sesquipedalis* in Latin means "foot and a half long", and this subspecies which arrived in the United States via Asia is characterised by unusually long pods, leading to the common names of yardlong bean, asparagus bean and Chinese long-bean.(Ensminger *et al.*, 1993). In West Africa, the plant is named niebe, wake or ewa. *Vigna unguiculata* (L.)Walp is traditionally grown in most parts of the savanna and forest belts of southern Nigeria. All cultivated cowpeas are grouped under *Vigna unguiculata* which is subdivided into four cultigroups namely *Biflora*, *Sesquipedalis* and *Textilis*(Westphal, 1974, Marechal *et al.*, 1978 and Ng and Marechal 1985). There has been no major contention on this classification since its adoption over 10 yearsago. The classification and nomenclature of the wild taxa within *V.unguiculata*however is complicated and could sometimes be confusing.More than 20 epithet names have been used in the past to designate wild taxa within *V.unguiculata* species complex. An extensive work on characterization of over 400 wild *V.unguiculata* accession was conducted at IITA (Ng and Padulosi1991;Padulosi,1993). This work coupled with surveys of live materials in the field and specimens in major herbaria in Europe and Africa, as well as cytological studies has led to the description of new taxa and a change of nomenclature of some species (Padulosi, 1993, Ng, 1995).

Linnaeus described *Dlichos unguiculata* L. later renamed *Vigna unguiculata* (L.) Walp in 1953.Between 1753 and 1845, more than 20 binomials were described from cultivated *V.unguiculata* specimens. These binomials were considered as conspecific and ranked at infraspecific levels during the second half of XIXth century. Now, cultivated forms are pooled in *V.unguiculata* SSP. *unguiculata* Var *.unguiculata* and wild annual forms in SSP. *unguiculata* Var.*spontanea* (Schweinf) Pasquet.Wild perennial forms are ascribed to ten subspecies (Pasquet, 1997, Pasquet.1993a, Pasquet, 1993b). Wild annuals are easily crossed with cultivated cowpeas (Ng, and N.O, 1995).Two opposing approaches have suggested for the classification of cultivated *V.unguiculata*. One was issued from Piper (1912) study of US cultivated forms.Three groups known since Linnean works (1763), were separated according to seed and or pod size.Later these groups were ranked at all the possible taxonomic levels; and Westphal (1974) finally used the cultigroup rank(group of cultivars) now widely accepted (Ng and N.Q, 1995). This classification is not always convenient. Cvgr *Biflora* is not easily separated from Cv *unguiculata* as pod orientation is tightly dependent on pod and seed weigth (Piper, 1912) Steele

(1974) recognized that Piper's system and Vercourt's (1970) classification were not fitted with African cultivated forms, proposed a fairly complex key, including seed testa texture and photosensitivity, later Steele admitted Piper's trilogy while highlighting opposition between photosensitive and photoindependent groups (Steele, 1980). Another concept, strangely not used is that of Chevalier (Chevalier *et al.*, 1944). Chevalier's classification considered the number of seeds per pod which was an important difference with respect to Piper's classification. Chevalier divided West African cowpeas into two subspecies according to seed number per pod.

The subspecies with low seed number per pod was divided into four groups. Wild cultivated with wrinkled testa seeds (var. *Melanophthalmus*) (DC) A. Chev) and cultivated with long inflorescence peduncles. Unfortunately, Chevalier's classification included Asian cowpeas (Piper classification in extensor) and West African cowpeas without discussing the connection between the two subspecies in West Africa.

Adanson (1763), a French botanist, was the first to put forward a plan for assigning numerical values to the similarity between organisms. Many characters as possible were used for the classification, and such classifications were recognized as Adansonian classifications. In recent years Adansonian principles have developed several new methods in taxonomy particularly after 1960, and these methods are all included under numerical taxonomy. Sokal and Sneath (1963) developed the concept and later elaborated them. They divided the field into phenetics in which classification are formed based on the pattern of overall similarities and cladists in which classification are based on the patterns of the estimated evolutionary history of the taxa. Numerical Taxonomy proposes to base classifications entirely on resemblance, defining natural classification as those yielding taxa whose members are in the same sense more similar to one another than they are to members of other taxa, It follows from the concept of naturalness which is based on the ideas of J.S.L. Gilmour, a Botanist at the University of Cambridge that a natural taxon will be most predictive than other characters in various grouping of the classification therefore should be more successful than if the taxon were based on few characters. Furthermore, it is likely that a classification based on a great variety of characters will be of general utility to biology as a whole whereas a classification on only a few characters is less likely to be generally useful except for the special purposes relevant to the chosen characters. Overall phenetic similarity is based on all available characters without any differential weighting of some characters over others. A substantial part of the controversy about numerical taxonomy has

centered on this point above. Although intended as an objective classification method in practice, the choice and implicit weighting of characters is of course influenced by available data and research interests of the investigator. Conventional taxonomists usually employ only a few characters in classification. Henshaw (2008) classified cowpea varieties into size categories based on their 100-seed weight. Varieties with seeds 10-15g are described as small, 15.1-20g as medium-sized seeds while large seeds have 20.1-25.0g. Seeds weighing over 25g are described as very large seeds. There are different varieties of cowpea and they vary greatly in their growth habits. Seed colour varies from white, cream, yellow, and red, purple, brown to black and may be smooth or wrinkled according to Arthur (2009). Some of the varieties as reported by the IITA include; TVX 3236 (Danknarda), IT82 E- 60 (Ezorowo), Ife Brown L-25, Vita 4, ER-7, CA-I, II, III, IV. Others are Texas Cream 40, IFE BPC, Kanannado, Moola, L-80 and IT86D-1010. These seeds differ in all physical properties which include hilum colour and seed coat texture. Seed shape varies from the typical kidney shape for beans to globose, ovoid and rhomboid shapes (Henshaw, 2008). Smartt and Hymowitz (1985) noted that “the cowpea can be regarded very much as the Old World counterpart of the *Phaseolus* beans. Its products, seeds and fruits, can be utilized in precisely similar ways.” These researchers also noted that the similarity between cowpea and common bean probably explains the ready acceptance of cowpea in the New World and common bean in the Old World. The cowpea is one of the mandated crops addressed by the International Institute of Tropical Agriculture, Ibadan, Nigeria.

A data that show differences from species to species are of taxonomic importance and thus constitute part of the information or evidence which may be used by taxonomists. This information can be obtained from diverse sources which will help in obtaining the best sort of natural classification. Taxonomy is a science without data of its own and therefore it utilizes the investigations of other branches of biology. Taxonomists do not often use this information from anatomical, chemical, cytological and other investigations because they are mostly from comparative studies and not from the systematic point of view. Taxonomist because of this often gather these informations, they bearing in mind the enormous number of species which they have to work on and their various characters. Taxonomists have because of necessity been very selective of the characters which they have chosen to study. Characters selected are those which are most easily observed and those which show promise as being reliable and discriminating in the taxon delimitation. This is not undesirable because it is most convenient if taxa are

delimited by obvious features rather than by cryptic (hidden) ones, but it is one of the major causes of the considerable degree of subjectivity in taxonomy.

In these modern times, many less obvious sorts of characters have been utilized by taxonomists. This is because previously they do not have the time or see the need to investigate them and also in many cases necessary apparatus are not there and the expertise too are lacking, then the necessity becomes available at least to taxonomist. These new techniques uncover more reliable or fundamental characters than old ones. Features such as chromosome number and morphology, pollen structure ability or inability to interbreed, stomata architecture, occurrence of secondary metabolites can now be studied as sources of taxonomic information but in the real sense none of these can be compared with gross morphology. Experimental studies of plants, both in the field and in the laboratory, have yielded information which appears to various investigators to bear directly on the nature and delimitation of taxonomic units. The classification of organisms on the basis of these experimental facts has been termed Experimental Taxonomy. Experimental taxonomy fully appreciates the value of morphological differences. In fact the cytologist has disclosed a fresh field for such investigation but it also seeks to show the causes which under these differences, are to ascertain their physiological, ecological, or genetical nature.

2.2 The use of morphology in plant taxonomy

Plant morphology or phytomorphology is the study of the physical form and external structure of plants. It is useful in the visual identification of plants. It represents a study of the development, form and structure of plants, and by implication, an attempt to interpret these on the basis of similarity of plan and origin. Four areas are necessary in plant morphology; these areas are comparative which compares different plants of the same or similar species.

Morphological characters are features of external forms or appearance. They currently provide the characters used for hypothesizing phylogenetic relationships. These features have been used for a longer time than the anatomical evidence in the beginning of plant systematic. Morphological characters are easily observed and obtainable and of practical use in keys and description. Plants are classified based on external morphological structures such as flowers, leaves, stems and fruits. Morphological data of plants are easily observable and obtainable and thus are used frequently in plant identification (Radford and Caddell, 1986). The external morphological evidence provides the basic language for plant characterization, identification,

classification and relationships. (Radford and Caddell 1986; Sharma, 1993) noted morphological characters as vegetative, phenological floral characters, seeds and fruit morphology thus morphological features of plants are those external diagnostic features of plants (Philipson, 1974). Some of these structures are not always available on plants because they are seasonal in production. The use of epidermal studies is one of such means of micro morphological classification. From available literatures, the use of leaf epidermal features in systematic botany is now popular just like the use of other markers like DNA sequence and chemical compositions (Edeoga and Ikem, 2001; Mbagwu and Edeoga, 2006). Epidermal characters are attributes of potential taxonomic significance both in genus and species level found on the leaves of plants. The morphology of stomata and surrounding epidermal cells of the leaf have long been regarded as useful tools (Cutler, 1984; Stace, 1984). Payne (1970), Dicher (1974) and Wilinon (1979) were among the early workers that observed the tendency of the cells surrounding the general cells of the stomata to form patterns which were constant within taxa and even families and proposed different terminologies for the description of these cells in recent times. Edeoga, (1991); Edeoga and Osakwe, 1996; Mbagwu and Edeoga, (2006) reaffirmed that epidermal and cuticular traits of plants could serve as vital in the systematic of the present day angiosperms. Shapes of the epidermal cells, subsidiary type and arrangement of stomata, size and shape of trichomes and number of vascular bundles are all vital in systematic botany (Nwachukwu and Mbagwu, 2006). The use of vegetative and floral morphology in the systematic grouping, characterization and classification of different taxa is no more a new event. This is because this aspect of identifying plants has great value and enables correlation of characters to be easily determined. Leaf characters such as arrangement, type, form, duration and venation are widely used in both classification and identification of plants (Sharma, 1993). In using leaf characters in *inulmus* and *Betula*, the species are delimited only on the bases of leaf characters (Singh, 2004) described that fruit characteristics were widely in identification. He further indicated that fruit characteristics are widely used in delimitation of species of the *Valerianella*. He also noted that seed characters were valueable in the identification of features in the genus *Vernonia* and in the construction of diagnostic keys in distinguishing species. Underground parts such as roots, tubers are also of taxonomic value in plants. Sharma, 1993 and Pandey, 2000 used morphological features in recognition of two solanaceae subfamilies of Solanoideae and Cestroideae. Edeoga *et al.*, (2000) used vegetative and floral characteristics to classify some species of *Dioscorea*. In a

similar way, Nwachukwu *et al.*, (2001) used morphological characters in the characterization of *Maesobotrya barteri var. bateri*. Furthermore Mbagwu *et al.*, (2006) did similar work in eight *Vigna* species.

There are large morphological diversity found within *Vigna unguiculata*, and the growth conditions and grower preferences for each variety vary from region to region. (Singh, *etal.*, 1997). Morphological characters of plants are commonly used in the description of varietal differences in leaves, the photosynthetic organs of the plant growth and seed yield. The flat leaf blade is specialized for catching sunlight. The leaves are usually alternate, pinnately compound or trifoliolate (King, 1963). Leaves vary greatly from plant to plant and are useful in classification and identification. Leaf appearance in crop plants is an important process involved in canopy development (Singh, *et al.*, 1997). There is wide range among the plant genotypes in shape, size and number of leaves produced by plants. In cowpea, the leaf shape is important for taxonomic classification and also for distinguishing cowpea varieties.

However, there isn't a central naming convention for cowpea leaves nor detailed descriptions of the leaf shapes, thus, many researchers name the leaf shapes differently. The two largest cowpea /germplasm agencies are the International Institute of Tropical Agriculture (IITA) and the United States Department of Agriculture (USDA). IITA, which houses 14,500 cowpea accessions from 65 different countries, classifies cowpea leaf shapes into four categories, sub-globose, sub-hastate, globose and hastate/lanceolate. The hastate leaf shape was reported to be dominant to the ovate leaf shape in several studies (Oluwatosin, 2002). This may indicate that the hastate shape is ancestral to the ovate leaf shape and the preponderance of the latter in most cultivated cowpea is due to direct or indirect selection by humans over time. Sawada has developed a leaf shape index (aspect ratio) and this has shown that this index can describe variations in soybeans (*Glycine max. L... marr*) leaflet shape. It is necessary to correctly evaluate the intra and interspecific variations for the efficient collection and preservation of genetic resources and leaf shape is one of the important characteristic to be evaluated. (Porter, *etal.*, 1974) reported that morphological variability in cowpeas (*Vigna unguiculata* (L.) Walp) abounds in the tropics. The loss of genetic diversity, in part is due to the conventional breeding programs associated with modern agricultural practices, and thus has been dramatic for many cultivated species (Wikes, 1983). As better yielding crop varieties are adopted by farmers and they shift to other crops which give better returns, cowpea landraces and diversity may be lost. Consequently, the narrow genetic

base of the elite germplasm has increased the potential of vulnerability to biotic and abiotic stress. Knowledge, access, and use of the available diversity in domesticated and wild relatives are essential in broadening the genetic base of cultivars to sustain improvement (Singh, 2001). Genetic diversity is the key to improvement and development of effective conservation strategies (Hodgkin, 1997). Traditionally, diversity is estimated by measuring variation in phenotypic or qualitative traits such as flower colour, growth habit, or quantitative agronomic traits such as yield potential and stress tolerance (Kameswara, 2004). Diversity has been used as a powerful tool in the classification of cultivars and also to study taxonomic status.

2.3 The use of anatomy in plant taxonomy

Anatomical characters are very useful in the determination of relationship in orders and genera and their features have played an increasingly important role in phylogenetic relationships (Sharma, 1993). Anatomical studies of the subfamily Papilionoideae have provided significant support for the taxonomic classification of the group (Metcalf & Chalk 1950; Krishnamurthy & Kannabiram 1970; Kannabiram & Krishnamurthy 1974; Kothari & Shah 1975; Lackey 1978; Crow *et al.* 1997; Teixeira & Gabrielli 2006; Fortuna-Perez *et al.* 2012). Some of these studies (e.g., Kothari & Shah 1975) have focused on the morphological diversity of specific characters, such as the types of stomata and trichomes in the tribe Hedysareae, and these have proven to be quite useful in generic grouping, despite their heterogeneity. In addition, studies of leaf anatomy have made significant contributions to the systematic classification of the Leguminosae. In the case of the Phaseoleae, detailed information concerning the leaflet anatomy within the tribe not only confirmed and expanded previous knowledge, but also highlighted the usefulness of the data in taxonomic classification (Lackey 1978). Despite the importance of anatomical detail in determining the taxonomy of some plant groups, the anatomical characteristics of the Leguminosae have not been fully explored. Among the few anatomical studies that are available for this genus, some were restricted to the leaf epidermis (Krishnamurthy & Kannabiram 1970; Kannabiram & Krishnamurthy 1974; Gill *et al.*, 1982), whereas others focused on the anatomy of the cell wall of the fruits (Roux *et al.*, 2011). Leaf anatomy provides various characters of taxonomic importance. Foliar anatomy has been used widely in several taxonomically different groups such as Euphorbiaceae, Cyperaceae and Poaceae of Angiosperms and Coniferae of Gymnosperms. Stace (1980) discovered that trichome anatomy is of immense significance in

classification at levels from the circumscription of the family down to the separation of species and even varieties. In particular it has led to an improved tribal classification within the largest genus *Combretum*. In Dioscoreaceae, certain anatomical features were used in the characterization of *Dioscorea alata* L. and *Dioscorea smilacifolia* L. (Edeoga, 2002). In Costaceae differences in features of vegetative anatomy suggested a separate specific status for *Costus afer* and *Costus lucanusianus* as opposed to the conspecific treatment given to them by previous researchers (Edeoga and Okoli, 1997). In Leguminosae-Caesalpinoideae, the unicellular and multicellular trichomes are described in certain species of *Senna tournex* Mill and *Senna hirsuta* was diagnostic in the acquisition of these two types of trichomes (Edeoga and Osakwe, 1996). In anatomical study of the transverse section of the root of *Capsicum* species homodominant anatomical features was reported which strengthens the affinity relationship in the genus *Capsicum* (Aziagba *et al.*, 2014). (Ezeabara *et al.*, 2013) also recorded affinity relationship in the study of stem anatomy of *Citrus* species. Type, size, shape, stellate patterns, vascular bundles, rays, parenchyma, epidermal and phloem cells are some of the basic anatomical characters of well established taxonomic value. (Sharma, 1993; Pandley, 2007). Other studies of interest relating to anatomy of different angiospermous groups are found in Curcubitaceae (Okoli, 1987), Dicotyledons as a whole (Metcalfe and Chalk, 1950) Leguminosae-Papilionoideae (Mbagwu and Edeoga, 2006). Leaf anatomy provides various characters of taxonomic importance (Sharma, 1993). Among the many taxonomically important features of stomata; arrangement of the surrounding epidermal cells (termed subsidiary cells if they are distinct from the normal epidermal cells) is the most valuable. In Acanthaceae, the stomata are diacytic, whereas in closely related Scrophulariaceae they are anomocytic; within the family Combretaceae, the stomata are paracytic in the subfamily Strephonematoideae and anomocytic in the subfamily Combretoideae (Stace, 1980). Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for elucidation of phylogenetic relationships. It was Bureau who for the first time used anatomical characters in plants taxa of various levels within the family Bignoniaceae. However anatomical data have been used extensively as a taxonomic tool only after the nineteenth century. Anatomical data has not only been useful at the higher levels but in certain instances have been successfully employed even at the specific level. Auguste Mathew is one of the pioneer taxonomists, who used features of wood anatomy in the description of forest plants in floras

florestiere. Later another Taxonomist Sloeder discussed the systematic value of anatomical structures in dicotyledons in his Classic book *Systematische Anatomie der Dicotyledonen* the English translation.

2.4 The use of cytology in taxonomy

Cytology is the study of cells. A cell is the basic unit of structure and function in all living organisms. It is a unit of protoplasmic matter which may or may not contain nucleus (Okoli, 1992). Chromosomes are strands or string-like structures found within the nucleus. It has been shown that the behaviour of the chromosomes during meiosis parallels the behaviour of Mendelian factors on genes during reproduction. It has therefore been proved that the genes which are the hereditary factors are located in the chromosomes. Variations occur in the structure, number and behaviour of chromosomes. Other changes frequently encountered include differences in staining with dyes, euchromatin-heterochromatin patterns and other morphological markers. Genetic principles especially aspects of cytogenetics have been very useful in taxonomy. The frequently cytogenetic data include the number, structure and behavior of chromosomes. Chromosome is the significant structure required in cytology. The number of chromosome is as important as the number of carpels, and the morphology or type of chromosome is to be considered in the same way as the shape of leaves. On the other hand they constitute a special type of information for chromosomal number and homology which largely determine pairing behavior at meiosis, which in part governs the level of fertility of hybrids and hence the breeding behavior and pattern of variation of populations (Stace, 1980).

The fact that chromosomes are the carriers of genetic information provided an impetus for their studies since the establishment of chromosomal theory of inheritance in the second decade of this century. The steady increased amount of information on chromosome and the development of cytogenetics concepts and methods demonstrated the importance of chromosomes in several fields of plant biology. Chromosome information also has been used in the study of floras particularly those regarding to chromosome number because it is the easiest to assess. Chromosomal features are being regarded as decision making characters in the study of phylogenetic affinities and evolutionary development, and as indications of appropriate classifications of several plant groups (Jones, 1978). Chromosome numbers indicate the occurrence of polyploidy and reflect differences in the basic chromosome numbers among plants

which may be reflected in their treatment in floras. Chromosome numbers are only known for 15-20% of angiosperm species (Moore, 1981). Other karyotype features, such as chromosome size, position of the centromeres and the presence of satellites in chromosomes of the karyotype are recorded in less than 1% of angiosperms. World chromosome numbers are recorded for about 36% angiosperm excluding monocotyledons (Moore, 1981). A diploid chromosome number $2n=28$ was recorded in *Asphodelus fistulosus* family Liliaceae with a small variation among the chromosomes. The number of chromosomes recorded resembles that of *Asphodelus fistulosus* from Egypt (Badr and Hamound, 1987) and Balearic Islands. *Astragalus eremophilus* species family leguminosae has $2n=16$ as the chromosome number. This number was also reported in several species of *Astragalus* from different parts of the world (Dahlgreen *et al.*, 1971). At Moor Plantation, Ibadan, some mutation work was initiated, firstly to provide a wider scope of genetic variation to supplement the existing breeding programme; secondly, to provide information on the mutation process in the crop, since none virtually exists. By definition (Rieger, *et al.*, 1968), chromosome mutation refers to structural changes which involve loss, gain or relocation of chromosome segments. Many such breaks in chromosome or chromatid arms rejoin in their original order and cause no visible structural changes in the chromosome. Often, the broken segments do not rejoin due to "stabilization" of breakage surfaces. Such breaks remain and produce structural changes in the chromosomes which can be detected cytologically. Magri-Allegra and Zannone (1965), using mitotic root tip investigations, observed the incidence of bridges, laggards, centric and acentric rings in Vetches treated with different mutagenic agents. Sjodin (1968) made a comprehensive survey of cytological changes in *Vicia faba* after treatment with ionizing radiation and chemical mutagens. Chromosome breakages and translocations were commonly observed, and the rates of occurrence varied with treatment agent and dose. These were confirmed in a similar study in *Vicia faba* reported by Savage (1968). Similar examples have been recorded on other crops by other workers especially (Bora, *et al.*, 1961) on *Arachis hypogea* and *Plantago ovata*. The diploid chromosome reported in the study of *Vigna unguiculata* by Olatunde (1971) recorded the diploid number of chromosome as $2n=22$ at the prophase stage. Other studies on diploid chromosome numbers by (Federov, 1969) recorded the following number of chromosomes for these species; *Erodium cicutarium* family Geraniaceae has $2n=18$. *Iris rostrata* family Iridaceae $2n=14$, *Plantago arabica* family Plantaginaceae $2n=12$, *Plantago cylindricalis* family Plantaginaceae $2n=10$, *Schouwia thebaica*

$2n=24$; these species were studied in the flora of Madinah region in Saudi Arabia (Ferdusov, 1969).

On the genomic level, Plant diversity correlation with a high degree of variation in overall genome sizes, ploidy level and chromosome number (Kellogg *et al.*, 2004) was reported. Much of this genomic variation is due to the action of transportable elements (Bennett and Leitch, 2003) leading to the potentially more functional permitting interspecific hybridization, polidization and genome change through meiotic and mitotic mechanism. Numerous types of chromosomal adaptation and ploidy alterations result from aberrations in the ubiquitous meiotic and mitotic processes, including whole genome duplications and chromosome rearrangements. Increasingly, these processes are found to provide underlying mechanism for plant speciation, particularly in response to environmental change (Leitch *et al.*, 2008).

2.5 Importance of phytochemical study in plant taxonomy

Plants are almost an exclusive source of drugs for the majority of the world population and man and animals depend on the plants for their very existence. This is because the emerging world wide interest in adopting and studying traditional systems and exploiting their potentials are based on different health systems. Thus in this regard one of those heritages are species belonging to *Vigna* genus, fabaceae family. This fabaceae is cultivated and used as nutraceutical in all over the world. The medicinal value of plants lies in the compound. In the traditional system of medicine, this genus is mainly used in the treatment of liver disorders, ulcers, to decrease the weight and also used in hormonal balancer (Anonymous, 1956). The nutritional, and pharmacology of plants are possible through chemical taxonomy. This findings will also assist in the systematic study of plant families.

Chemical taxonomy is the method of investigating the distribution of chemical compound in plants, thus needed for obtaining the real chemical constituents which usually differ much in different organs and parts of plants. Methods of chemical taxonomy are simple in principle and are based in the investigation of the distribution of chemical compounds or groups of biosystematically related plants. Sharma (1993) enumerated some of the major classes of the chemical evidence as flavonoids, alkaloids, amino acids, fatty acids, terpenoids, polysaccharides and aromatic compounds. Singh (2004) listed phenolics, glucosinolites and terpenes among others as chemical characters. The chemotaxonomic studies include the investigations of the

pattern of distributions of the compounds existing in plants, and in all the individual parts, such as leaves, stem, roots, barks and wood. These chemical constituents usually differ much in different organs and its investigations will help to obtain the real evidence for the relationship of plants (Sofowora, 1993). Chemotaxonomy is used in all groups of plants. From early 1960 onwards, the phytochemical characters have been used in taxonomy (Waterman, 1998). Recently the development of new and powerful analytical techniques contributed much in the field of Phytochemistry. Various studies confirm that phytochemical characters correlate quite well with other plant characters. Chemotaxonomy is a major source of new characters and information. The chemical characters are more important when they show a high degree of correlation with other features. The plants classified by taste, colour, smell etc. were the practice of ancient people. Modern methods of chemical identification of plant products like chromatography and spectroscopy, gained important status in phytochemistry. The taxonomical studies in relation to chemistry involve the study of the distribution of chemical compounds in related families of plants. The compounds exist in individual parts of plants such as bark, wood, leaves, roots etc. With the development of improved techniques for studying biological molecules, especially proteins and nucleic acids, the knowledge in phytochemistry has greatly increased. Chemical information is used to improve classification. Man classified plants as edible and inedible which is based on their chemical differences. A large variety of chemical compounds are found in plants. The biosynthetic pathways responsible for these compounds also differ from one taxonomic group to another. The distribution of these compounds and their biosynthetic pathways correspond well with the existing taxonomic arrangements based on more traditional criteria such as morphology. In some cases, chemical data contradicted existing hypothesis. This necessitates a re-examination of the problem. Chemical data provide informations in situations where other forms of data are insufficient (Singh, 2000). The distribution of serological characteristics of seed proteins with primulaceae is studied and the results obtained are in accordance with the proposed subdivisions of the family made on morphological basis (John, 1978). The leaf phenolics of the leaves of the 11 species of Fouquieriaceae studied reveal that there is no variation among the species in the phenolic distribution. All species contained ellagic acid, isoquercitrin, rutin, caffeic acid and scopolin (Scogin, 1978). The taxonomy of *Primula* species has been in dispute due to the high morphologic variability and several hybridizations. Three morphological features of the trichome, size and dimensional ratio of stalk,

neck and gland head were studied. These three trichome elements are found to be typical for each species of *Primula* – *Primula auricula*, *Primula daonensis* and *Primula hirsuta*. The morphological characters treat the 3 species differently. The studied flavonoids are different in the three species. Three different flavonoid profiles were obtained. Phytochemical investigations of the flavonoid composition of leaves are taxonomic markers. Thus both the morphological and taxonomic markers support the separation of the three species (Fico *et al.*, 2007).

Apocynaceae members show anti-leukemia activity and this is attributed to the frequent occurrence of indole alkaloids as major anti-tumor active constituents in many of the genera, *Catharanthus*, *Rauvolfia*, *Tabernaemontana*, *Thevetia*, *Alstonia*, *Cerbera*, *Holarrhena*, *Chilocarpus*, *Kopsia*, *Hunteria* (Cragget *et al.*, 2006). Many of the 8 medicinally important plants such as *Alstonia scholaris* (L.) R. Br., *Catharanthus pusillus* (Murray.) G. Don., *Catharanthus roseus* (L.) G. Don., *Cerbera odollam* Gaertn., *Holarrhena pubescens* (Buch-Ham.) Wall. ex Don., *Plumeria alba* L., *Plumeria rubra* L., *Rauvolfia serpentina* (L.) Benth. ex Kurz, *Rauvolfia tetraphylla* L., *Tabernaemontana alternifolia* L., *Tabernaemontana divaricata* (L.) R. Br., *Wrightia tinctoria* (Roxb.) R. Br. belongs to the family Apocynaceae. The plants are used in many of the crude as well as purified drugs in both traditional and modern systems of medicines. Hence there is a need for standardizing the raw drug. There still exists a lacuna in the field of Apocynaceae members. George (1800 – 1884) and sir Joseph Dalton Hooker (1817 – 1911) jointly published the *Genera plantarum* in 1862-1883 in three volumes. Apocynaceae is included in the cohort Gentianales which is in the series of Bicarpellatae in their work. *Bicarpellatae* is placed in the sub-class Gamopetalae of the class Dicotyledons.

Tannin is a general descriptive name for a group of polymeric substances capable of tanning leather or precipitate gelatin from a solution, a property known as astringency (Harborne, 1973). Tannins are incompatible with alkalis, gelatin, heavy metals, iron, lime water, strong oxidizing agents and zinc sulphate. They have been traditionally considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. Tannins if ingested in excessive quantities inhibit the absorption of minerals such as iron which may, if prolonged, lead to anaemia (Chavan *et al.*, 1995). Many physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and wide range of anti-infective action have been assigned to tannins (Okwu and Okwu, 2004). (Adamu *et al.*, 2007) reported the presence of tannins, saponins, phenol, flavonoids, and glycosides in the root and

bark of *Detarium microcarpium* and *Ziziphus mucronata*. Cowman, 1999; Soetan and Aiyelaagba, 2009 and Adesokan *et al.*,(2009) , reported that the medicinal properties of plants could be based on the antioxidant, antimicrobial and antipyretic effects of the phytochemicals in them.

Alkaloids rank among the most efficient and therapeutically significant plant substances (Okwu, 2005). Some 5, 5000 alkaloids are known and they comprise the largest single class of secondary plant substances which contain one or more Nitrogen atoms, usually in combination as part of a cyclic structure (Harborne, 1973). Alkaloid production is a characteristic of all plant organs. They exhibit marked physiological activity when administered to animals (Okwu and Okwu, 2004).Furthermore, alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs. (Harbone 1973, Okwu 2005, Okigbo *et al.*,2009).

Saponins are glycosides of both triterpenes and steroids that are characterized by their bitter or astringent taste, foaming property, hemolytic effect on red blood cells and cholesterol binding properties (Okwu, 2005; Okigbo *et al.*, (2009).

Saponins have been shown to possess both beneficial (lowering cholesterol level) and deleterious (cytotoxic and permeabilization of intestinal epithelium) properties and to exhibit structure dependent biological activity. In medicine, it is used to some extent as an expectorant and an emulsifying agent(Harborne, 1973).They can be used as diuretics healing agents and analgesics(George, 1999). They are harmless when taken orally in small quantity (Finar, 1996). Some of the general properties of saponin include formation of forms in aqueous solution, hemolytic activity and cholesterol binding properties and bitterness (Sodipo *et al.*,2000).They are responsible for imparting a bitter taste and astringency,properties to raw of *Vigna unguiculata* (Iwe,2003).

Flavonids are widely distributed in plants.The widespread distribution of flavonoids, and their relatively low toxicity, compared to other plant metabolites (such as alkaloids) result that many animals including humans ingest significant quantities in their diet (Close and McArthur ,2002).Naturally occurring flavonids are potentially anti-allergic, anti-carcinogenic, antiviral and antioxidants(Close and McArthur 2002).They show anti-inflammatory and anti cancer activities.(Okwu, 2005). Consumers and food manufacturers have become interested in

flavonoids for their medicinal properties, particularly their roles as potentially important dietary, cancer chemoprotective and cardiovascular disease prevention (Okwu and Emenike, 2006).

Steroid is a terpenoid lipid characterized by a carbon skeleton with four fused rings, generally arranged in a 6-6-6-5 fashion. They vary by the functional groups attached to these rings and the oxidation state of the rings. All steroids are made in cells either from the sterol Lanosterol (animals and fungi) or from the sterol cycloartenool Plants (Moss, 1989).

The rate-limiting step of steroid synthesis is the conversion of cholesterol to pregnenolone which occurs in the mitochondrion (Rossier, 2006). Anabolic steroids were first isolated, identified and synthesized in the 1930's, and are now used therapeutically in medicine to stimulate bone growth and appetite, induce male puberty and treat chronic wasting conditions, such as cancer and AIDS Clifford *et al.* (1973). Excessive dose of anabolic steroids has harmful effects like causing ache, liver damage and high blood pressure (Rossier, 2006). The phenolic compounds of higher plants may act as antioxidants or as agents of other mechanism contributing to anti-carcinogenic or cardio-protective actions (Okwu, 2004, 2005). Generally non toxic phenolic compounds are believed to play vital roles in the development of adverse flavours, colour reactions and odours of oilseed protein sources (Iwe, 2003).

The quantities of nutrient contents in plants vary and the nutrient contents in food also vary a great deal (Wardlaw, 2003). Among these nutrients are carbohydrates, proteins, fats, fibre, ash, and carbohydrates which are the major constituents and provide a major source of fuel for the body. Proteins are body building supplement which comprise of phosphorus and sulphur sometimes, but mainly carbon, hydrogen and nitrogen. (USDA, 2007).

Dietary sources of protein include nuts, grains, legumes and dairy products such as milk and cheese (WHO, 2001). The building block of protein is amino acids of which eleven are non-essential and nine essential amino acids. They, like carbohydrates contain four kilocalories per gram as opposed to lipids which contain nine kilocalories. Digestion of protein typically begins in the stomach when pepsinogen is converted to pepsin by the action of hydrochloric acid. (WHO, 2001). Protein nourishes the body and its deficiency leads to kwashiorkor and mental degradation.

The ash content of a plant is basically the mineral elements present in the ash which has been decomposed and passed off in the form of gases as a result of excess heat. It is shown to contain

the following elements; calcium, iron, sulphur, phosphorus, chlorine and sodium. The ash content of plant tissue varies from a fraction of 1-15% or more of the dry weight (Dutta, 2004).

Fat is an essential class of the four major biomolecules. It functions to insulate and act as a major source of fuel for the body's metabolic processes. (Pichon *et al.*, 1998).

Moisture content of a plant refers to the difference in weight of a fresh sample of plant and one dried in the oven. Moisture which is the liquid component of the plant is essential for most of the chemical reactions taking place in the course of the plants metabolism. Deficiency of moisture may lead to chlorosis of leaves and stunted growth.

Fibers are those parts of fruits, vegetables and grains that are neither absorbed nor digested. Fiber comes in two forms, soluble and insoluble fiber. (Agarwal, 1986). The soluble fiber absorbs water in the intestines, mixes the food into a gel and thereby slows the rate of glucose digestion and consequent absorption in the blood stream. A diet high in soluble fibre lowers blood level of the harmful type of cholesterol without lowering the good cholesterol level (Agarwal, 1986).

Insoluble fibres are found mainly in plant leaves, peels, skins and coverings of whole grains. Insoluble fibres mainly cellulose in skin of fruits and vegetables and the husk of grains help to prevent constipation. It has also been discovered that fibre protects the humans against colon cancer (Boutwell, 1998). Carbohydrates are the most abundant of the four classes of biomolecular constituents of plants. They play major roles in storage and transport of energy, and their derivatives in the working process of the immune system, fertilization, blood clotting and so on (Maton *et al.*, 1993). Phytate is found in plant seeds. It serves as the main storage form of phosphorus in the seeds. All edible seeds, legumes, grains and nuts contain it in varying quantities and small amounts are also found in roots and tubers. Leguminous plants synthesize in their cells a great variety of phytochemicals particularly Isoflavones, flavonoids, phenolic compounds, lignans, alkaloids and cyanogenic glycosides (Okwu, 2004, 2005). The phytochemicals present in the plants endow them with medicinal properties. The antioxidant properties of many plants are mainly contributed by the phenolic compounds present in them (Brown and Rice-Evans, 1998 and Krings and Berger, 2001). Phenols and flavanoids are active antioxidant compounds showing many other medicinal properties. Most of the phytochemicals are known to have therapeutic properties such as insecticidal (Kambu *et al.*, 1982), antibacterial, antifungal (Lemos *et al.*, 1990) and anticonstipative (Ferdous *et al.*, 1992) activities. The plants thus find their medicinal values due to the presence of respective phytochemical constituents. The presence of

various phytochemicals in plants reveals that these plants may be a good source for production of new drugs for various ailments. Isoflavones which are phytoestrogens effectively and efficiently modulate estrogen levels in humans. They are of clinical value in low estrogen states like menopause or imbalanced and toxic estrogen sensitive conditions such as breast, uterine and prostate tumor growth (Okwu and Omodamino 2005, Okwu, 2005). Phytochemicals regulate, protect and control cancer and semen quality in men. They prevent breast cancer, cystic ovaries and endometriosis among women (Verger and Leblanc, 2003). It is now well recognized that people who consume traditional diets rich in fermented soil foods and beans (mainly Leguminosae) experience less breast, uterine and prostate cancers and increase semen quality in men. Lignans are weak phytoestrogens that are found in seeds and grains especially flaxseed. They have antiviral, anti-bacterial, antifungal, antioxidant and immune enhancing properties (Okwu, 2005). Lignins on the other hand are non-carbohydrate dietary fiber that along with polysaccharides occurs in the cell wall of plants. The grain is rich in phytochemicals which are vital in health protection; disease prevention and drug production. Phytochemicals act as antioxidants, stimulate the human system, induce protective enzymes in the liver or block damage to genetic materials (Okwu, 2004).

Their amino acid complements those of cereals (Fashokin and Fansaya, 1988; Fashokin and Ojo, 1988; Asumugha, 2002). Their mineral contents: calcium and iron are higher than that of meat, fish and egg and the iron content equates that of milk; the vitamins- thiamin, riboflavin, niacin (water soluble) and their levels compare with that found in lean meat and fish (Platt, 1962; Adams, 1984; Rachie *et al.*, 1985; Achuba, 2006) which make them very useful in blood cholesterol reduction (Johnson *et al.*, 1983; Anderson, 1985). Many researchers including Anderson (1985), Adaji *et al.* (2007) and Adeniji (2007) have shown that daily consumption of 100–135gm of dry beans reduces serum cholesterol level by 20%, thereby reducing the risk for coronary heart diseases by 40% (Anderson, 1985; Ofuya, 1993). Besides its health related benefits, beans are inexpensive, considerably cheaper than rice or any other dietary fibre type (Ayenlere *et al.*, 2012). It is a good food security item as it mixes well with other recipe (Singh and Rachie, 1985; Muoneke *et al.*, 2012).

2.6 The potentials and role of cowpea in food security

Cowpea is predominately a hot weather crop. It is more tolerant to drought, water logging, infertile soils, and acid stress than the common bean, an indication that Cowpea can be grown quite successfully under conditions that are totally unsuitable for the common bean. However, cowpea is much less tolerant to cold soils than common bean and it grows well on poor soils with more than 85% sand and less than 0.2% organic matter and low level phosphorus (Singh, 1997). It is one of the most important pulse crops native to central Africa. Arthur (2009) mentioned that cowpea is the second most pulse crop after groundnut. It is grown extensively in the low lands and mid latitude regions of Africa (particularly in the dry savanna) sometimes as sole crop but more often intercropped with cereals such as sorghum or millet (Agbogidi, 2010a). It is shade tolerant, and this makes cowpea an important component of traditional intercropping systems, especially in the complex and elegant subsistence farming systems of the dry savannasin sub-Saharan Africa (Blade *et al.*, 1997). Cowpea fixes atmospheric nitrogen through symbiosis with nodule bacteria (Shiringani and Shimeles, 2011). When used with cereal crops it can help restore soil fertility (Sanginga, 2003). Therefore, cowpea can play an important role in the development of agriculture. World production of cowpea was estimated to be 2.27million tons of which Nigeria produces about 850,000 tones (FAO, 2002; Adaji *et al.*, 2007). Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics (FAO, 2002).This is why cowpea is called ‘poor man’s meat (Diouf, 2005) because the seed protein content ranges from 23% to 32% of seed weight rich in lysine and tryptophan compared to animal proteins. Therefore, cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins and contains a substantial amount of mineral and vitamins (folic acid and vitamin B necessary for preventing birth defect during the pregnancy stage (Neilson *et al.*, 1993). Cowpea shoots and leaves are rich sources of calcium, phosphorous and Vitamin B. (Maynard, 2008). In many parts of West Africa, cowpea hay is also critical in the feeding of animals during the dry season (West *et al.*, 1982). Cowpea can be used at all stages of growth as a vegetable crop. The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Immature snapped pods are used in the same way as snapped pods are used,often being mixed with other foods.Green cowpea seeds are boiled as a fresh vegetable,or may be canned or frozen. Dry mature seeds are also suitable for boiling and canning.It is also used as a green mature crop, and nitrogen fixing crop through

symbiosis with nodule bacteria (Shiringani and Shimeles, 2011) and erosion control. It does well and most popular in the semiarid area of the tropics where other food legumes do not perform well (Sankie *et al.*, 2012). It is an extremely resilient crop and cultivated under some of the most extreme agricultural conditions in the world (Owolade *et al.*, 2006; Muoneke *et al.*, 2012). In Nigeria cowpea is majorly produced in the North in the savannah belt. Its yield in the South is affected by some environmental factors including rainfall, hence it is seasonal. The high demand for this leguminous multipurpose crop is not met in the Southern part of Nigeria. Yield being a complex trait, is influenced by many other important yield contributing characters controlled by polygenes and also environmental factors. The overall effect of plant breeding on genetic diversity has been a long standing concern in the evolutionary biology of crop plants (Simmonds, 1962). The loss of genetic diversity has been dramatic for many cultivated species (Wikes, 1983). Better cultivation of Cowpeas are mostly for their edible beans, although the leaves, fresh peas and fresh pea pods can also be consumed, meaning the cowpea can be used as a food source before the dried peas are harvested . (Ehlers *et al.*, 1997). As well as an important source of food for humans in poor arid regions, the crop can also be used as feed for livestock. This predominately occurs in India, where the stock is fed cowpea as forage or fodder. (Singh *et al.*, 1997). When used with cereal crops, it can help restore soil fertility (Sanginga, 2003); therefore cowpea can play an important role in the development of agriculture. The nitrogen fixing ability means that as well as functioning as a sole-crop, the cowpea can be effectively intercropped with sorghum, millet, maize, cassava or cotton. (Blade *et al.*, 1997) Islam *et al.* (2006) emphasized that all parts of the plant used as food are nutritious, providing protein and vitamins. Immature pods and peas are used as vegetables meat while several snacks and main dishes are prepared from the grains (Duke, 1981; Bittenbender *et al.*, 1984). Apart from this, cowpea forms excellent forage and it gives a heavy vegetation growth and covers the ground so well that it checks the soil erosion. It is a most versatile pulse crop because of its smothering nature, drought tolerant characters, soil restoring properties and multi-purpose uses. As a pulse crop, cowpea fits well into most of the cropping systems. Egho (2009) reported that Nigeria is the second greatest consumer of cowpea in the whole world. Among the legumes, cowpea is the most extensively grown, distributed and traded food crop consumed, more than 50% (Philip and McWalters, 1991; Ogbo, 2009; Agbogidi, 2010a). This is because the crop is of considerable nutritional and health value to man and livestock (Agbogidi, 2010b). They form a major staple in the diet in Africa and

Asian continents (Awe, 2008). The seeds make up the largest contributor to the over all protein intake of several rural and urban families hence Agbogidi (2010b) regarded cowpea as the poor man's major source of protein

The recent annual global production of cowpea approximates to 3.3 million tons, Central and West Africa region are the major areas of its cultivation (CGIAR, 2014), while it is chiefly a vegetable and grain crop for human who values it as a nutritional supplements to cereals and an extender of animal proteins, it serve as a very safe fodder for livestock animals. Cowpea has vast utility in the food culture of both man and animal (Tarawali *et al.*, 2002; Diouf and Hilu, 2005).

Knowledge of the genetic similarity of breeding materials could help to maintain genetic diversity and sustain long term selection gain. Insects are a major factor in the low yields of African cowpea crops, and they affect each tissue component and developmental stage of the plant. In bad infestations, insect pressure is responsible for over 90% loss in yield (Bagheri, 1996). The legume pod borer, *Maruca (testulalis) vitrata* is the main pre-harvest pest of the cowpea. It causes damage to the flower buds, flowers and pods of the plant. Other important pests include pod sucking bugs, thrips and the post-harvest weevil *Callosobruchus maculatus*. (Booth *et al.*, 1990). The production of cowpea all year round in all parts of Nigeria is expected to boost production, thereby improving nutrition, and contribute to food security as well as increase revenue of the producers and create employment opportunities by enhancing the efficiency of the utilization of labour. *Vigna unguiculata* is a very important crop species worldwide. The seeds also have deworming and diuretic properties, and promote stomatic health; when powdered and burned, they alleviate insect bites. Roasted seeds are used to treat neuritis, insomnia, weakness of the memory, Indigestion, dyspepsia, sensation of pins and needles in limbs, periodic palpitation and congestive cardiac failure. It is an excellent medicine for stomatitis, corneal ulcers, coleic diseases, Kwashiokor, Marasmus. The leaves and seeds are made into compresses to treat blisters. The root also has medicinal properties and is used as a cure for snake bites and as medicines for epilepsy, chest pain and dysmennorrhoea. The plant is also used as animal feed. In Nigeria, the fibres of the penuncles are used to make fishnets and paper (Tohoue *et al.*, 2009).

Legume seeds are important source of protein supplement. This is not only for protein but for minerals and vitamins of B complex, the legumes also provide additional nicotinic acid and minerals. Legumes together with cereals are the main plant source of proteins in human diet. They

are also rich in dietary fibre and carbohydrates,(Rochfort *et al.*,2007). There are anti nutrient content in legume seeds that can cause harm if not treated well before use.*Vigna unguiculata* seeds contain these anti nutrients Phytate acid, oxalate, trypsin are some of the chemical content in the seed of *V.unguiculata*.Dehulling decrease the amount of phytic acid. It is also recommended for cowpea before cooking where people are using them in great amounts, as in developing contries, Moreover when such legumes are used for baby foods, dehulling helps to prevent mineral deficiency. Trypsin inhibitor is one of most important anti-nutritional factors present in plants particularly in legumes and reported to affect the nutritive value and protein digestibility.Significant ($P < 0.05$) observed between the rawcowpea flour and de-hulled defatted cowpea seed flour in their trypsin inhibitor activity of dehulled defatted cowpea seed flour was 16.640TIU/g on dry weighth basis .18850 TIU/g were found for raw cowpea (Griffiths, 1984); 9035TIU/g for cowpea flour (Rangel *et al.*,2003) and 1113 TIU/g for raw vegetable cowpea seeds (*Sesquipedalis*) (Udensi *et al.*,2007) .Analysis of 18 pea and five bean varieties for trypsin inhibitor activity gave values ranging from 0.15 -4.62 TIU/mg (Griffiths,1984). Their research concluded that de-hulled defatted cowpea has considerably lower levels of trypsin inhibitor activity than other peas. The levels of trypsin reduced showing that the anti-nutritional quality of cowpea can be improved by dehulling, heat treatmentor supplementationof dietswith enzymes and finally by plant breeding (Balail *et al.*, 2014).

Factors contributing to poor protein quality of cowpea include poor digestibility, deficient of sulfur amino acids and present of anti-nutritinal factors (phytate, polphenols) enzyme inhibitors (trypsin, chymotrypsin) Several studies have shown that physical treatments,including de-hulling, soaking,cooking,thermal treatments,irradiation and protein fractionation can help to reduce anti-nutritional compounds as germination and fermentation can also assist.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of the Study

The study area covered Anambra State where the plant was available. Anambra State is located in the lowland rain forest of Southern Nigeria. (Ewuim, 2006). The climate is an equatorial rainforest type characterized by two main seasons; the dry and rainy season. The experimental site was Nnamdi Azikiwe University (Unizik) Botanical Garden, where the plant was cultivated which lies between Latitude $06^{\circ} 15.14'N$ and Longitude $007^{\circ} 06.695'E$.

3.2 Collection of Specimens

The specimens (seeds) for this study were sourced from various markets in Anambra state and Ministry of Agriculture. Tags bearing numbers and alphabets were used for the identification of the places and varieties collected. This was authenticated by a Plant Taxonomist Prof C.U. Okeke of the Department of Botany, Nnamdi Azikiwe University Awka. The specimen (voucher number NAUH NO 14) was deposited at the Herbarium in the Department of Botany Nnamdi Azikiwe University, Awka.

3.3 Planting and Harvesting

The seeds were planted in a 30cm plastic bucket and watered adequately. Planting of the seed was between the months of August and September 2013 and 2014 respectively. Planting was done in rows at the depth of 1 - 3cm deep ensuring good seed and soil contact. This was on complete randomized design (CRD).

Harvesting was done at three stages of maturity

1. Green snaps
2. Green mature
3. Dry

3.4 Morphological Study

The varieties of *Vigna unguiculata* (L.) Walp collected from the experimental garden was studied using morphological data such as phyllotaxy, length, width, leaf shape and venation. Each

study was replicated three times and analysed. The third or fourth fully opened leaf from the tip was used for the study at the flowering stage of the plant. The root, stem types, height and petiole of the varieties as well as the colour, shape, size, length and weight of the pods were studied. Measurements were made using a meter rule and photographs taken using DX WC 230 Sony Camera (USA). Epidermal characteristics were made from fresh peels of the specimens and were stained with safranin. Photomicrographs were made with Olympus microscope model No: N52-405 and DXWC 230 Sony digital camera (USA).

For height measurement; a ruler was used directly while for the width a thread was folded round the sample and placed against a ruler.

For Pod study; a ruler was used to measure directly. Horticultural properties of the cowpea seeds namely, seed shape, seed coat colour and hilum colour were described after visual examination.

3.5 Anatomical Studies

The roots, stems, leaves and petioles of *Vigna unguiculata* varieties under study were collected and preserved in vials containing formaldehyde, glacial acetic acid and alcohol in the ratio of 1:1:8 respectively. The specimens were dehydrated in ethanol series (30% 50% 70% and 95%) each for 2 hours. Complete dehydration of specimens was effected by storing the specimens in absolute (99.6%) ethanol overnight. The specimens were then cleared in 3:1, 1:1 and 1:3 ethanol/chloroform each for 3 hours. Anatomical Wax was melted at 70°C in an oven. The cleared specimens were then put in molten anatomical wax and alcohol and allowed to stay at 70°C for 24 hours for effective infiltration of wax into the specimen to replace the chloroform which was gradually lost by evaporation. Embedding was carried out after infiltration. This was done by smearing the inside of clean molds with glycerin, pouring the molten wax into the molds with appropriate orientation in a position suitable for the type of section to be cut. The Wax in the mould was allowed to cool into a block. The wax blocks were freed from the mould stuck on wooden holder and labeled, pending sectioning.

Wax blocks already freed from the holder and trimmed were affixed on the sledge microtome and sectioned at 15-20 microns thickness. The thin sections were fixed on clean slides already smeared with a thin film of egg albumen. They were stretched by passing them over hot plate. This also made them to become attached to the slides. Slides bearing sections were arranged in a

slide rack and placed in an oven at 70°C to melt off wax from the sections. This lasted for 12 hours.

For staining, sections were dehydrated by passing slides across xylene and xylene/absolute ethanol series (3:1, 1:1 and 1:3 v/v), absolute ethanol, and 95, 70, 50 and 30% ethanol. Slides were briefly immersed in water, then stained with 0.1% alcian blue and counter stained with 1% safranin. Stained slides were rinsed briefly in tap water dehydrated through the ethanol series and cleared across the xylene /absolute ethanol series.

Mounting was carried out by placing one drop of Canada balsam on a clean slide and carefully covering the sections with the cover slip in a way that the Canada balsam spread and covered the specimen sections overlaid by the cover slip. They were studied and photomicrographs taken. This study was carried out using the method of AOAC (2005).

3.6 Cytological Studies

Procedure for mitotic studies

The seeds were germinated and when several roots have grown, about 1-8cm of the root tips were cut off with the use of fine forceps. The soil particles were washed off using fine forceps. The soil particles were washed off and the root tips separated. The roots were transferred to a corked bottle containing Carnoy's fluid and left overnight at room temperature to fix the material. This was done to coagulate the components of the cell without solution and disintegration of their internal or external spacing. It also reduces the staining of the cytoplasm while allowing the chromosomes to take up stain readily thus improving the optical contrast of the cell's components. The root tips were removed with forceps by grasping the cut end of the root, transferred to a petri dish containing distilled water and washed for few minutes to remove fixative. The root tips were then transferred to a test tube containing 18% hydrochloric acid for 2 minutes. This process loosens the cementing substance between cells and allows the cells to spread out during squashing. The root tips were removed from the acid and transferred into a dish containing 70% alcohol and washed to remove the acid.

Using a mounted needle, 2cm portion of the root tip was cut off onto a clean slide. One drop of F.L. Porcin stain was dropped on the specimen. A thin cover slip was laid on top of the specimen and the material squashed by gently and briskly tapping the cover slip with the blunt end of a biro. The tapping continued until the material was spread out properly and was hardly

visible. The slide was placed between a large filter paper on a hard smooth table surface and thumb pressure applied cautiously on top of the cover slip. Lateral movement of the cover slip was avoided during the process. This technique is called the “squash” technique.

Excess stain was drained off with filter paper and the slides were examined to see the chromosomes under a high power microscope (Model No: N 52-405) and photomicrographs taken (DXWC 230 Sony camera). This procedure was as outlined by Okoli (1992).

3.7 Phytochemical Analyses

3.7.1 Qualitative Phytochemical Analysis

The fresh plant parts stems, leaves, seed, and root of *Vigna unguiculata* were oven dried for 2 days at 70°C. Stem, root and leaves were sliced before drying. The dried samples were ground to a fine powder using corona grinding machine. The dried powdered samples were used for the analysis for qualitative and quantitative determination.

For the qualitative analysis, the following keys was used

+ = present,

++ = strongly present

- = not present.

Test for the presence of alkaloids

The presence of alkaloids in each sample was investigated using the method described by Harborne (1973). An alcoholic extract was used and obtained by dispersing 2 g of the powdered sample in 10 ml of ethanol. The mixture was thoroughly shaken before filtering using whatman No 40 filter paper. In a test tube 2 ml of the filtrate was added and 3 drops of pirovic acid was mixed with it. The formation of light green colouration indicates the presence of alkaloid

Test for the presence of flavonoid

The determination of the presence of flavonoid in the sample was carried out using the acid alkaline test described by Harborne (1973). In a test tube 2 ml of the aqueous extract was added and a few drops of Bench concentrated ammonia (NH₄) were also added. The formation of a yellow colouration shows presence of flavonoid. Confirmatory test was carried out by adding few drops of concentrated hydrochloric (HCL) into the yellow solution which turned colourless.

Test for the presence of saponin

The presence of saponin in the test samples was investigated using the Harborne (1973) method. Two tests are involved in the investigation, the froth test and emulsion test. In the froth test, 2 ml of the aqueous extract was mixed with 6 ml of distilled water in a test tube. The mixture was shaken well and the formation of froth indicated the presence of saponin.

For the emulsion, 3 drops of vegetable oil was added into the test-tube containing an aqueous extract of the test sample. The mixture was shaken well and observed for the presence of stable emulsion. The formation of a stable emulsion indicates the presence of saponins.

Test for the presence of steroid

The presence of steroid in the test sample was carried out using the method described by Harborne (1973). In a test tube 2 ml of acetic anhydride was added to 5 ml ethanol extract of each sample with 2 ml sulphuric acid (H_2SO_4). The colour changed from violet to blue or green in the sample indicates the presence of steroids.

Test for the presence of tannin

The determination of the presence of tannin in the test sample was carried out using the ferric chloride test described by Harborne (1973). In a test tube 2 g of the powdered sample was added into 10ml of distilled water. The mixture was shaken for 30 minutes in a mechanical shaker and the filtrate used as aqueous extract. 2 ml of the aqueous extract was added into a test tube and 3 ml of distilled water added to it. After shaking to mix well, 2 drops of dilute ferric chloride ($FeCl$) was added to the mixture. The formation of a very dark precipitate indicated the presence of tannin.

Test for Phenol

2ml of the powdered sample was added in 2ml of distilled water. This mixture was added by 10% of $FeCl$ solution. Bluish black colour indicates the presence of phenol.

3.8 Quantitative Phytochemical Analysis**Determination of Alkaloids**

The gravimetric method (Harborne, 1973) was adopted. About 5.0g of each sample was dispersed in 50ml of acetic acid solution in ethanol. The mixture was shaken and shaken and allowed standing for four hours before it was filtered. The filtrate was evaporated to one quarter its original volume. Concentrated NH_4OH was added drop-wise to precipitate the alkaloids. The

filtrate was evaporated to one quarter its original volume and concentrated NH_4OH added dropwise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH_4OH solution, the filtering was done with a weighed filter paper. The precipitate in filter paper was dried in the oven at 600° for 30mins and reweighed, by weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample weighed analyzed. It was given thus:

W = weight of sample

W_1 = weight of empty filter paper

W_2 = weight of paper + precipitate

Flavonoid Determination

The flavonoid content of the sample of the plant was determined by the gravimetric method as was described by Harborne (1973). 5g of the powdered sample was placed into a conical flask and 50ml of water and 2 ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture allowed cooling before it was filtered through what man filter paper (No 42). 10 ml of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed what man filter paper was used to filter the second (ethyl-acetate layer); the residue was then placed in an oven to dry at 60°C . It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:-

W_1 = weight of empty filter paper

W_2 = weight of paper + Flavonoid extract

Determination of Saponin

The method used was described by AOAC (2005). In an extractor thimble 5.0g of dry ground sample was weighed inside and transferred into the soxhlex extractor chamber fitted with a condenser and a round bottomed flask. Some quantity of acetone, enough to cause a reflux was poured into the flask, the sample was exhaustively extracted of its lipid and interfering pigments for 3hours by heating the flask on a hot plate and the solvent distilled off. This is the first

extraction. For the second extraction a pre-weighed round flat bottom flask is fitted unto the soxhlex apparatus (bearing the sample containing thimble) and methanol should be enough to cause the reflux, the saponin is then exhaustively extracted for 3hours by heating the flask on a hot plate after which the difference between the final and initial weight of the flask represents the weight of saponin extracted.

Determination of Tannins

The follin –Denis spectrophotometric method was used. The method was described by Pearson (1976). Inside a test tube 1.0g of the sample was dispersed in 10ml distilled water and agitated. This was left to stand for 30mins at room temperature being shaken every 5mins at the end of the 30mins, it was centrifuged and the extract gotten 2.5ml of the supernatant extract was dispersed into a 50ml volumetric flask similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask 1.0ml follin denis reagents was measured into each flask followed by 2.5ml of saturated to mark in the flask (50ml) and incubated for 90mins at 150m temperature, the absorbance were measured at 250nm in a Genway model 6000 electronic spectrophotometer readings were taken with the reagent blank at zero. The tannin content was given as follows

Where; an absorbance of test sample

A_s = absorbance of standard solution

W = weight of sample used

Total volume of extract analyzed

Determination of Sterol

The sterol content of the specimen plant was determined using the method described by Harborne (1973) filtered using Whatmans filter paper No42. The filtrate was transferred to a separating funnel. Equal volume of ethyl acetate acid was added to it, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) recovered while the aqueous layer was discarded. The extract was dried at 1000c for 5minutes in a steam bath. It was then heated with concentrated amly alcohol to extract the steroid. The mixture becomes turbid and a weighed whatman filter paper No42 was used to filter that mixture properly. The dry extract was then cooled in adessicator. The process was repeated two or more times and an average obtained. The concentration of steroid was determined and expressed as a percentage, thus

$$\% \text{ Steroid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:-

W_1 = weight of filter paper

W_2 = weight of filter paper + steroid

Determination of Pytic acid

Phytic acid was determined using method described by Oberlease *et al.*, (1978) the sample was first extracted with 0.2 NH₄Cl; 0.5ml of the extract solution was pipette into a test tube fitted into a ground glass stopper. 1ml of Ferric solution was added and the tube heated in a boiling water bath for 30 minutes a 3000rpm. 1ml of the supernatant was transferred to another tube and 1.5ml of 2,2 bipyridine solution was added and the absorbance was measured at 519nm against distilled water.

Calculation

Pytic acid =

Where

=represents total vol. of extract

= vol. of extract used.

= W =weight of sample used

=PPm off curve

Determination of Total Phenol

Phenol content in the plant extract was determined byFolin-Ciocalteu reagent method with slight modifications Adedapo *et al.*, 2009; Koncic *et al.*, 2001; McDonald *etal.*, 2001 and Nabavi *et al.*,2008). One gram of the sample was extracted with 10 ml of 80% methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and evaporated to dryness. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank

was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. A standard curve was prepared using gallic acid. Several dilutions of gallic acid in 80% methanol were prepared viz. 20, 40, 60, 80, 100 µg/ml (Lin. and Tang, 2005). One ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water. After this 2.5 ml Folin-Ciocalteu's reagent was added. This was followed by the addition of 2.5 ml of 7.5 % NaHCO₃ in each test tube. The resulting mixture was left to stand for 30 minutes at room temperature. Absorbance of the standard was measured at 765 nm using UV/VIS Spectrophotometer against blank. Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Total phenol content = GAE x V x D /m,

Where GAE is the gallic acid equivalence (mg/mL); V is the volume extract (ml),

D is dilution factor and m is the weight (g) of the pure plant extract.

Determination of oxalate

The titrimetric method of Day and Underwood (1986) was used in the determination of oxalate in the seven varieties. In a test tube, 150 ml of 15 N H₂SO₄ was added to 5 g of the pulverized *Vigna* samples and the solution was carefully stirred intermittently with a magnetic stirrer for 30 minutes and filtered using Whatman No 1 filter paper, after which 25 ml of the filtrate was collected and titrated against 0.1 N KMnO₄ solution until a faint pink colour appeared that persisted for 30 seconds.

3.9 Proximate Analysis

Determination of moisture content

Moisture content was determined by gravimetric method described by James, (1995). In a moisture can 5 grams of the sample was weighed in it. The can and its content were dried in the oven at 105⁰c for 3 hours in the first instance; it was cooled in a desiccator and reweighed. The weight was recorded while the sample was retained in the oven for further drying. The drying,

cooling and weighing was continued repeatedly until a constant weight was obtained. The moisture content was calculated as shown below,

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

W_1 = Weight of empty moisture can

W_2 = Weight of empty can + sample before drying

W_3 = Weight of can + sample after drying to a constant weight

Determination of Total Ash

Ash content was determined using incineration gravimetric method (AOAC, 2005). A measured weight (5g) of sample was put in a previously weighed porcelain crucible. The sample in crucible was put in a muffle furnace and set at 550^oc and allowed to burn for 2-3 hours (until the sample became a grey ash). The sample in the crucible was carefully removed from the furnace and cooled in a desiccator. It was reweighed by difference, the weight of ash was obtained and the percentage. It was given by the formula

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where

W_1 = Weight of empty crucible

W_2 = Weight of empty crucible + Ash.

Determination of crude protein

This was determined by Kjeldahl digestion method described by James (1995). The total nitrogen was determined and multiplied with the factor 6.25 to obtain the protein.

About 0.5g of the sample was mixed with 10mls of concentrated sulphuric acid in a Kjeldahl digestion flask. A tablet of selenium catalyst was added to it and the mixture was digested under fume cupboards until a clear solution was obtained. The acid and other reagent were digested but without sample to form a blank control. All the digests were carefully transferred to a 100ml volume flask using distilled water and made up to the mark in the flask. A 100ml portion of each

digest was mixed with equal volume of 45% NaOH solution in kjeldahl distilling unit. The mixture was distilled and distillate collected into 10ml of 4% boric acid solution containing three (3 drops mixed indicator cresol green and methyl red). A total of 50ml distillate was obtained and titrated against 0.02N H₂SO₄ solution. The end point is from the initial green colour to a deep red point. The nitrogen content was calculated as shown below where

1ml OF N H₂SO₄ = 14mg

Protein (%) = N₂ (%) × 6.25

$$N_2 (\%) = \frac{100 \times N \times 14 \times V_t}{W \times 1000 \times V_a} \times TB$$

W = Weight of sample analyzed (0.5g)

N = Normality of Titrant (0.02 NH₂ SO₄)

V_t = Total volume of digest (100ml)

V_a = Volume of digest analysed (10ml)

T = Titre value

B = Blank titre value.

Determination of fat content

Fat content of the sample was determined by the continuous solvent extraction method using a soxhlex apparatus. The method was described by Pearson, (1976). In a porous paper 0.5grammes of the sample was wrapped (whatman No 1 filter paper). The wrapped sample was put in a soxhlex reflux flask containing 200ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electrothermal heater, it vapourizes and condensed into the reflux flask. The wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over thus carrying oil extract from the sample down to the boiling flask. This process was allowed on repeatedly, for about 4 hours before defatted sample was removed and reserved for crude fibre analysis. The solvent was recovered and the extracting flask with its oil content was recovered and the extracting flask with its oil content was dried in the oven at about 600°C for 3 minutes to remove any residual solvent.

After cooling in dessicator the flask was reweighed. By difference the weight of fat oil extraction was determined and expressed as percentages of the sample weight. It was calculated as

$$\text{Fat (\%)} = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100$$

W_1 = Where of empty extraction flask

W_2 =Flask and oil extract

Determination of crude fibre

Crude fibre was determined by the Wende method (James, 1995). During fat analysis, 5g of the sample was defatted. After that, the sample was boiled in 200ml of 1.25% H_2SO_4 solution under reflux for 30 minutes after that, the sample was washed with several portions of hot (boiling) water using a two-fold muslin cloth to trap the sample particle. The washed samples were carefully transferred quantitatively back to the flask and 20ml of 1.25% NaOH solution was added to it again, the sample was boiled for 30 minutes and washed as before with hot water. Then they were very carefully transferred to a weight porcelain crucible and dried in the oven at 1050c for 3 hrs after cooling in a desiccator, then reweighed (w_2) and then put in a muffle furnace and burn at 5500c for 2hrs until they become ash. Again they were cooled in a dessicator and reweighed. The crude fibre content was calculated gravimetrically as

$$\% \text{ crude fibre} = \frac{W_2 - W_1}{W_1 \text{ of sample}} \times \frac{100}{1}$$

Where

W_2 = Weightof crucible after washing and drying in an oven

W_3 = Weight of crucible +sample as ash

Determination of carbohydrates

The carbohydrate content was calculated using a method described by James (1995).Forty five milliliter of each of the sample extracts was diluted to 450ml with diluted water. One milliliter of each of the diluted filtrate was pipetted into different test tubes while 1 ml of glucose into a test tube as a standard. To each of the test, 5 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. Each tube was labeled and placed in a test tube rack both the test tubes and the rack were placed in attest tube rack both the test tubes and the rack were placed in water bath (30%) for 12min, removed and cooled to room tempreture.The absorbance of the samples and the standard were read from a spectrophotometer

at 630nm against the blank. The green colour which shows the presence of glucose was stable for about 2 hours. Total available carbohydrate as percentage glucose is calculated as shown below;

$$\text{Glucose (\%)} = \frac{25A_1}{X} \times 100$$

$$X \times A_2$$

X = Weight of sample (g)

A = Absorbance of diluted sample

A₂ = Absorbance of diluted standard

Determination of minerals/ vitamins

The sample for the determination of the element was subjected to acid digestion and subsequently the different element were determined using the methods as described below by James (1995)

Digestion

Into an evaporation dish 10mm volume of the sample was dispersed and treated with 15ml of conc HNO₃. The mixture was evaporated to 50ml on a water bath and transferred quantitatively to a 100ml standard volume flask. It was made up to volume with de-ionized water.

3.10 Statistical Analysis

The quantitative data obtained was statistically analysed by calculating the mean of the replicates followed by calculation of the sum of squares, Variance, Standard Deviation, Standard error, ANOVA and Duncan's multiple test. SPSS software version 20 and data were expressed as mean \pm standard deviation of triplicate determinations. Text of significance was measured using Duncan's multiple Range Test (DMRT). Statistical methods showed the variety that has the highest phytochemical and proximate constituents. The intra and inter-specific relationship was used to check the differences, similarities and stabilities of the taxonomic characters in the varieties studied.

Vigna unguiculata var. "kafanji" showing the herbaceous habit ,climbing stem,green colour of leaf, trifoliolate leaf type,arcuate venationand purple and zygomorphic type of flower (Plate 1).



Plate 1: *Vigna unguiculata* Var. 'Kafanji'.

Vigna unguiculata Var. Ifebrown showing alternate leaf arrangement, trifoliolate leaf shape, hastate leaf base, a long petiole and a purple colour and zygomorphic type of flower(Plate 2).



Plate 2: *Vigna unguiculata* var. 'Ifebrown'

Vigna unguiculata Var. 'Sokoto guzo' in its habitat showing hastate leaf base, green colour leaves, a long petiole and a petioleous, an alternate leaf arrangement, trifoliate leaf shape and a prostrate stem (Plate 3).



Plate 3: *Vigna unguiculata* Var. 'Sokoto guzo'.

Vigna unguiculata var. 'Oloka' showing the green colour of leaves, trifoliate leaf shape, cylindrical pod shape and a zygomorphic type of flower (Plate 4).

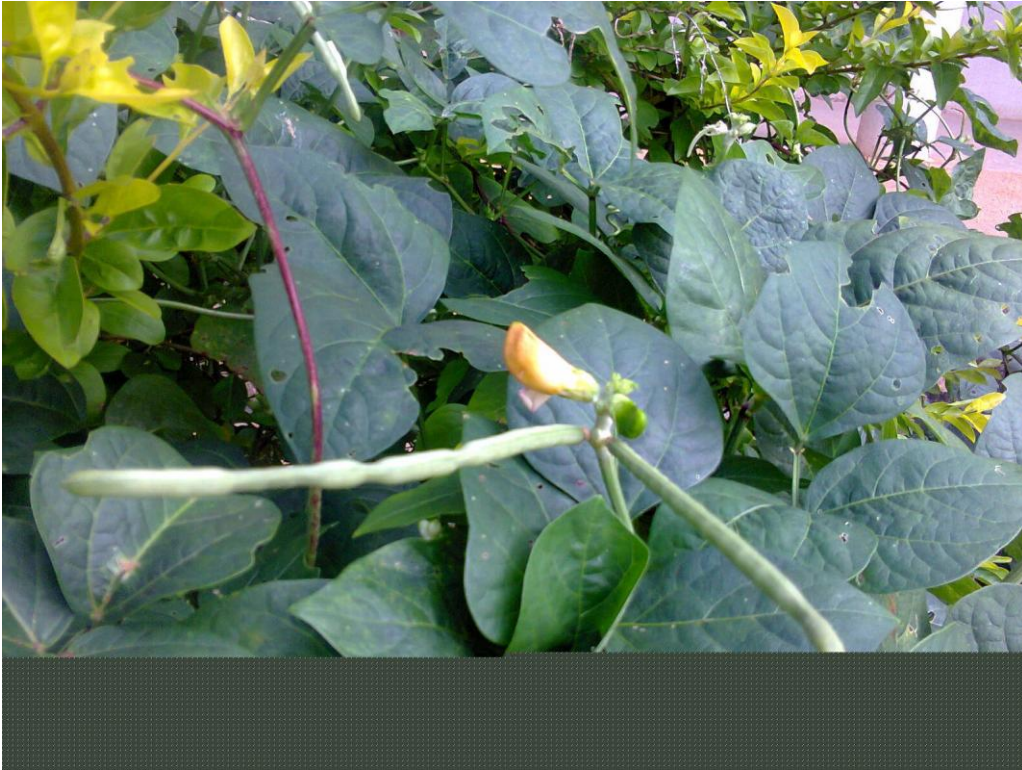


Plate 4: *Vigna unguiculata* Var. 'Oloka'

Vigna unguiculata var. Crowderpea in its habitat showing prostrate stem, trifoliolate type of leaf, purple colour and zygomorphic type of flower, long petiole and a green colour of leaf (Plate 5).



Plate 5 : *Vigna unguiculata* Var. Crowderpea.

Vigna unguiculata Var. 'Potiskum' in its habitat showing the prostrate stem, trifoliate type of leaf, a long petiole, petioleous, green leaf colour, zygomorphic type of flower which is purple in colour (Plate 6).



Plate 6: *Vigna unguiculata* Var. 'Potiskum'

Vigna unguiculata Var. 'Iron beans' in its habitat showing green colour leaf, prostrate stem, alternate leaf arrangement, long petiole, petioelouse and trifoliate leaf shape (Plate 7).



Plate 7: *Vigna unguiculata* var. 'Iron beans'

Leaflets characteristics of var.'Ifebrown' (A) var.'Potiskum' (B) and var.'Sokoto guzo' (C) showing the subglobose type of leaf and a trifoliate leaf shape with long petiole (Plate 8).



Plate 8: Leaflets Characteristics of the varieties of *Vigna unguiculata* showing sub-globose leaf Ifebrown (A), 'Potiskum' (B) and 'Sokoto guzo' (C)

Leaflets characteristics of var. 'Ironbeans' (D) and var. 'Kafanji' (E) showing the subglobose type of leaf and trifoliate leaf shape with long petiole (Plate 9).



Plate 9: leaflets characteristics of *Vigna unguiculata* showing sub-globose leaf shape of varieties Iron beans (D) and 'Kafanji' (E).

Leaflets characteristics of var. 'Oloko' (F), and var. Crowderpea (G) showing subglobose type of leaf and trifoliate leaf shape with a long petiole (Plate 10).



Plate 10: Leaflets of *Vigna unguiculata* sub-globose leaf shape showing the varieties 'Oloko' (F) and Crowder peas (G).

Leaf Characteristics of Seven Varieties of Beans

The result of the leaf characteristics of seven varieties of *Vigna unguiculata* showed that 'Ifebrown' have the highest leaf area ($261.67 \pm 4.133 \text{ cm}^2$) while 'Oloka' gave the lowest leaf area ($6.01 \pm 0.011 \text{ cm}^2$), Iron beans gave the highest petiole length ($12.20 \pm 0.005 \text{ cm}$) while 'Oloka' gave the least petiole length ($7.00 \pm 0.004 \text{ cm}$), Iron beans gave the highest petioleous length ($2.52 \pm 0.024 \text{ cm}$) while 'Oloka' gave the least petioleous length ($0.50 \pm 0.014 \text{ cm}$). 'Kafanji' gave the highest terminal leaf length ($10.81 \pm 0.007 \text{ cm}$) while 'Oloka' gave the least terminal leaf length ($5.03 \pm 0.035 \text{ cm}$), 'Kafanji' gave the highest terminal leaf width ($6.69 \pm 0.014 \text{ cm}$) while 'Oloka' gave the least terminal leaf width (3.41 ± 0.129). The seven varieties of beans showed a significant difference in their leaf area, petiole length, petioleous length, terminal leaf length and terminal leaf width ($p < 0.05$) (Table 2)

Table 2: Leaf characteristics of seven varieties of beans

Varieties	Leaf Area (cm^2)	Petiole length (cm)	Petioleous length (cm)	Terminal leaf length (cm)	Terminal leaf width (cm)
Kafanji	234.49 ± 0.267^c	10.50 ± 0.000^c	0.50 ± 0.014^e	10.81 ± 0.007^a	6.69 ± 0.014^a
Potiskum	247.01 ± 0.371^b	10.31 ± 0.007^d	2.21 ± 0.014^c	7.20 ± 0.004^d	5.20 ± 0.000^b
Ifebrown	261.67 ± 4.133^a	12.01 ± 0.016^b	2.51 ± 0.007^a	7.52 ± 0.021^b	4.37 ± 0.099^c
Black bean	203.82 ± 5.528^e	8.80 ± 0.000^f	2.30 ± 0.003^b	7.30 ± 0.003^c	4.31 ± 0.016^c
Iron beans	259.49 ± 1.051^a	12.20 ± 0.005^a	2.52 ± 0.024^a	7.50 ± 0.000^b	5.21 ± 0.007^b
Sokoto Guzo	210.59 ± 0.233^d	10.21 ± 0.014^e	2.21 ± 0.007^c	6.50 ± 0.003^e	4.22 ± 0.028^c
Oloka	6.01 ± 0.011^f	7.00 ± 0.004^g	1.80 ± 0.000^d	5.03 ± 0.035^f	3.41 ± 0.129^d
p-value	**	**	**	**	**

Flower of Var.'Ifebrown' showing zygomorphic type and purple colour of flower and an immature pod (Plate 11).



Plate 11: flower of *Vigna unguiculata* Var. 'Ifebrown'

Flower of Var. 'Oloka' showing the purple colour flower and a zygomorphic type and purple colour flower with an immature pod beside the flower (Plate 12).



Plate 12: flower of *Vigna unguiculata* Var. 'Oloka'

Flower of Var 'Potiskum' showing the purple colour flower and a zygomorphic type of flower and tender pods (Plate 13).



Plate 13: flower of *Vigna unguiculata* Var. 'Potiskum'

Flower of Var.'Ironbeans' showing the purple colour flower and a zygomorphic type of flower with a tender pod (Plate 14).



Plate 14: flower of *Vigna unguiculata* Var. Iron beans

Flower of Var. 'Sokoto guzo' showing the purple colour flower and a zgomorphic type of flower (15).



Plate 15: flower *Vigna unguiculata* Var. 'Sokoto guzo'

Flower of Var. 'Kafanji' showing purple colour flower and a zygomorphic type of flower (Plate 16).



Plate 16: *Vigna unguiculata* Var. 'Kafanji'

Flower of Var.Crowderpea showing the purple colour flower and zygomorphic type of flower (Plate17).



Plate 17: flower of *Vigna unguiculata* Var, Crowder pea.

Seed of Var. 'Ifebrown' showing large seed size, brown colour of seed and a rough seed texture (Plate 18).



Plate 18: Seed of *Vigna unguiculata* 'Ifebrown'

Seed of Var. 'Potiskum' showing medium size seed, white colour seed and a rough seed texture (Plate 19)



Plate 19: Seeds of *Vigna unguiculata* 'Potiskum'

Seed of Var.'Oloka' showing medium size seed and a multiple seed colour (mixed white and brown) and a rough texture (Plate 20).



Plate 20: Seeds of *Vigna unguiculata* 'Oloka'

Seed of Var. 'Ironbeans' showing large seed size, white seed colour and rough seed texture (Plate21).



Se

Plate 21: Seeds of *Vigna unguiculata* Iron beans

Seed of Var. 'Kafanji' showing small seed size, brown colour seed and a smooth texture seed (Plate 22).



Plate 22: Seeds of *Vigna unguiculata* 'Kafanji'

Seed of Var. 'Sokoto guzo' showing small seed size, white seed colour rough texture seed (Plate 23).



Plate 23: Seeds of *Vigna unguiculata* 'Sokoto guzo'

Seed of Var.Crowderpea showing meduim size,black colour seed and a rough texture (Plate 24).



Plate 24:Seeds of *Vigna unguiculata*Crowder pea

The physical characteristics of seven varieties of *Vigna unguiculata* showing the seed size of all the varieties, colour of the seeds of all the varieties and seed texture of the seven varieties (Table 3).

Table 3: Physical characteristics of the seven varieties of cowpea (*Vigna unguiculata*L. Walp)

Varieties	Size of seed	Colour of seed	Texture of seed
Kafanji	Small	Brown	Smooth
Ifebrown	Large	Brown	Rough
Potiskum	Medium	White	Rough
Crowderpea	Medium	Black	Rough
Sokoto Guzo	Small	White	Rough
Oloka	Medium	Mixed white and	Rough
Iron beans	Large	brown	
		White	Rough

Pods of Var 'Ifebrown' showing cylindrical shape, green colour fresh pod and dry pod (Plate 25).



Plate 25: Pods of *vigna unguiculata* Var. 'Ifebrown'

Pods of Var.'Oloka' showing cylindrical shape ,green colour fresh pod and brown dry pod (Plate 26)



Plate 26: Pods of *vigna unguiculata* Var.'Oloka'

Pods of Var. 'Kafanji' green pod with cylindrical shape (Plate 27)



Plate 27: Pods of *Vigna unguiculata* Var. 'Kafanji'

Pods of Var. 'Potiskum' showing fresh pods and dry pods with cylindrical shape (Plate 28).



Plate 28; Pods of *Vigna unguiculata* Var. 'Potiskum'

Pods of Var.'Ironbean' showing fresh pods and dry pods with cylindrical shape (Plate 29)



Plate 29: Pods of *Vigna unguiculata* Var. Iron beans

Pods of Var. 'Sokoto guzo' showing green pods with cylindrical shape (Plate 30).



Plate 30 Pods of *vigna unguiculata* Var. 'Sokoto guzo'

Pods of Var.Crowderpea showing green fresh pod and a brown pod with cylindrical shape (Plate 31).



Plate 31: Pods of *Vigna unguiculata* Var.Crowderpea

The pod width and pod length of seven varieties *Vigna unguiculata* shows that Var. “Ironbeans” has the highest pod width (2.80 ± 0.001) and Var.”Kafanji” has the lowest pod width (1.50 ± 0.000) . Var.Ifebrown has the highest pod length (15.70 ± 0.012) while Sokoto guzo has the lowest pod length (10.60 ± 0.006) (Table 4).

Table 4: Pod Width and Length of Seven Varieties of *Vigna unguiculata*

Varieties	Pod Width (cm)	Pod Length (cm)
Ifebrown	2.70 ± 0.001^b	15.70 ± 0.012^a
Kafanji	1.50 ± 0.000^f	12.20 ± 0.000^e
Oloka	2.20 ± 0.003^c	10.80 ± 0.030^f
Potiskum	2.70 ± 0.003^b	12.80 ± 0.001^d
Crowderpea	1.60 ± 0.004^e	13.20 ± 0.003^c
Iron Bean	2.80 ± 0.001^a	14.50 ± 0.020^b
Sokoto guzo	2.00 ± 0.003^d	10.60 ± 0.006^g
p-value	**	**

**p<0.05

Root nodules of Var. 'Ironbeans' in various numbers at different locations in the roots (Plate 32).



Plate 32: Root nodules found in *Vigna unguiculata* Var. 'Iron beans'

Root nodules of Var. 'Ifebrown' in various numbers and in various positions in the root (Plate33).



Plate 33: Root nodules found of *Vigna unguiculata* Var. 'Ifebrown'

Root nodules of Var. 'Kafanji' found in the root (Plate 34).



Plate 34: Root nodules of *Vigna unguiculata* Var. 'Kafanji'

Root nodules of Var. 'Sokoto guzo' in various positions in the root (Plate 35).



Plate 35: root nodules of *Vigna unguiculata* Var 'Sokoto guzo'

Root nodules of Var. 'Potiskum' in various numbers and positions.(Plate 36).



Plate 36: root nodules of *Vigna unguiculata* Var. 'Potiskum'

Root nodules of Var. 'Oloka' found in various positions in the root (Plate 37).



Plate 37; root nodules of *Vigna unguiculata* Var. 'Oloka'

Root nodules found in Var.Crowderpea in various sizes (Plate 38).



Plate 38:root nodules of *Vigna unguiculata* Var.Crowder pea.

Comparison of the Morphological Characteristics of Seven Varieties of cowpea

The leaf area of 'iron beans' was the highest (82.85cm²) while that of 'oloka' was the lowest (21.75cm²). Analysis of variance showed a significant difference in the leaf area between cowpea variety ($p < 0.05$) (Fig 1)

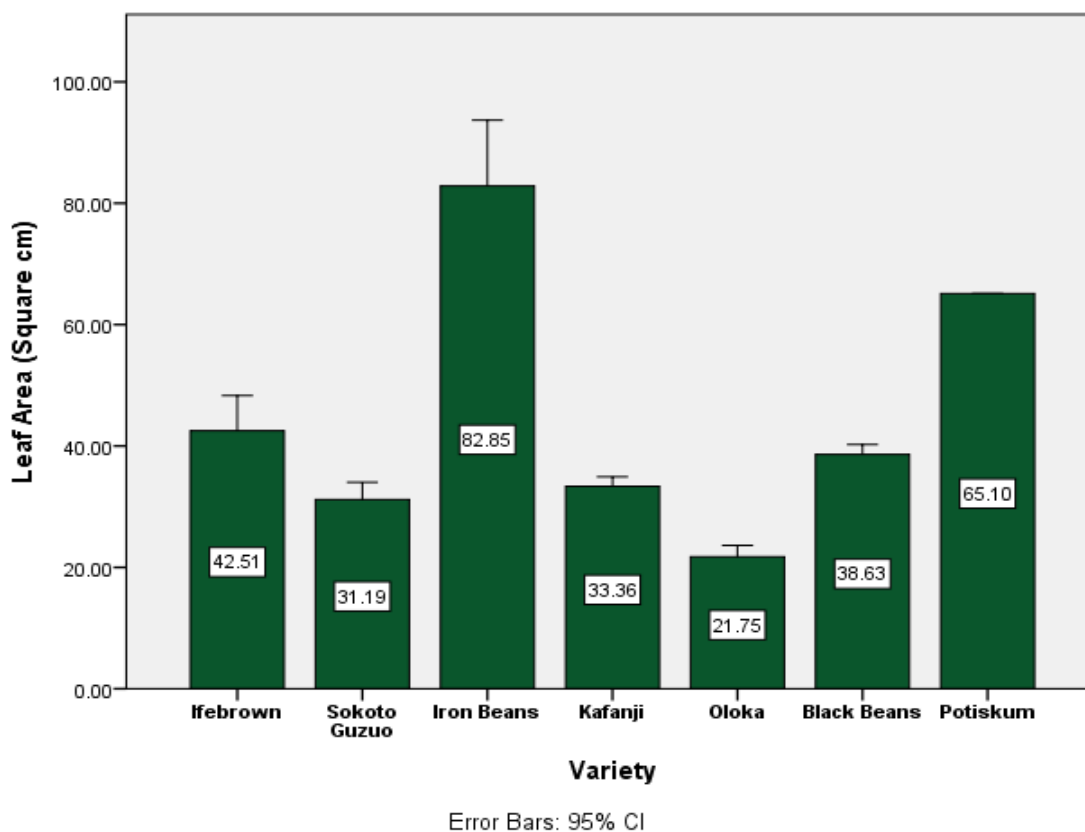


Figure 1: Showing the leaf area (cm²) of seven varieties of *Vigna unguiculata*

Var. Crowderpea and var. 'Potiskum' showed the highest length of petioluose (0.6cm) while 'oloka' showed the shortest length of petioluose (0.37cm). Analysis of variance showed a significant difference in the length of petioluose between cowpea variety ($p < 0.05$) (Fig 2).

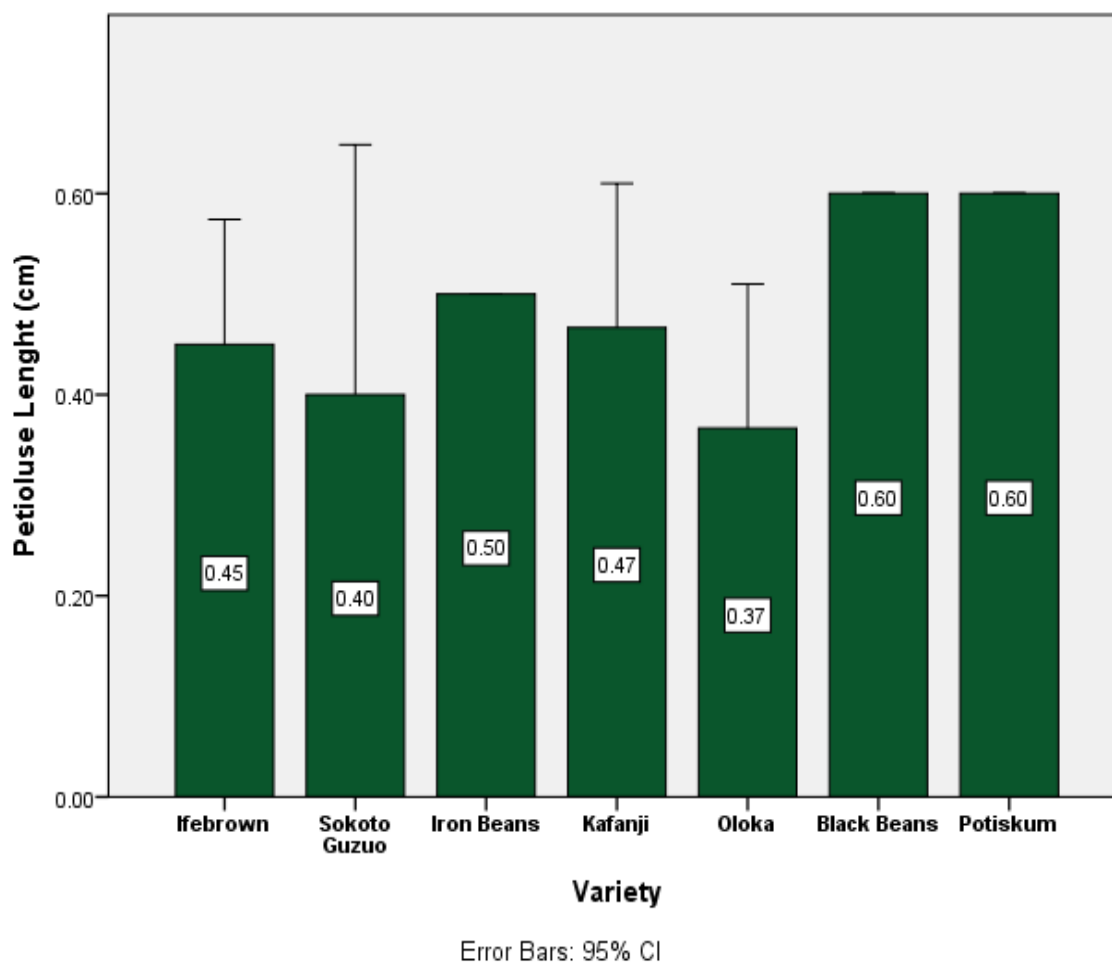


Figure 2: Showing the length of petioluose (cm) of seven varieties of *Vigna unguiculata*

Var. 'Iron beans' showed highest length of petiole (2.20cm) while Var. 'Oloka' showed the shortest length of petioles (1.47cm). Analysis of variance showed a significant difference in the length of petiole between cowpea variety ($p < 0.05$) (Fig 3).

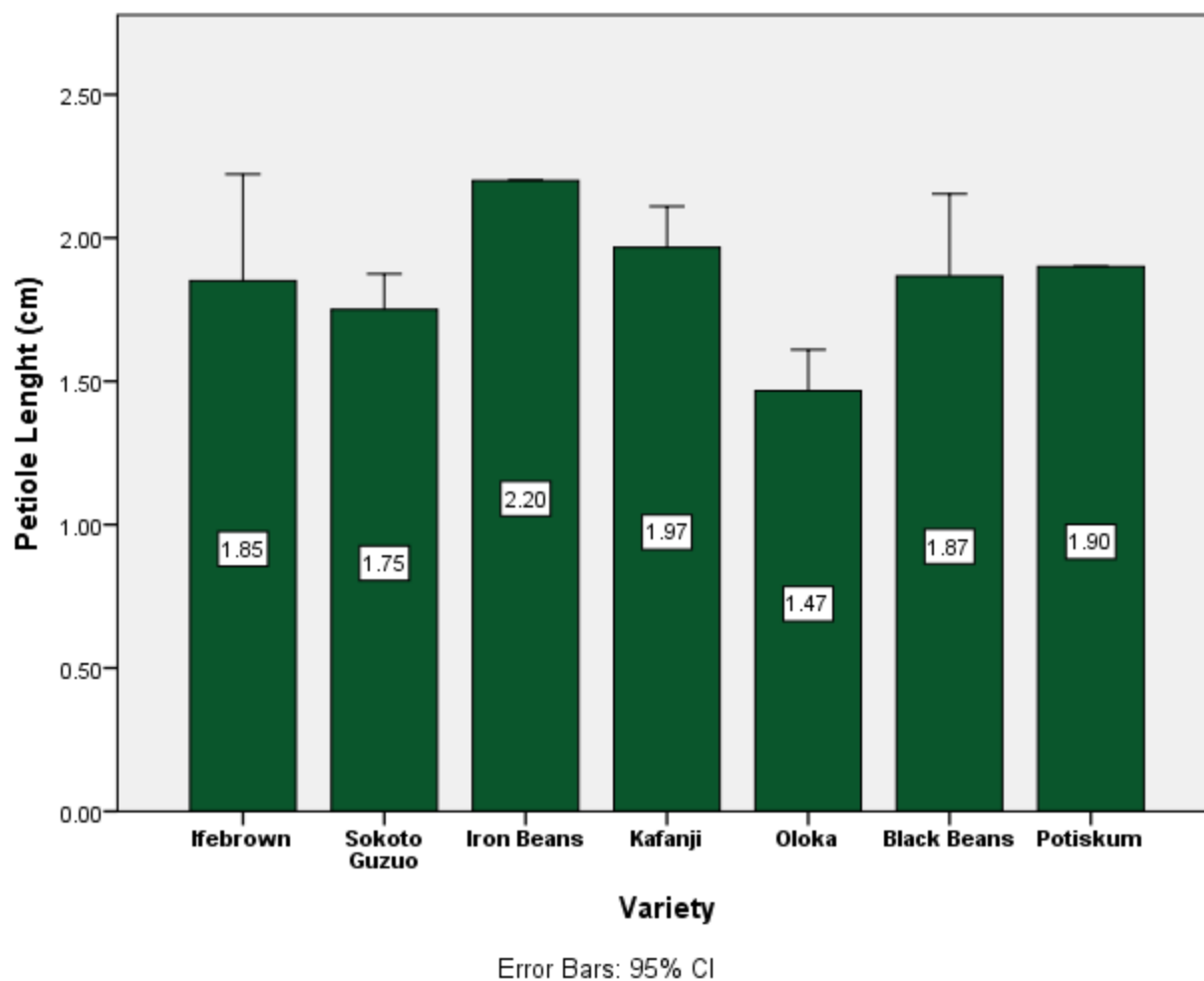


Figure 3: Showing the petiole length (cm) of seven varieties of *Vigna unguiculata*

Var. 'Iron beans' and Var. 'potiskum' showed the highest stem length (10cm) while 'oloka' showed the least stem length (7.67cm). Analysis of variance showed a significant difference in the stem length between cowpea variety ($p < 0.05$) (fig 4)

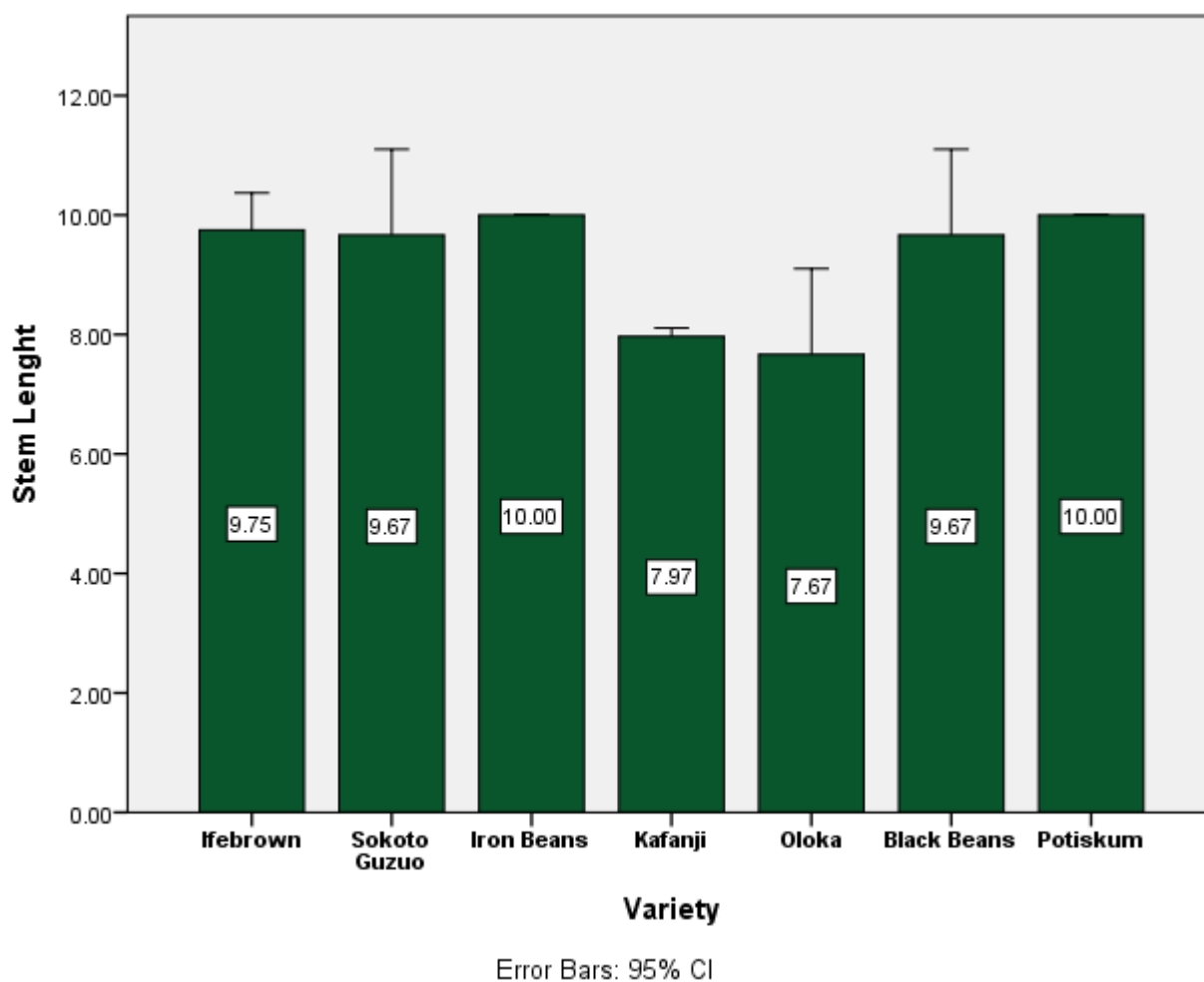


Figure 4: Showing the stem length (cm) of seven varieties of cowpea.

Var.'Ifebrown' showed the highest pod weight (3.93g) while Var.'Kafanji' showed the lowest pod weight (1.36g) (Fig 5).

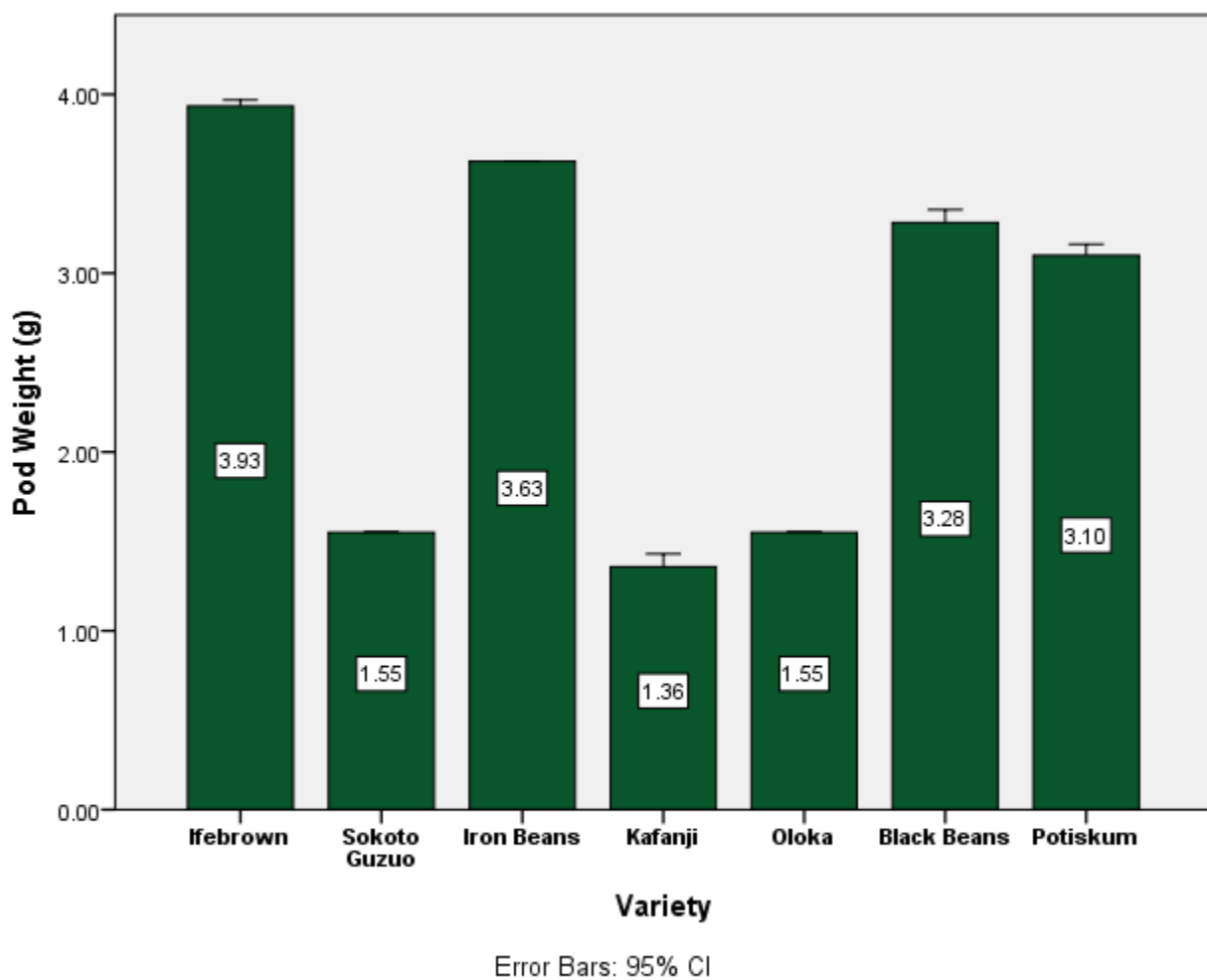


Figure 5: Showing the pod weight(g) of seven varieties of *Vigna unguiculata*.

Canonical discriminant functions based on their morphological features. Var 'Kafanji', 'Oloka' and 'Sokoto guzo' were grouped to one cluster indicating similarity in their morphological features while 'postikum', 'iron beans', crowderpea and 'Ifebrown' were grouped into a different cluster Fig 6.

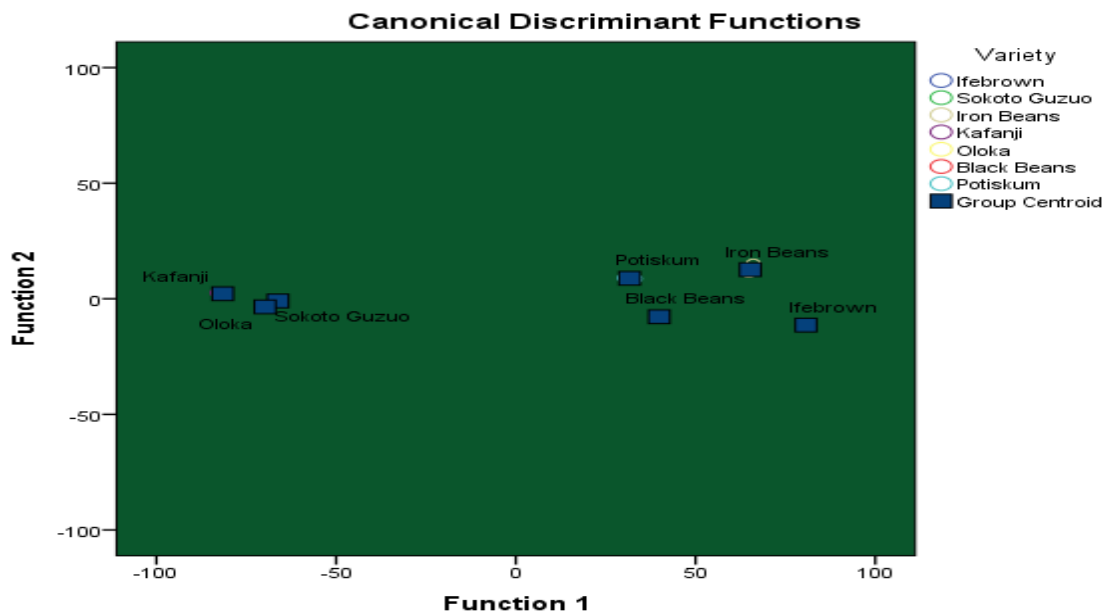


Figure 6: Showing canonical discriminant plot of the seven varieties of *Vigna unguiculata*.

4.2 Anatomical characters

Transverse section of the leaf of *Vigna unguiculata* “Ifebrown”, showed single layer of epidermis on both the upper and lower sides of the blades. Starch grains were visible in the cells. There were presence of angular collenchyma with small inter connected spaces. Parenchyma was thin walled with regularly shaped cells. Spongy and palisade tissues were also visible (Plate39).

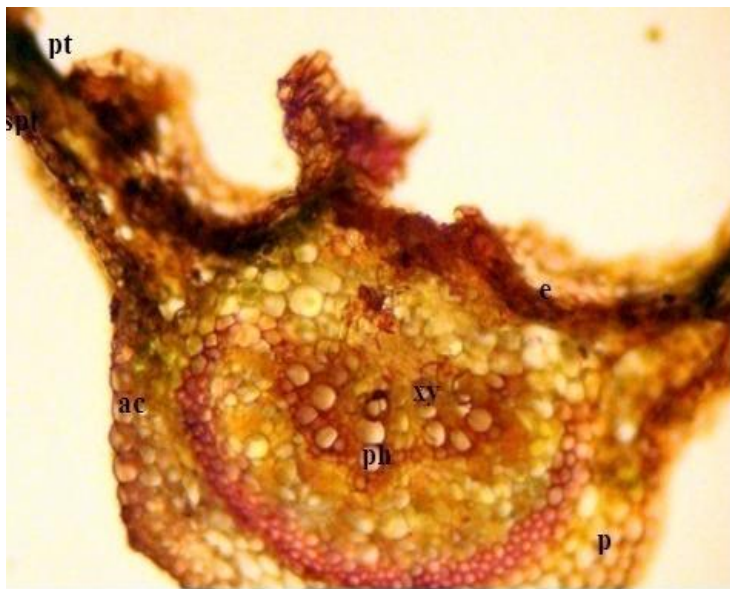


Plate 39: Transverse section of leaf of *Vigna unguiculata* “Ifebrown” × 40

pt = palisade tissue, spt = spongy tissue, ac = angular collenchymas, e = epidermis, ph = phloem
p = parenchyma, xy = xylem

Transverse section of the leaf of *Vigna unguiculata* var. 'Kafanji'; revealed the presence of a single layer epidermis on both sides of the blades. Inside the midrib was the presence of Parenchyma cells which were thin walled with regularly shaped cells. Collenchyma was angular with large and well inter connected spaces, and vascular bundles were also visible (plate 40).



Plate 40: Transverse section of leaf of *Vigna unguiculata* 'Kafanji' $\times 40$

ac =angular collenchyma, p =parenchyma, pt = palisade tissue, spt =spongy tissue, pi = pith v =vessels, sg = starch grains , e = epidermis

Transverse section of the leaf of *Vigna unguiculata* var. Crowder pea (black beans): showed single layered epidermal cells on both upper and lower sides of the blades, also visible were Palisade and spongy tissues. An angular collenchyma with large and well inter-connected spaces was present. Starch grains in the cells were visible. Parenchyma cells inside the midrib were thin walled with regularly shaped cells. The vascular bundles were visible (Plate41).



Plate 41: Transverse section of leaf of *Vigna unguiculata* Crowder pea (Black beans) ×40

Spt =spongy tissue, pt = palisade tissue e = epidermis, pi = pith, ph =phloem, xy = xylem, ac = angular collenchyma

Transverse section of the leaf of *Vigna unguiculata* var. "Sokoto guzo": showed single layered epidermal cells on both upper and lower sides of the blades. Starch grains in the cells were visible. Parenchyma cells inside the midrib were thin walled with regularly shaped cells. An angular collenchyma with large and well inter-connected spaces were observed. The vascular bundles were visible Also visible were spongy and palisade tissues (Plate42).

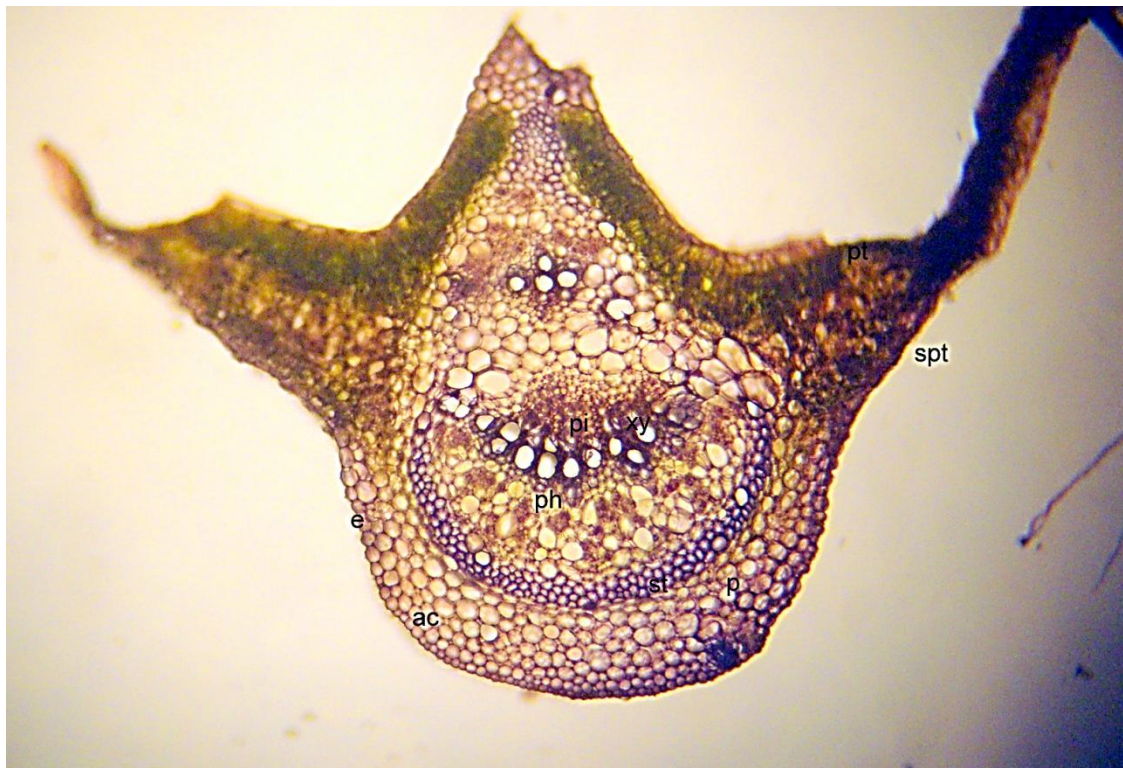


Plate 42: Transverse section of leaf of *Vigna unguiculata* "sokoto guzo" ×40

Spt = spongy tissue, pi = pith, pt = palisade tissue, ph = phloem , xy =xylem ,p =parenchyma,e = epidermis.

Transverse section of the leaf of *Vigna unguiculata* “Oloka: showed a single layerd epidermis on both sides of the blades. Parenchyma cells were thin layered with regularly shaped cells. It has an angular type of Collenchyma with small inter-connected spaces (plate43).



Plate 43: Transverse section of leaf of *Vigna unguiculata* “Oloka” ×40

Spt =spongy tissue, e = epidemis ,ac =angular collenchyma, ph =phloem, xy = xylem, pt = palisade tissue, p = parenchyma

Transverse section of the leaf of *Vigna unguiculata* "Potiskum": showed a single layered epidermal cell on both upper and lower sides of the blades. Starch grains in the cells were visible. Parenchyma cells inside the midrib were thin walled with regularly shaped cells. An angular collenchyma with small and well inter-connected spaces was found. The vascular bundles were visible Also visible were spongy and palisade tissues on the upper and lower blades (Plate 44).

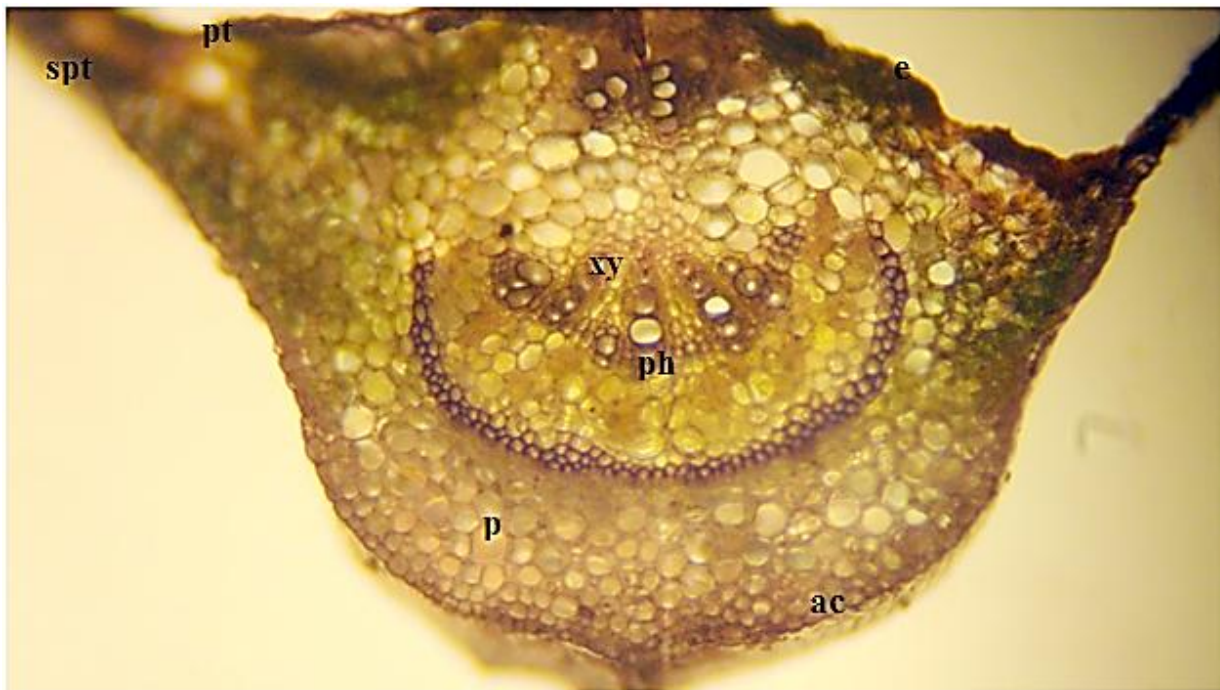


Plate 44: Transverse section of the leaf of *Vigna unguiculata* "Potiskum" ×40

Spt =spongy tissue, pt = palisade , ph = phloem, xy = xylem ac = angular collenchyma, p = parenchyma, e = epidermis

Transverse section of the leaf of *Vigna unguiculata* Iron beans, showed a single layered epidermal cells on both sides of the blades. Starch grains were visible stained in the cells. Parenchyma cells were thin layered with regularly shaped cells. Collenchyma cells were angular with well inter-connected spaces (Plate45).

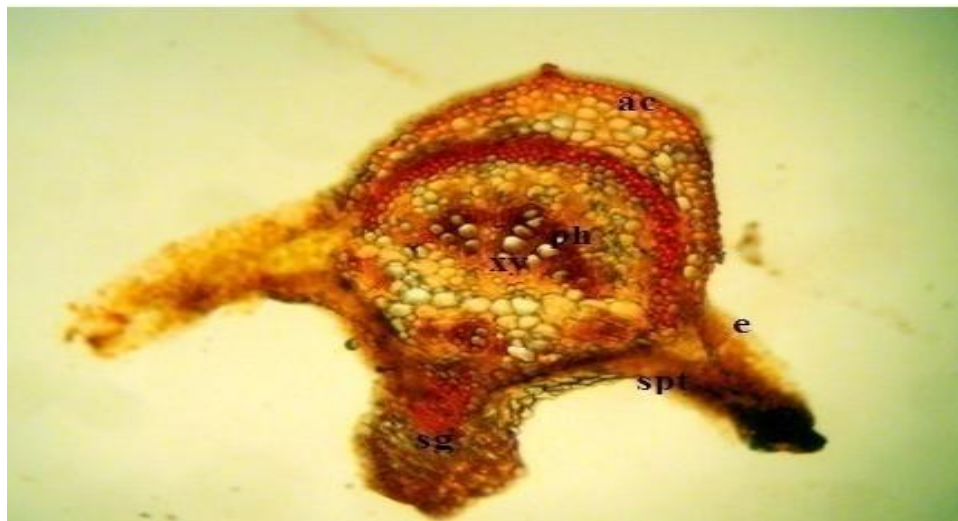


Plate 45: Transverse section of the leaf of *Vigna unguiculata* Iron beans $\times 40$

ac = angular collenchyma, ph = phloem, xy = xylem spt = spongy tissue ,sg = starch grains e = epidermis.

Transverse section of the stem of *Vigna unguiculata* "Ifebrown"; showed one layer of tearing epidermis. One layer of collenchyma was angular in shape having intercellular spaces which were in chains. Ring pores were present indicating growth. The pores were scattered (Diffuse) and round in shape. The arrangements of the pores were exclusively solitary. Apotracheial parenchyma was reticulate and the paratracheial parenchyma was banded. The rays were narrow and aggregate. The rays were non storied. The pith was large with vascular bundles present as in a typical dicotyledonous cell (Plate 46).

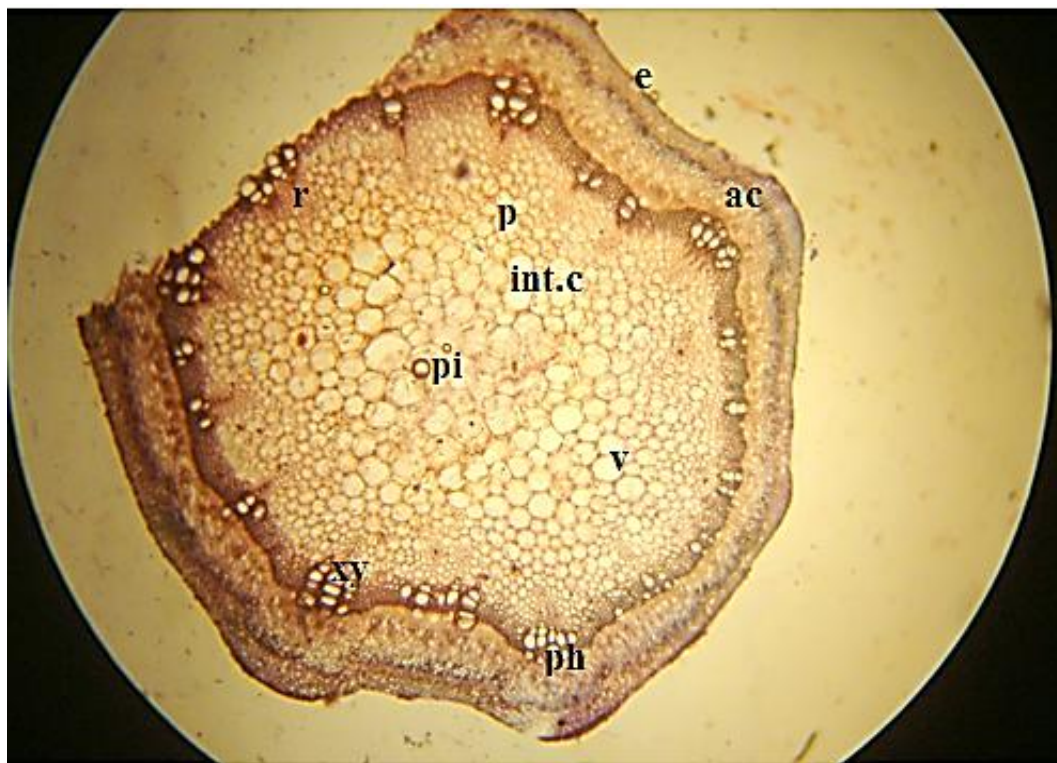


Plate 46: Transverse section of stem of *Vigna unguiculata* "Ifebrown" ×40

e = epidermis, pi = pith, int.c = intercellular spaces, p = parenchyma, v = vessels ph = phloem, xy = xylem, e = parenchyma, ac = angular collenchymas, r = ray

Transverse section of the stem of *Vigna unguiculata* 'Kafanji'; showed the epidermal layer as one layer, and an angular collenchymas present. Ring pores revealed growth. The pores were round in shape and diffusely arranged. Apotrachial parenchyma types were in diffuse aggregate. Paratrachial parenchyma was banded. Rays were narrow and in aggregate. Vascular bundles present were arranged in rings, also there was presence of large pith (Plate 47).



Plate 47: Transverse section of stem of *Vigna unguiculata* 'Kafanji' ×40
 rp = ray pores, pi = pith, ac = angular collenchymas, e = epidermis, xy - xylem

Transverse sections of the stem of *Vigna unguiculata* Crowderpea; there were presence of single layered epidermis and two layers of angular collenchyma. Ring pores of small sizes were scattered, indicating growth in the cells. The pores were angular in shape and arranged in radial multiples. Apotrachial parenchyma types were banded and the paratrachael parenchyma was also banded. Rays were more than half width of pores. Intercellular canals were narrow. Xylem and phloem alternates with one another (Plate 48).

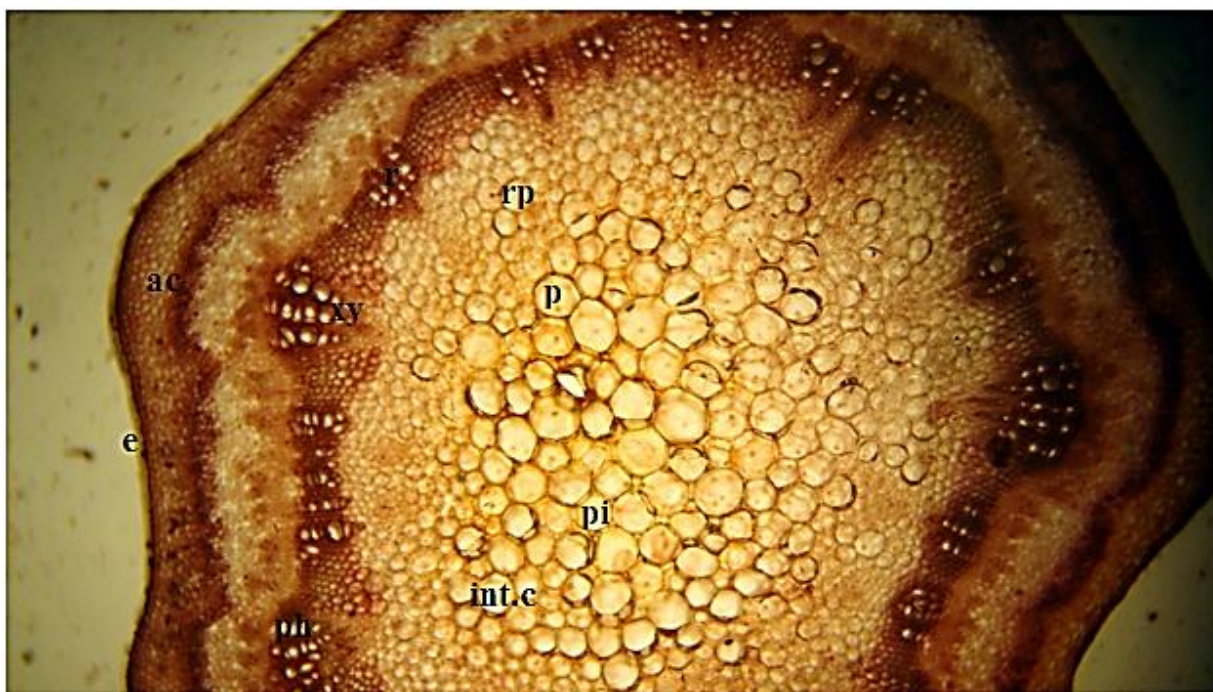


Plate 48: Transverse section of *Vigna unguiculata* “Crowderpea” ×40

e = epidermis, int.c = intercellular spaces, pi = pith, p = pararenchyma, rp = ray pores, ac = angular collenchyma, xy = xylem, ph = phloem

Transverse section of stem of *Vigna unguiculata* 'Potiskum' There was presence of one layer of epidermis. Ring pores were round and arranged in multiple radial, scattered in the cell indicating growth. Apotrachial parenchyma was banded and paratrachial were also banded. Ray sizes were in aggregate and storied. Normal intercellular canals were present. Collenchyma was present near the epidermis and the vascular bundles were arranged in rings with crushed protoxylem (Plate 49).

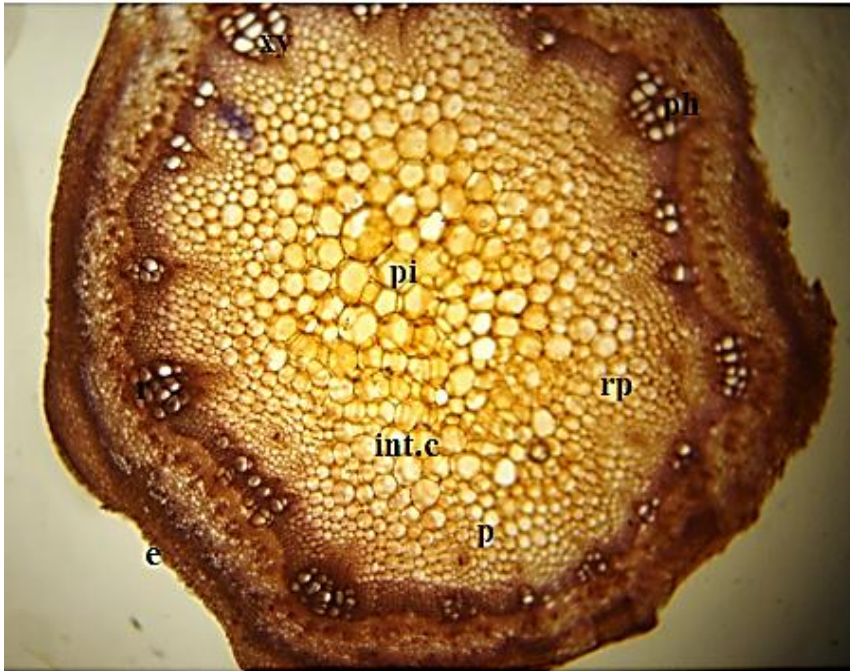


Plate 49: Transverse section of stem of *Vigna unguiculata* 'Potiskum' ×40

Xy = xylem, int.c = intercellular, p = parenchyma, rp = ray pores, e = epidermis, pi = pith

Transverse section of stem of *Vigna unguiculata* ‘Sokoto guzo’; the epidermal cells were single layered and the collenchymas was also one layer supporting the tissues. There were growth rings and pores that indicated growth. These pores or vessels were round in shape and arranged in multiple radial. The Apotracheal parenchyma types were terminal. The paratracheal parenchyma types were banded. Rays were in aggregates (transverse) and storied. Included phloem were visible; intercellular canals in cells were narrow and aggregate and crushed protoxylem were also visible (Plate50).

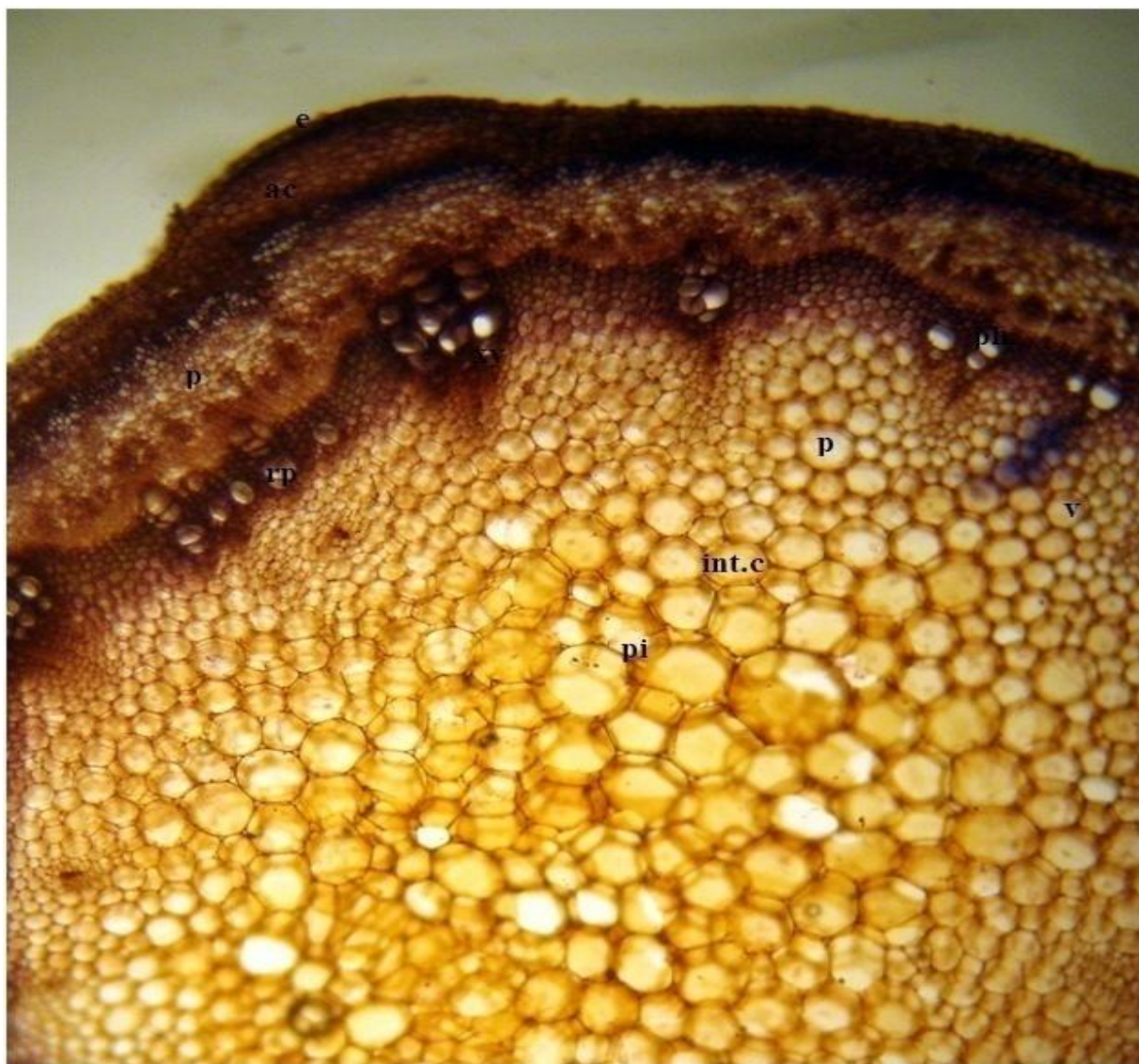


Plate 50: Transverse section of stem of *Vigna unguiculata* ‘Sokoto guzo’ ×40

P =parenchyma, int.c = intercellular spaces pi = pith, v = vessels, ac =angular collenchymas, e = epidermis, xy = xylem, ph = phloem.

Transverse section of the stem of *Vigna unguiculata* "Oloka": showed the presence of single layer epidermis, and a protective cork cell. Cortex was seen between the vascular system and a layer of collenchyma cell. There was presence of semi- ring pores indicating growth rings of various sizes in a diffuse form. Pores were angular in shape and in a multiple radial arrangement. Apotracheal parenchyma were diffuse in aggregate. The paratracheal parenchyma was banded. The rays in this variety were very transverse, in aggregates and storied. The intercellular canals were normal .There were presence of primary as well as conducting xylem and phloem cells. There were also presence of ridges and furrows round the epidermal layer (Plate51).

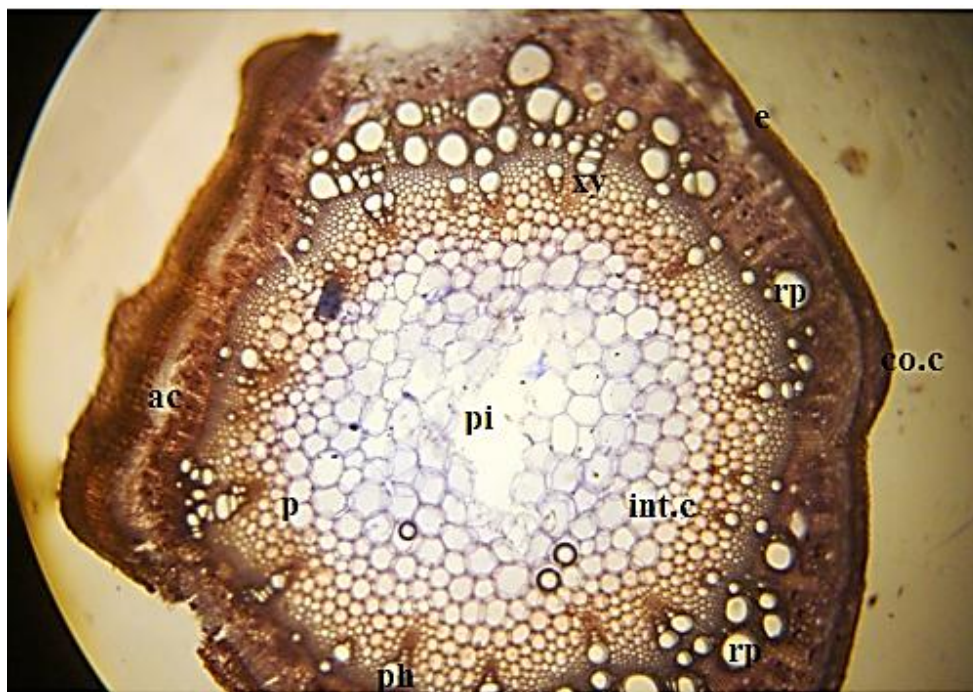


Plate 51: Transverse section of the stem of *Vigna unguiculata* "Oloka" ×40

int.c =intercellular spaces, pi =pith,ph =phloem,co.c =cork cell,rp =ring pores
e =epidermis,ac = angular collenchyma, xy = xylem

Transverse section of the stem of *Vigna unguiculata* Iron beans: showed single layer of epidermal cell and a layer of collenchyma. There was presence of ring pores of various sizes indicating growth. The pore arrangements were in radial multiple forms. The shapes of the pores were round and the rays were aggregate (transverse) and storied. The pith was crushed with and xylem and phloem alternating with each other. The apotraechial parenchyma was banded and also paratraechial parenchyma was banded and in aggregates (Plate 52).

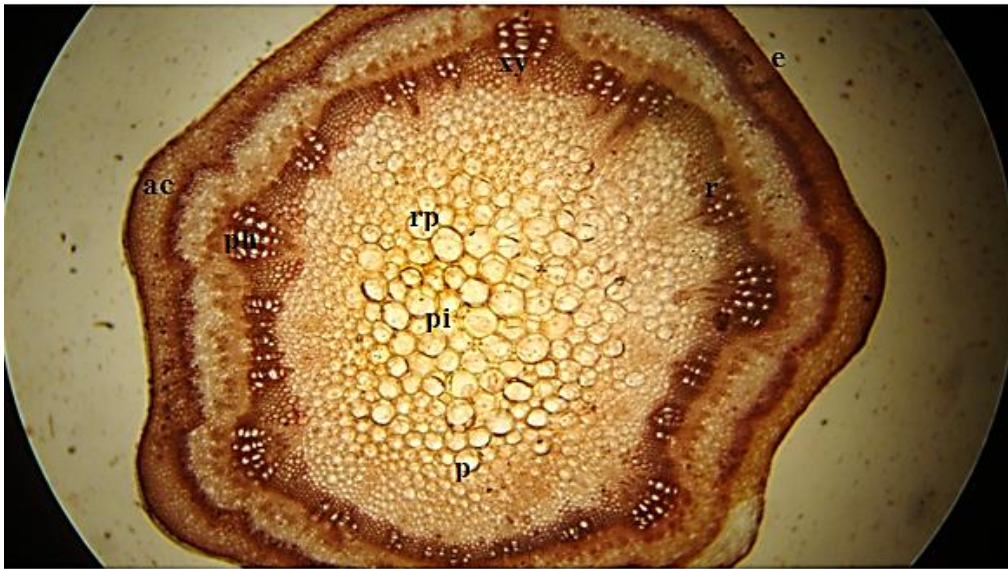


Plate 52: Transverse section of stem of *Vigna unguiculata* 'Iron beans' ×40

ac =angular collenchymas, pi =pith, p =parenchyma, e = epidermis,ac = angular collenchyma,ph
phloem, xy= xylem

Transverse section of petiole of *Vigna unguiculata* 'Ifebrown' showed single layered epidermis covered with a thickened cuticle. Angular collenchymas were observed immediately under the epidermis with 1- 2 layers of cells. The cortical parenchyma cells exhibited small intercellular spaces. Xylem and phloem were also present in the cortex (Plate 53).

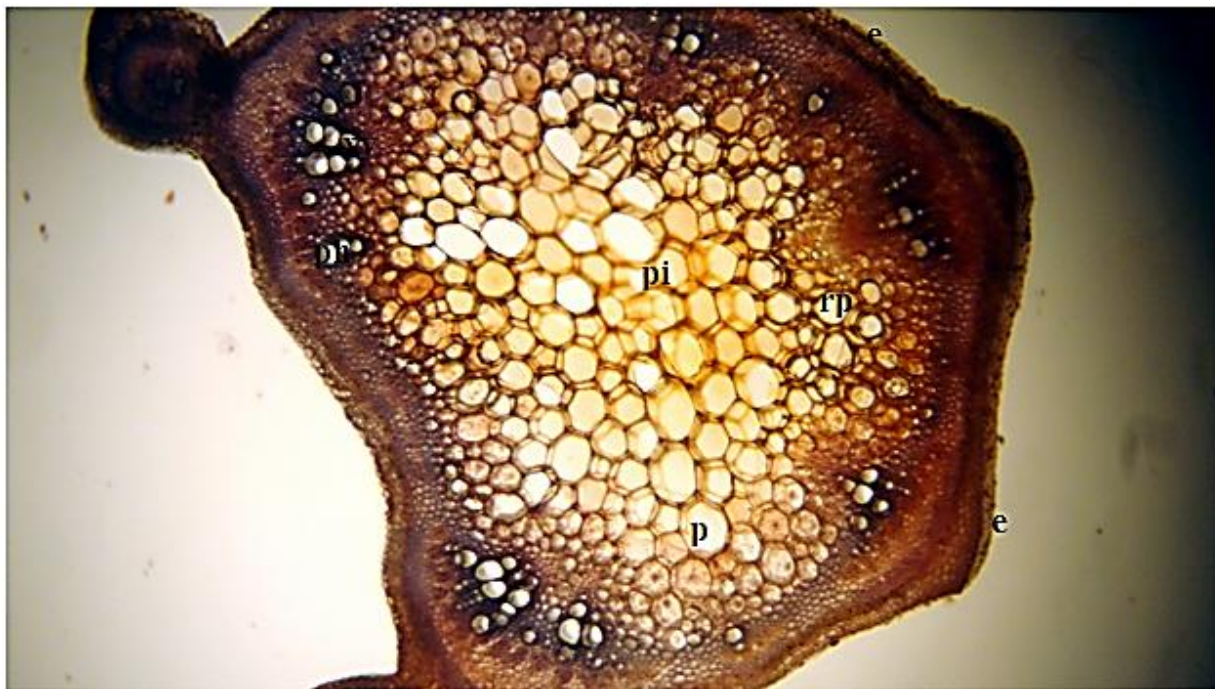


Plate 53: Transverse section of Petiole of *Vigna unguiculata* 'Ifebrown' × 40

e =epidermis, p =parenchyma, pi = pith, xy =xylem, ph =phloem, rp =ring pores

Transverse section of the petiole of *Vigna unguiculata* 'Kafanji' showed one layer of epidermal cells with 2-3 layers of cells. There were growth rings indicating growth. Angular collenchyma were also visible under the epidermis. Vessels were larger in sizes. Apotrachial and paratrachial parenchyma were arranged in diffuse aggregate and banded with narrow intercellular spaces. Xylem and phloem were observed alternating with one another (Plate 54).

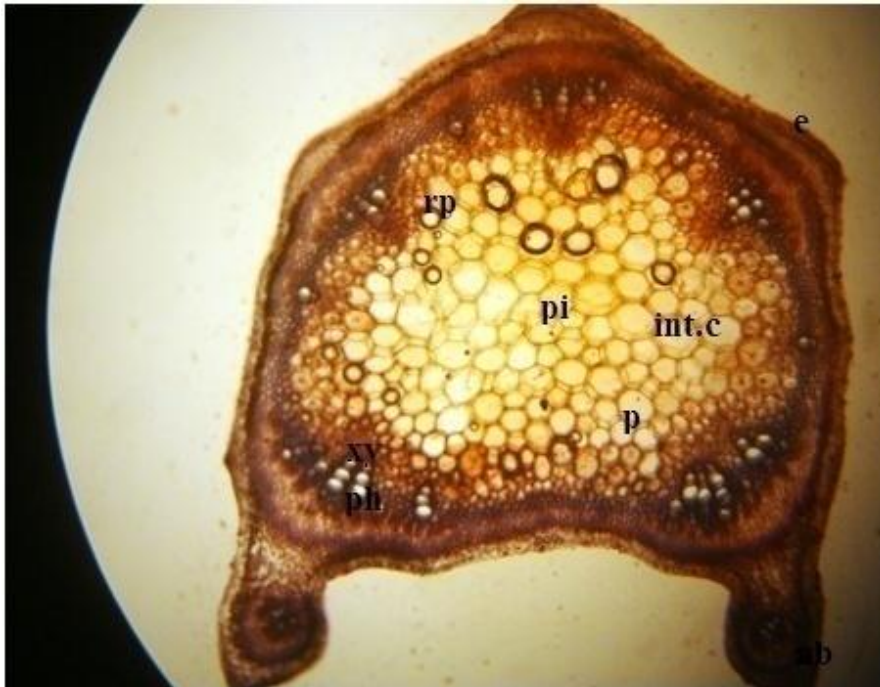


Plate 54: Transverse section of Petiole of *Vigna unguiculata* 'Kafanji' × 40

e = epidermis, rp = ring pores, int.c = intercellular spaces, p = parenchyma, rp = ring pores, ab = abaxial epidermal, pi = pith

Transverse section of petiole of *Vigna unguiculata* Crowderpea showed epidermis covered by thickened cuticle. This is followed by 2-3 layers of angular collenchymas. The external cortical parenchyma cells showed intercellular spaces of various dimensions. Xylem and phloem cells were also present (Plate55).

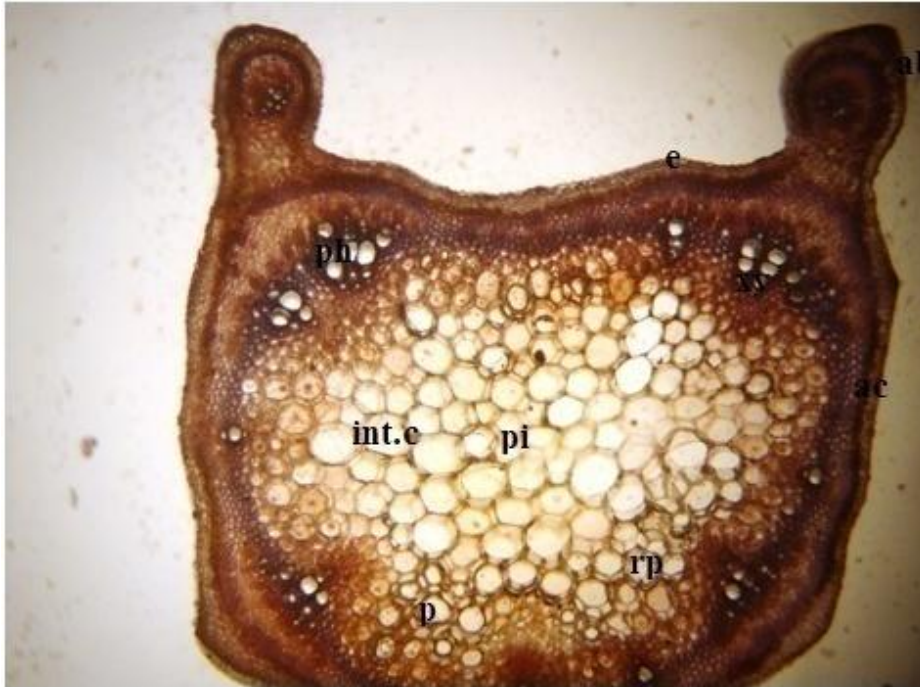


Plate55: Transverse section of Petiole of *Vigna unguiculata* Crowderpea $\times 40$

e =epidermis, int.c =intercellular spaces, pi =pith, rp = ring pores, xy =xylem, ac = angular collenchymas, p =parenchyma, ph phloem, ab =abaxial

Transverse section of petiole of *Vigna unguiculata* “Potiskum” showed a single layer of epidermis. Pore shape was round and pores arranged in radial multiple. Growth rings present indicated growth. Angular collenchyma was seen immediately under the epidermal cells. Vascular bundles present were arranged in rings. Paratrachial parenchyma and apotrachial were banded (Plate56).

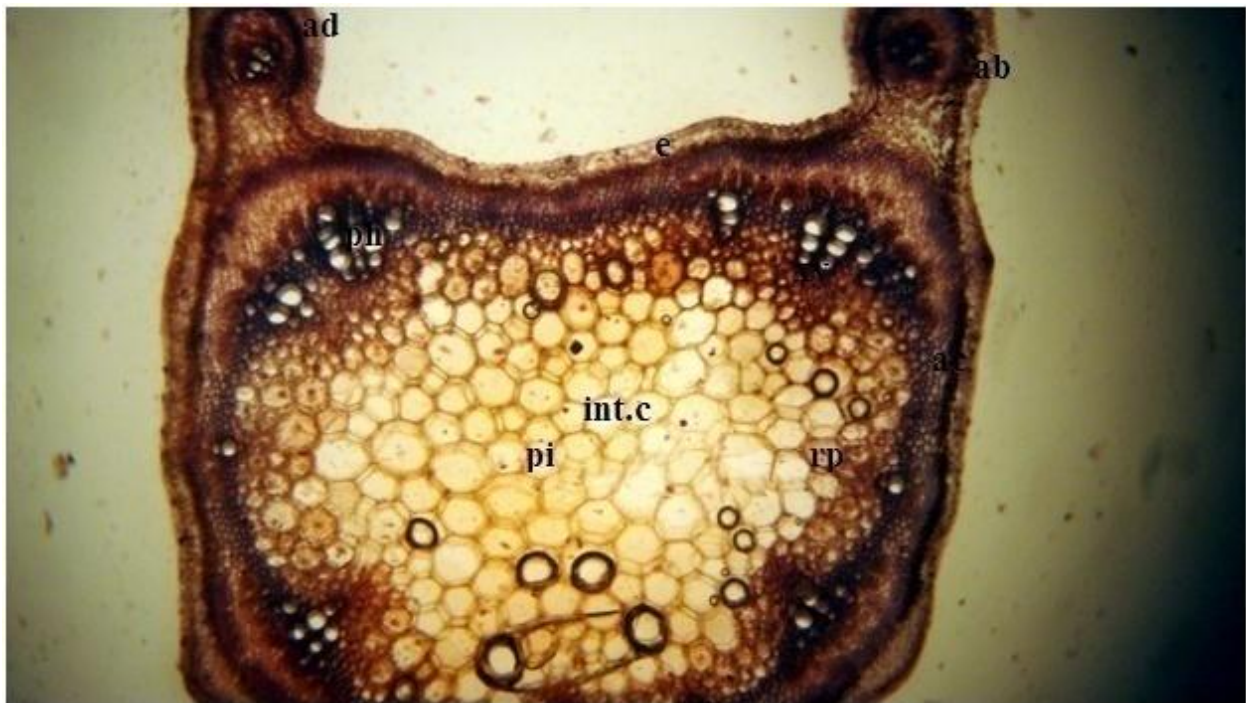


Plate56: Transverse section of Petiole of *Vigna unguiculata* ‘potiskum’ ×40

ab =abaxial epidermal, ad= adaxial, int.c =intercellular spaces, rp =ring pores, pi =pith, ph=phloem, e = epidermis, ac= angular collenchyma

Transverse section of petiole of *Vigna unguiculata* 'Sokoto Guzo'; showed a single layer of epidermis covered with a thickened cuticle. Growth rings were scattered in the cortex indicating growth. Angular collenchyma was present just below the epidermal cells. Paratrachial and apotrachial are banded. Intercellular canal was normal. Phloem and xylem were also present (Plate 57).



Plate 57: Transverse section of petiole of *Vigna unguiculata* Sokoto Guzo $\times 40$

Ph =phloem, xy =xylem, rp =ring pores, p =parenchyma,ac =angular collenchymas, pi =pith, e =epidermis,ab =abaxial epidermal, ad = adaxial epidrmal

Transverse section of petiole *Vigna unguiculata* Iron beans; showed the presence of single celled epidermal layer covered by a thickened cuticle. This was followed by 1-2 layers of angular collenchyma. The cortical parenchyma cells showed normal intercellular spaces of various dimensions and rays were aggregate and storied (Plate 58).

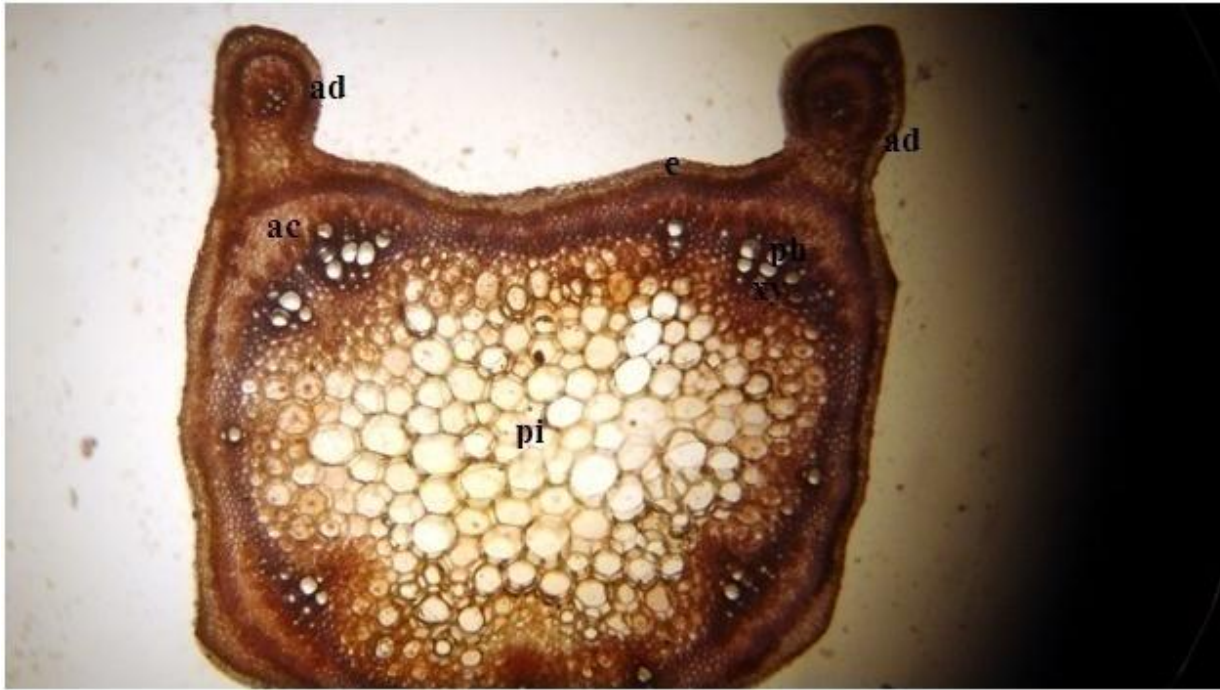


Plate 58: Transverse section of petiole of *Vigna unguiculata* Iron beans $\times 40$

ad =adaxial ,ab =abaxial, e =epidermis, ph =phloem, ac = angular collenchyma, xy =xylem ,e =epidermis

Transverse section of petiole of 'Oloka'; showed the presence of one layer of epidermal cell. Trichome was visible. Under the epidermal cell were the presence of two layers of angular collenchyma. Intercellular spaces were of various dimensions. Rays are arranged in aggregate with xylem and phloem visible in the pith (Plate 59).



Plate 59: Transverse section of petiole *Vigna unguiculata* 'Oloka' ×40

ad = adaxial, ab = abaxial, ac = angular collenchymas, xy = xylem, ph = phloem, p = parenchyma, pi = pith

Transverse section of the root of *Vigna unguiculata* "Ifebrown"; showed the presence of single epidermis. Presence of terminal parenchyma. The ring pores indicate growth. The pores were angular in shape and arranged in clusters. The parenchyma apotraechial was banded. Paratracheal parenchyma was banded. Sizes of the rays were transverse and storied. Pith was large and crushed (Plate 60).

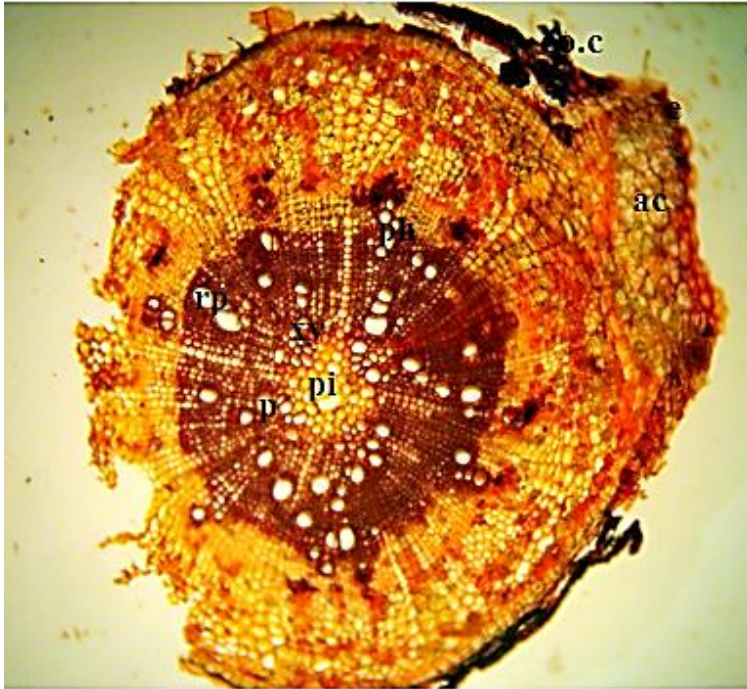


Plate 60: Transverse section of Ifebrown root $\times 40$

Co.c =cork cell, pi =pith, rp =ring pores, p =pararenchyma, ac = angular collenchymas, e =epidermis

Transverse section of the root of *Vigna unguiculata* 'Kafanji'; showed Presence of one layer of epidermis and an angular collenchyma and parenchyma cells having intercellular spaces. Ring pores were seen indicating growth. The growth rings were formed and diffuse porous. The shapes of the pores were round and the arrangements were exclusively solitary. Parenchyma was apotrachial and in diffuse aggregates while parenchyma of paratrachial types were banded. The ray sizes were more than half width of pores and storied (Plate61).



Plate 61: Transverse section of 'Kafanji' root $\times 40$

rp= ring pores, ac = angular collenchyma, pi = pith, xy= xylem, ph= phloem, p = parenchyma

Transverse section of the root of *Vigna unguiculata* Crowderpea showed the presence of one layer of epidermis. Angular collenchyma was present immediately after the epidermis. The growth rings which indicated growth were round and exclusively solitary. Apotrachial parenchyma was diffuse and aggregate. Paratrachial parenchyma present was broad and conspicuous. Rays were wider than half width with traumatic intercellular canals (Plate 62).



Plate 62: Transverse section of root Crowder pea $\times 40$

Ac=angular collenchymas, p= parenchyma, ph= phloem rp= ring pores, pi=pith e=epidermis

Transverse section of the root *Vigna unguiculata* 'Potiskum' showed single layer of epidermis with an angular collenchyma just below the epidermal layer. Growth rings inside the cortex were of various dimensions. Pore size was round and pores were exclusively solitary. Apotrachial parenchyma was in diffuse aggregate. Paratrachial parenchyma were broad and conspicuous. Ray sizes were more than half width of the pores with intercellular canals (Plate 63).

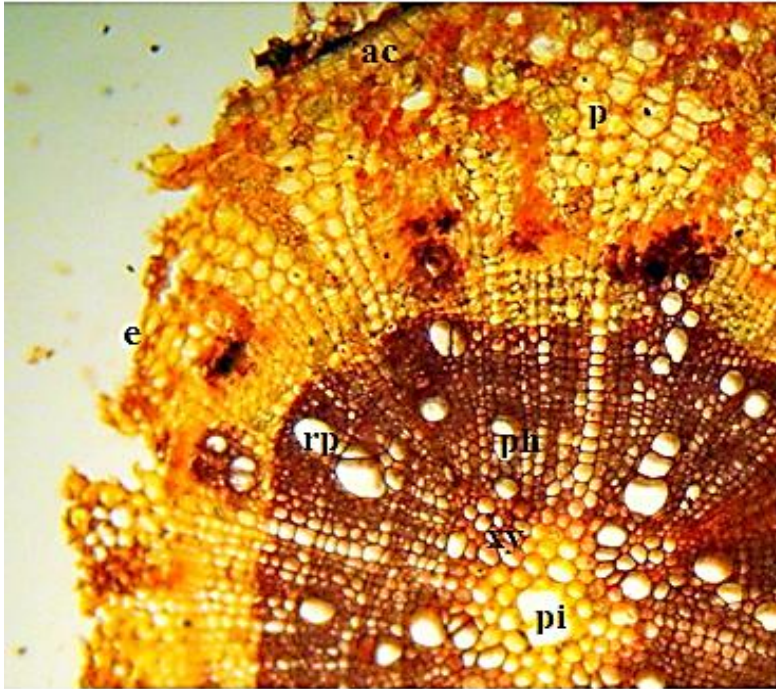


Plate 63: Transverse section of 'Potiskum' root $\times 40$

e = epidermis, rp = ring pores, ac = angular collenchyma, ph = phloem, xy = xylem, pi = pith

Transverse section of root of 'Sokoto guzo': showed one layer of epidermis. Collenchyma was 1-2 layers and angular in shape. Growth rings are indicated growth. Arrangement of pores was in radial multiple. Apotrachial and paratrachial were banded. Ray sizes were aggregate and storied. Intercellular canals are normal (Plate 64).

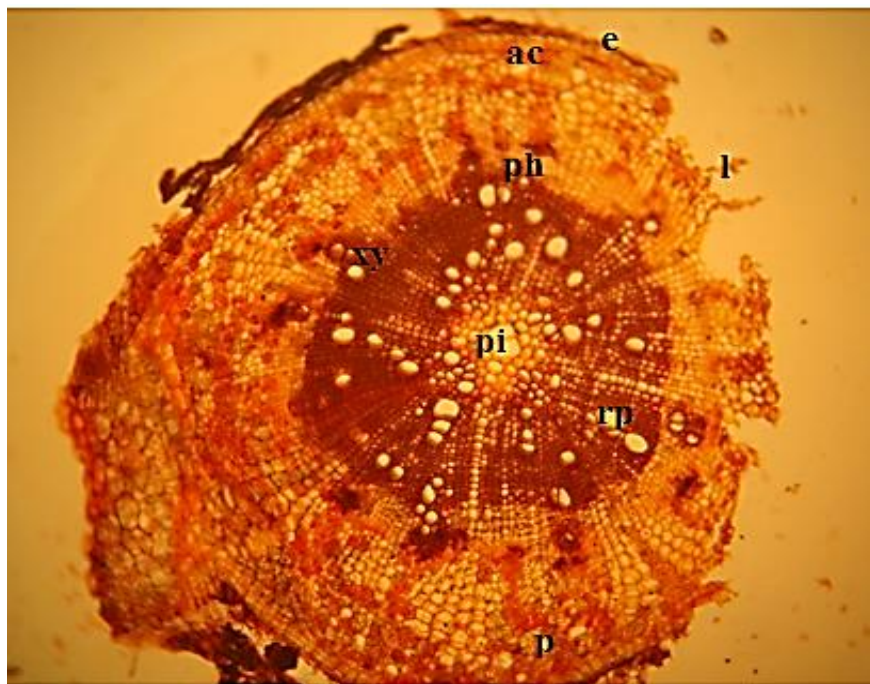


Plate 64: Transverse section of root of 'Sokoto guzo' ×40

e =epidermis, ac=angular collenchymas, rp=ring pores, p=parenchyma, pi=pith, xy=xylem,
ph=phloem, l = lenticel

Transverse section of root of “Oloka” showed a single layer of epidermis. Collenchyma was angular. Growth rings present were round indicating growth. Inside the pith were vessels of different dimensions. Arrangement of pores was exclusively solitary. Apotrachial parenchyma was reticulate and paratrachial parenchyma was scanty and vasicentric. Ray sizes were wider than pores and intercellular spaces were normal (Plate 65).

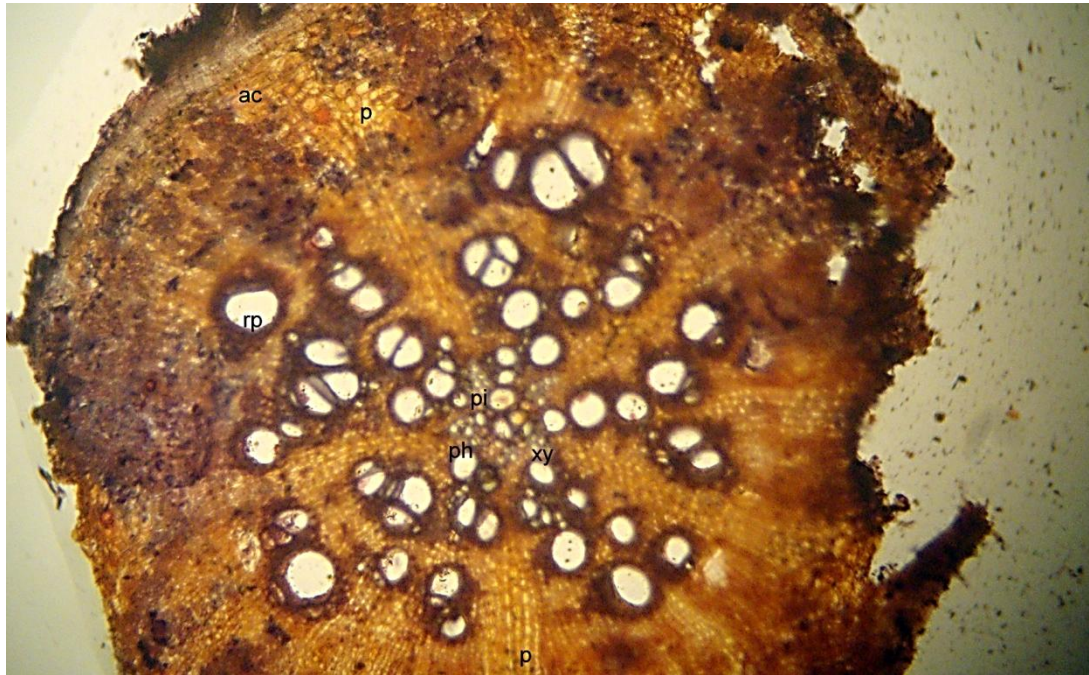


Plate 65: Transverse section root of ‘Oloka beans’ ×40

ac=angular collenchymas,p=parenchyma, ph=phloem, xy=xylem, pi=pith,e=epidermis

Transverse section of root of Iron beans; showed one layer of epidermal layer. Collenchyma was angular. Growth rings present indicated growth. Pores were round and exclusively solitary. Apotarchial parenchyma was diffuse in aggregate and paratrachial parenchyma was broad and conspicuous. Ray sizes were more than half width of pores. Intercellular canals were normal (Plate66).



Plate 66: Transverse section of root of “Iron beans’ ×40

e = epidermis, P= Parenchyma, Co.c=Cork cell, rp=ring pores,Pi=Pith, ac=angular collenchyma

Epidermal Studies

Results of the leaf epidermal features of the seven varieties of *Vigna unguiculata* studied showed similarities and differences both at the adaxial and abaxial surfaces. Summary of the cell shapes, epidermal wall types and the stomatal characteristics with their ranges were presented.

The most common stomatal type among the seven varieties studied were paracytic and anisocytic types of stomata at the abaxial and adaxial surfaces of the epidermal peels. The seven varieties also had irregular cell shape and anticlinal cell wall (Table 5).

Table 5: Stomata types, index and shape of epidermal cells

PLANT		STOMATAL TYPE	STOMATAL INDEX (%)	SHAPE OF EPIDERMAL CELLS
IRON	Upper	PARACYTIC	8.0 U.E	IRREGULAR
BEANS	Lower	PARACYTIC	8.82 L.E	IRREGULAR
OLOKA	Upper	PARACYTIC	8.57 U.E	IRREGULAR
	Lower	PARACYTIC	23.08 L.E	IRREGULAR
KAFANJI	Upper	PARACYTIC	8.85 U.E	IRREGULAR
	Lower	PARACYTIC	17.81 L.E	IRREGULAR
POTISKUM	Upper	PARACYTIC	9.09 U.E	IRREGULAR
	Lower	PARACYTIC	15.39 L.E	IRREGULAR
IFE BROWN	Upper	ANISOCYTIC	13.04 U.E	IRREGULAR
	Lower	PARACYTIC	15.39 L.E	IRREGULAR
CROWDER	Upper	PARACYTIC	16.67 U.E	IRREGULAR
PEAS	Lower	PARACYTIC	22.58 L.E	IRREGULAR
SOKOTO	Upper	PARACYTIC	16.69 U.E	IRREGULAR
GUZO				
	Lower	PARACYTIC	24.24 L.E	IRREGULAR

U.E: Lower epidermis

L.E: Lower epidermis

The abaxial or the upper surface of 'Ironbeans' epidermal shows stomatal index of 8.0% U.E, an rregular cell shape, paracytic stomata type, a moderate cell wall thickness and a curved contour (Plate67).

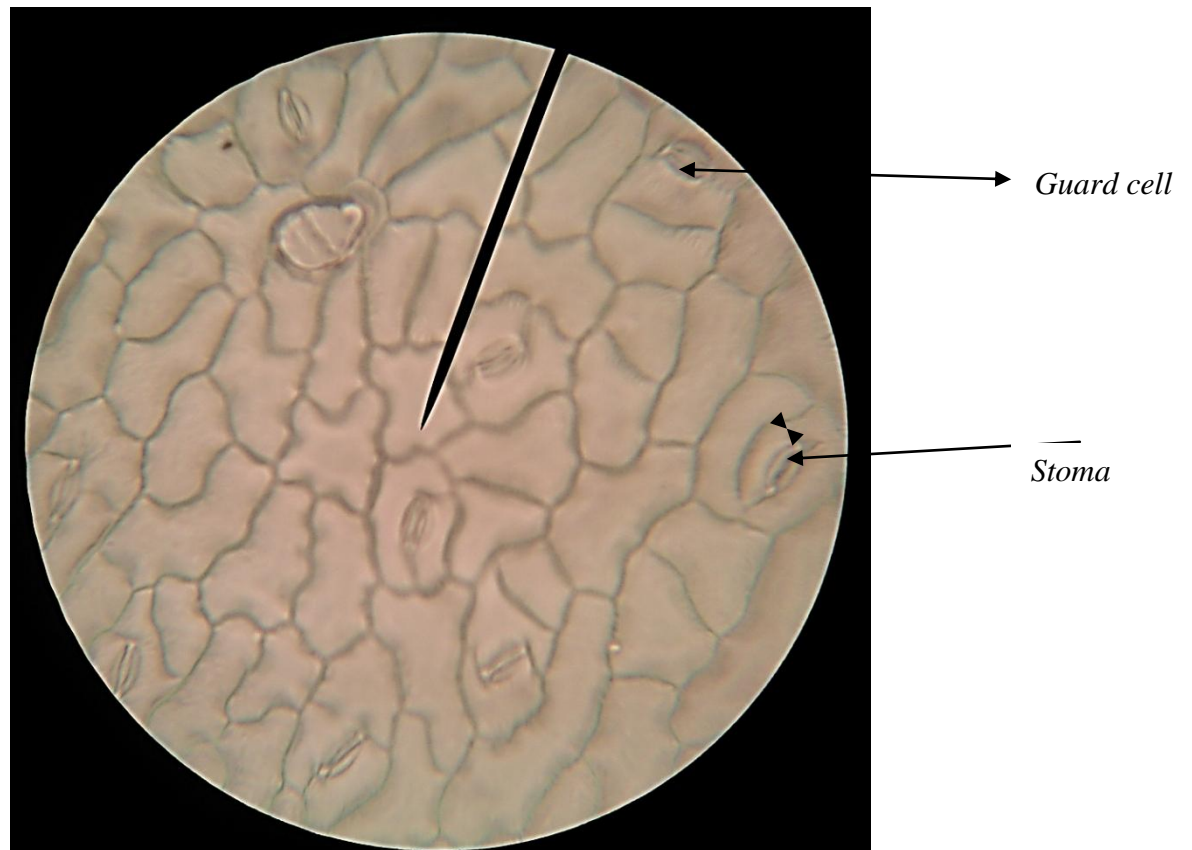


Plate 67: Abaxial or upper surface of Iron beans $\times 400$

The adaxial or lower surface of Ironbeans epidermal revealed 8.82% L.E stomatal index, irregular cell shape, paracytic stomata type, moderate cell wall thickness and a curved epidermal contours (Plate 68).

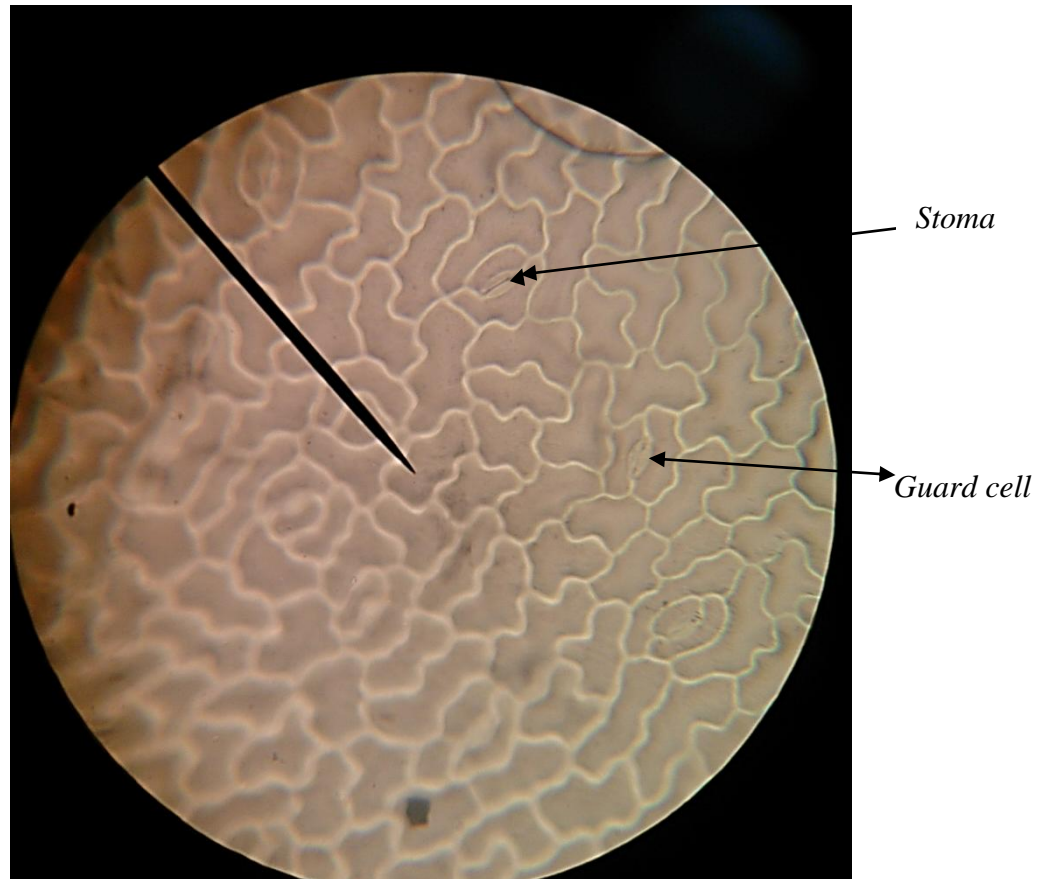


Plate 68: Adaxial or lower surface of Iron beans $\times 400$

The abaxial surface or upper surface of 'Oloka' had 8.57.04% U.E stomatal index, an irregular cell shape, paracytic stomata type, a moderate cell wall thickness and an epidermal curved contour (Plate 69).

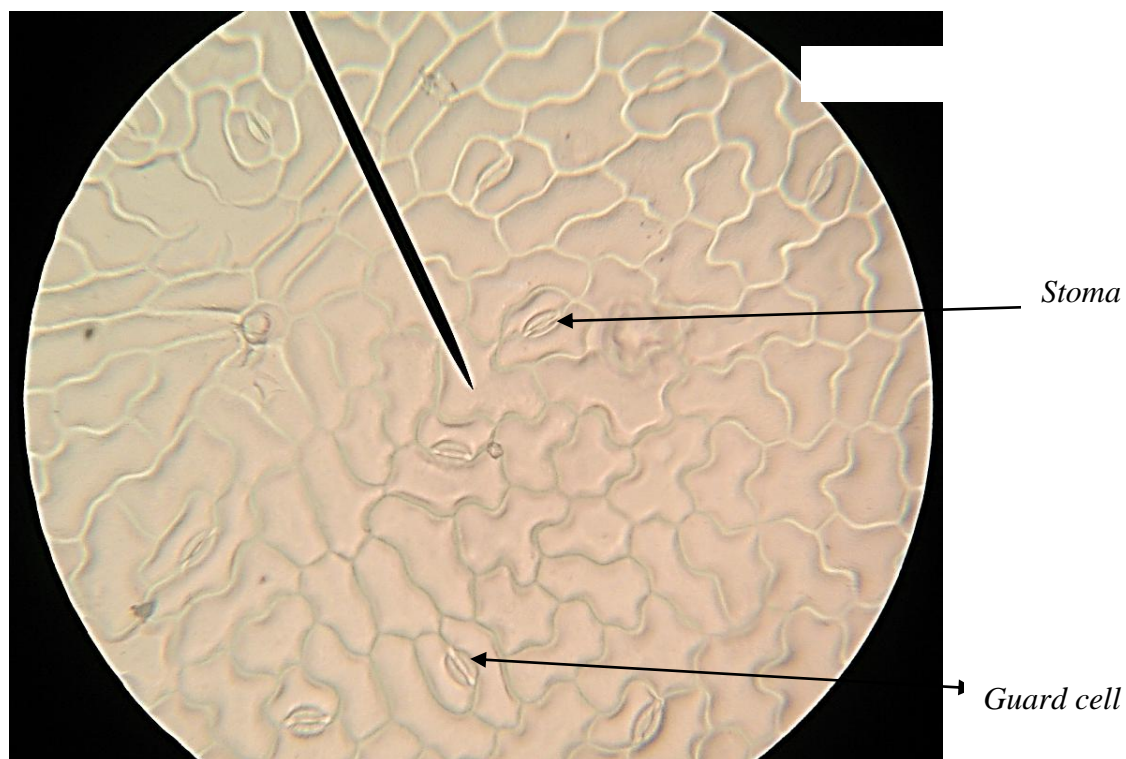


Plate 69: Abaxial or upper surface of 'Oloka' beans $\times 400$

The adaxial or the lower surface of 'Oloka' epidermal revealed 23.08% L.E stomatal index, an irregular cell shape, paracytic stomata type, moderate cell wall thickness and curved epidermal contours (Plate 70)

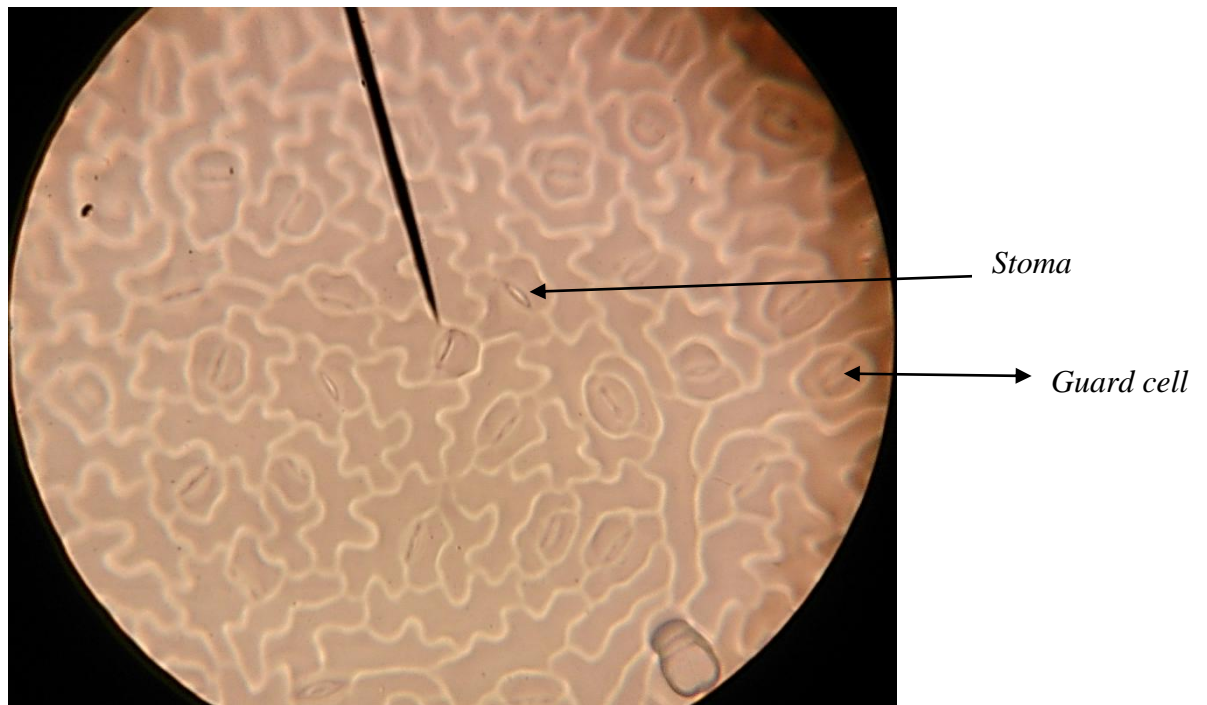


Plate 70: Adaxial or lower surface of 'Oloka' beans $\times 400$

The abaxial or upper surface of 'Kafanji' revealed the presence of paracytic stomata type 8.85% U.E stomatal index, irregular epidermal cell shape, a moderate cell wall thickness and a curved epidermal contour (Plate 71).

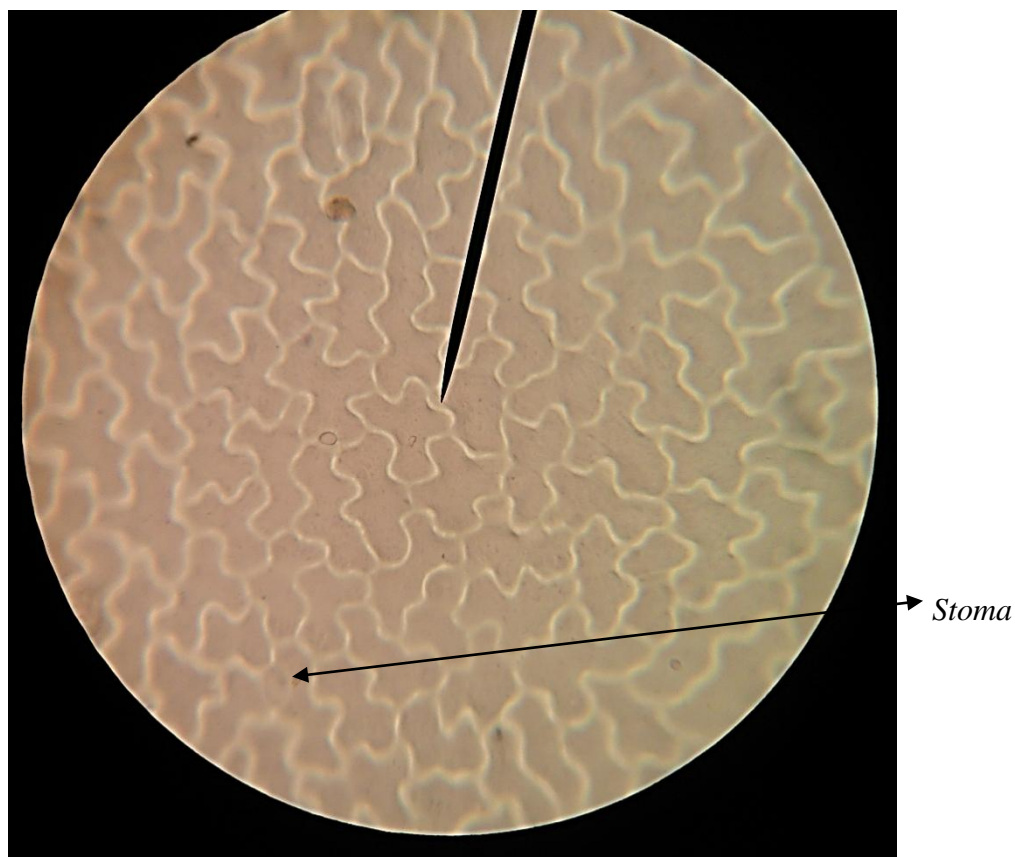


Plate 71: Abaxial or upper surface of 'kafanji' beans $\times 400$

The adaxial or lower surface of Var. 'Kafanji' revealed the presence of stomatal index as 17.81% L.E ,an irregular epidermal cell shape,paracytic type of stomata,a moderate cell wall thickness and a curved epidermal contour (Plate 72).

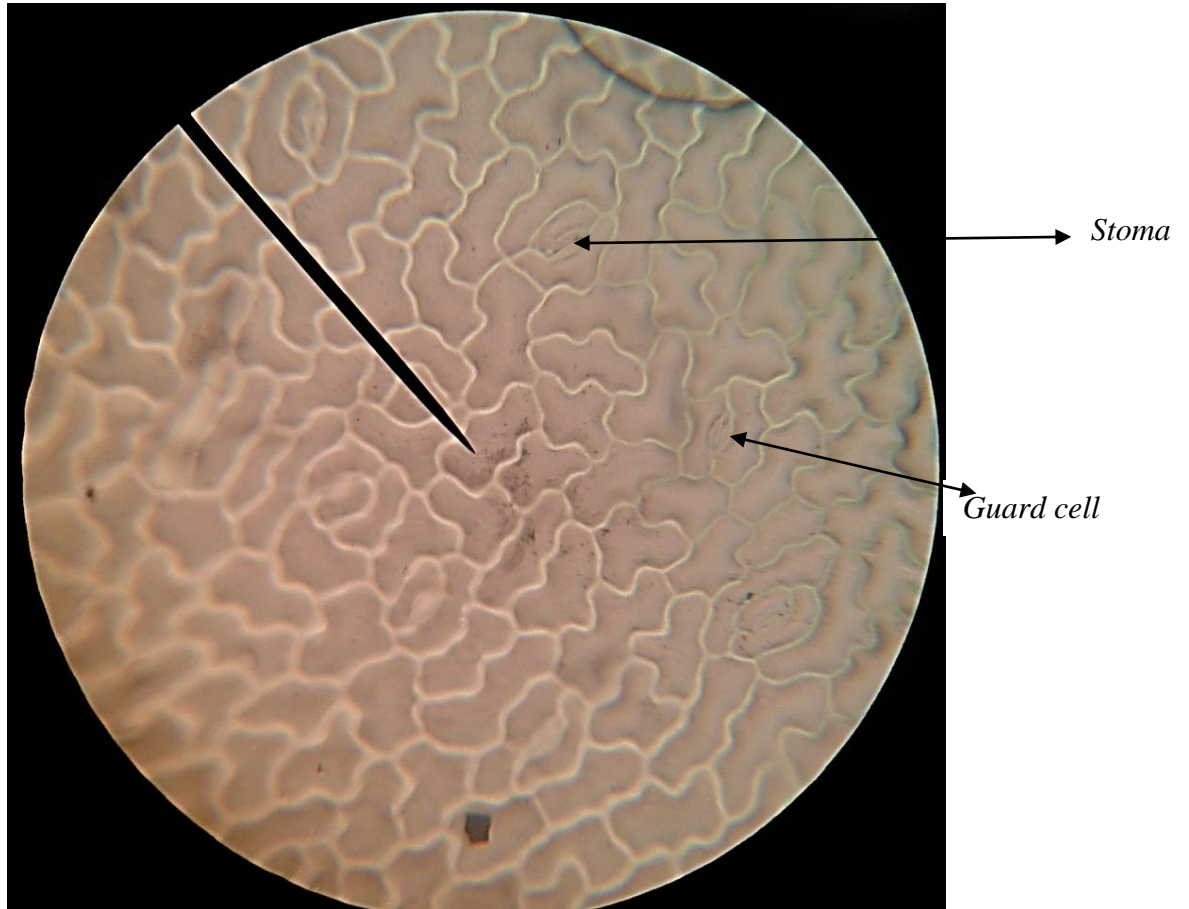


Plate 72: Adaxial or lower surface of 'Kafanji' beans $\times 400$

The abaxial or the upper surface of 'Potiskum' leaf epidermal shows the presence of 9.09% U.E stomatal index, an irregular epidermal cell shape, and paracytic stomata. A moderately cell thickness and an epidermal curved contours (Plate 73).

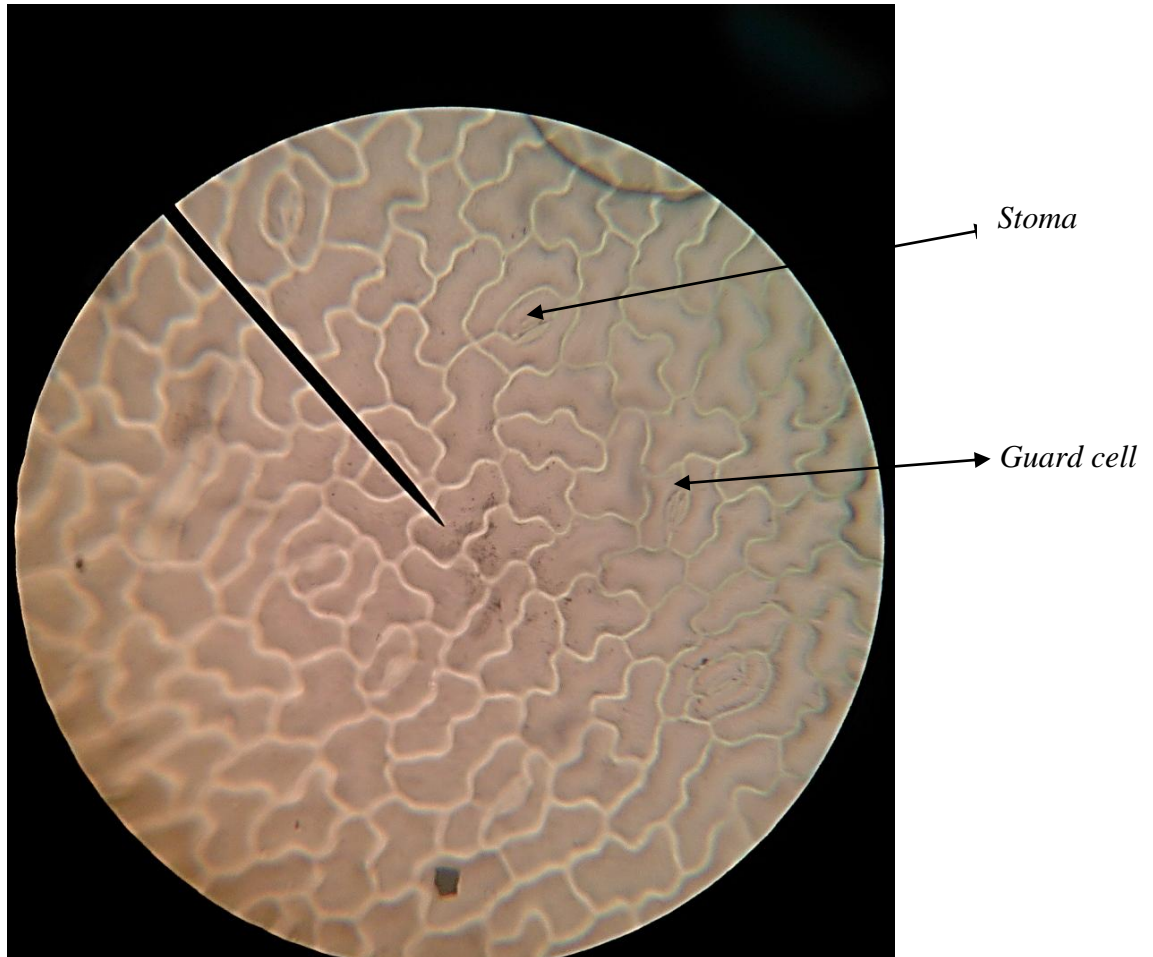


Plate 73: Abaxial or upper surface of 'Potiskum' beans. $\times 400$

Adaxial or the lower surface of 'Potiskum' shows the presence 15.39% L.E stomatal index, an irregular epidermal cell shape, paracytic stomata types, moderately cell wall thickness and epidermal contours (Plate 74).

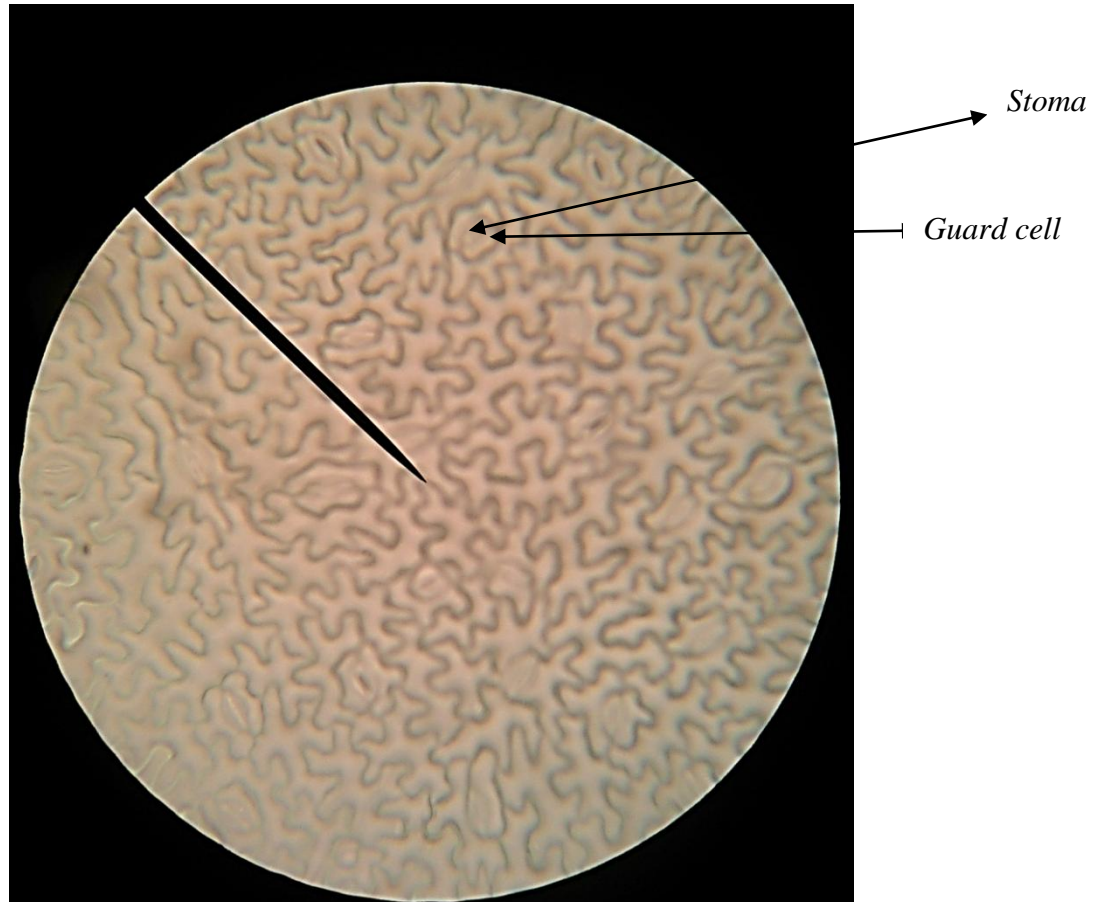


Plate 74: Adaxial or lower surface of 'Potiskum' beans $\times 400$

The abaxial or the upper surface of 'Ifebrown' revealed the presence of 13.04% U.E stomatal index, irregular epidermal cellshape, anisocytic stomata type which is unique in all the varieties, amoderate thick cell wall and curved epidermal contours (plate 75).

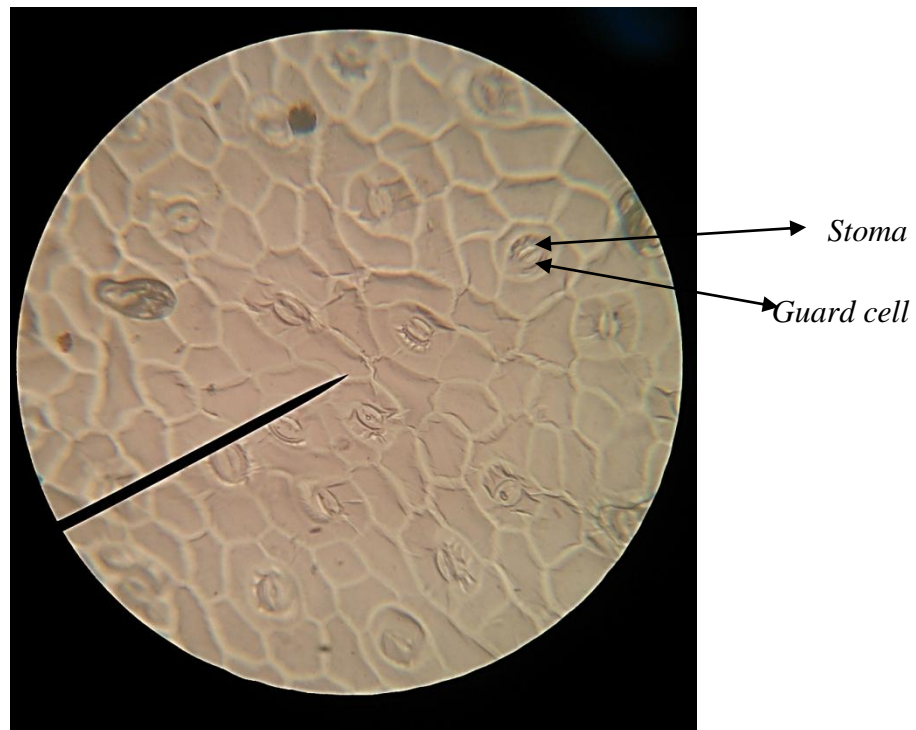


Plate 75: Abaxial or upper surface of 'Ifebrown' beans $\times 400$

The adaxial or lower surface of 'Ifebrown' revealed the presence stomatal index as 15.39% L.E, irregular epidermal cell shape, paracytic stomatal type, a moderately cell wall thickness and a curved epidermal contour (Plate 76).

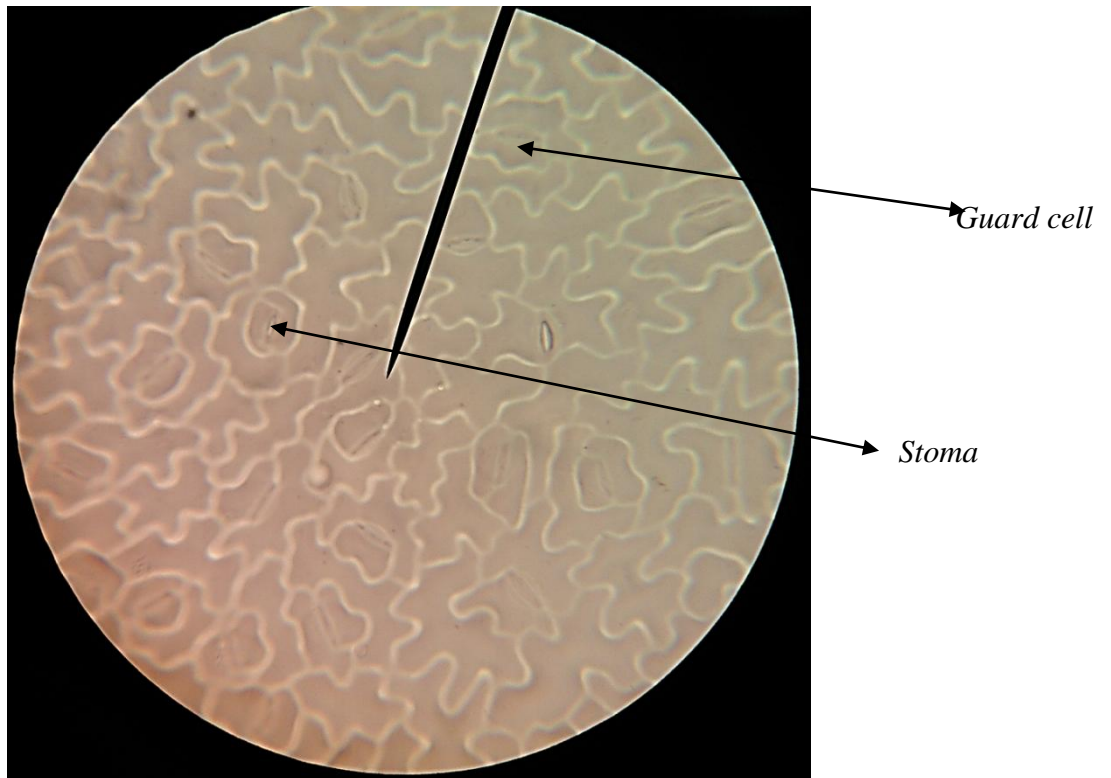


Plate 76: Adaxial or lower surface of 'Ifebrown' $\times 400$

The abaxial or upper epidermal of 'Sokoto guzo' revealed the presence of stomatal index as 16.69% U.E,irregular epidermal cell shape, paracytic type of stomata,moderately cell wall thickness and an epidermal curved contour (Plate 77)

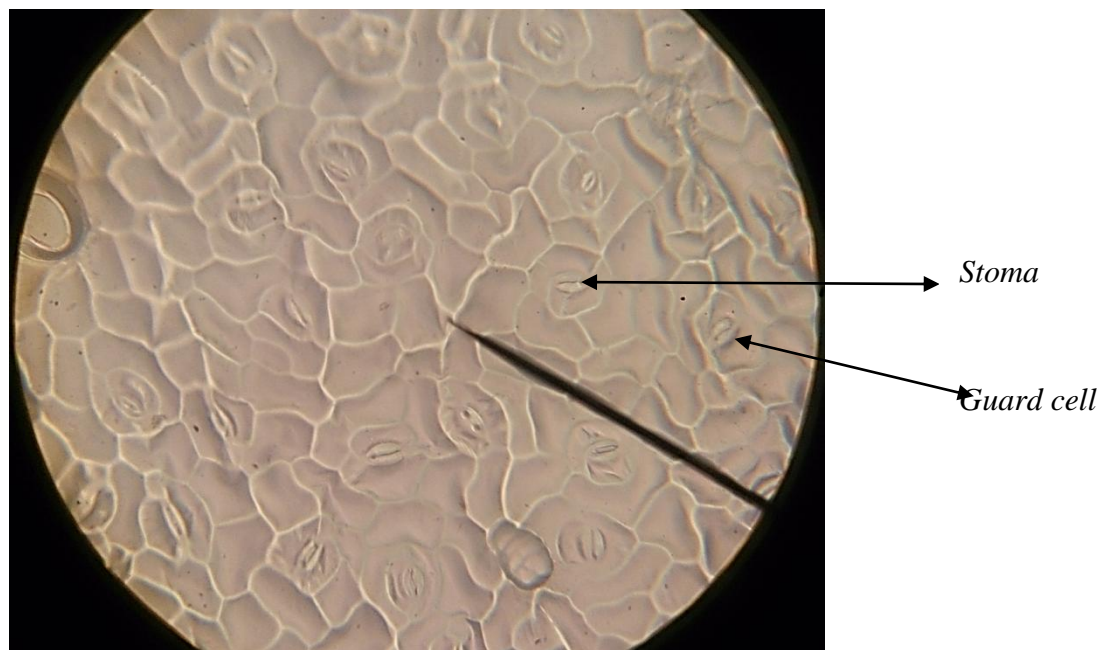


Plate 77: Abaxial or upper surface of 'Sokoto guzo' × 400

The adaxial or the lower epidermal of 'Sokoto guzo' revealed the presence of stomatal index as 24.24% L.E, irregular cell shape, paracytic type of stomata. The thickness of the cell wall was moderate and a curved contour epidermal (Plate 78)

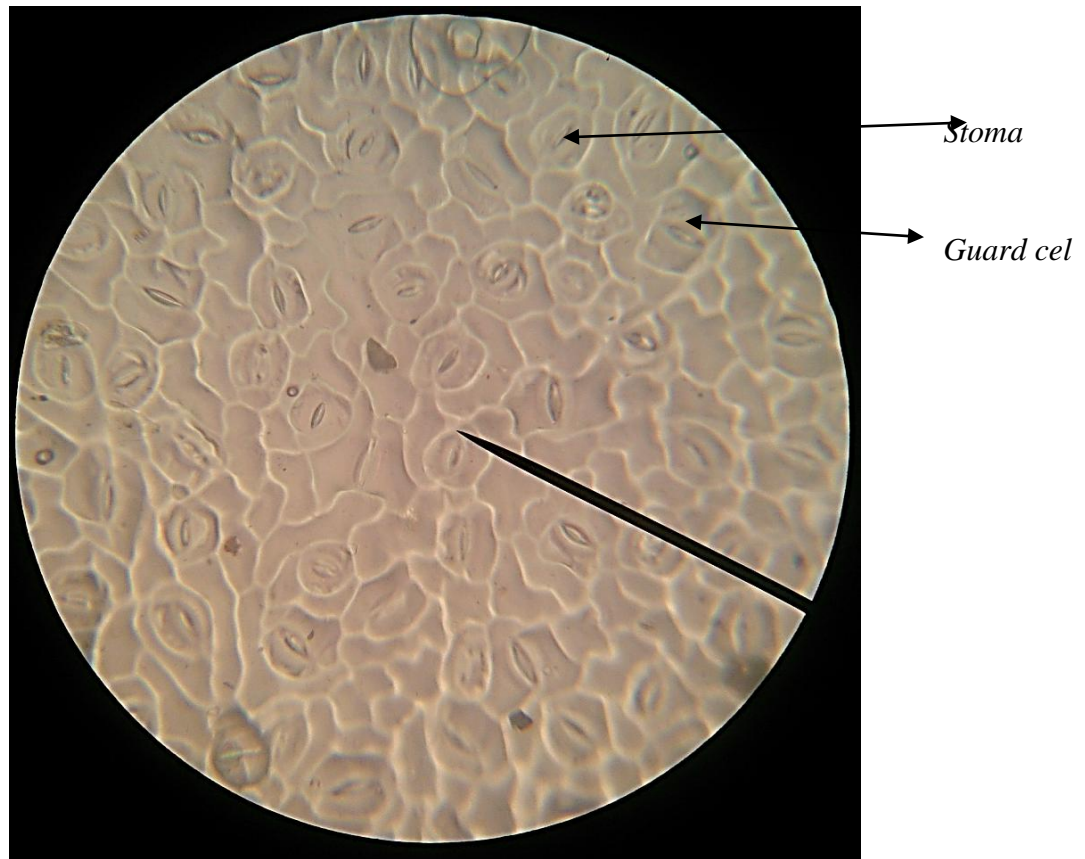


Plate78: Adaxial surface of 'Sokoto guzo' beans $\times 400$

The abaxial or the upper epidermal of Crowderpea revealed the presence of stomatal index as 16.69% U.E, irregular epidermal cell shape,paracytic type of stomata.The cell wall thickness was moderate(Plate 79).

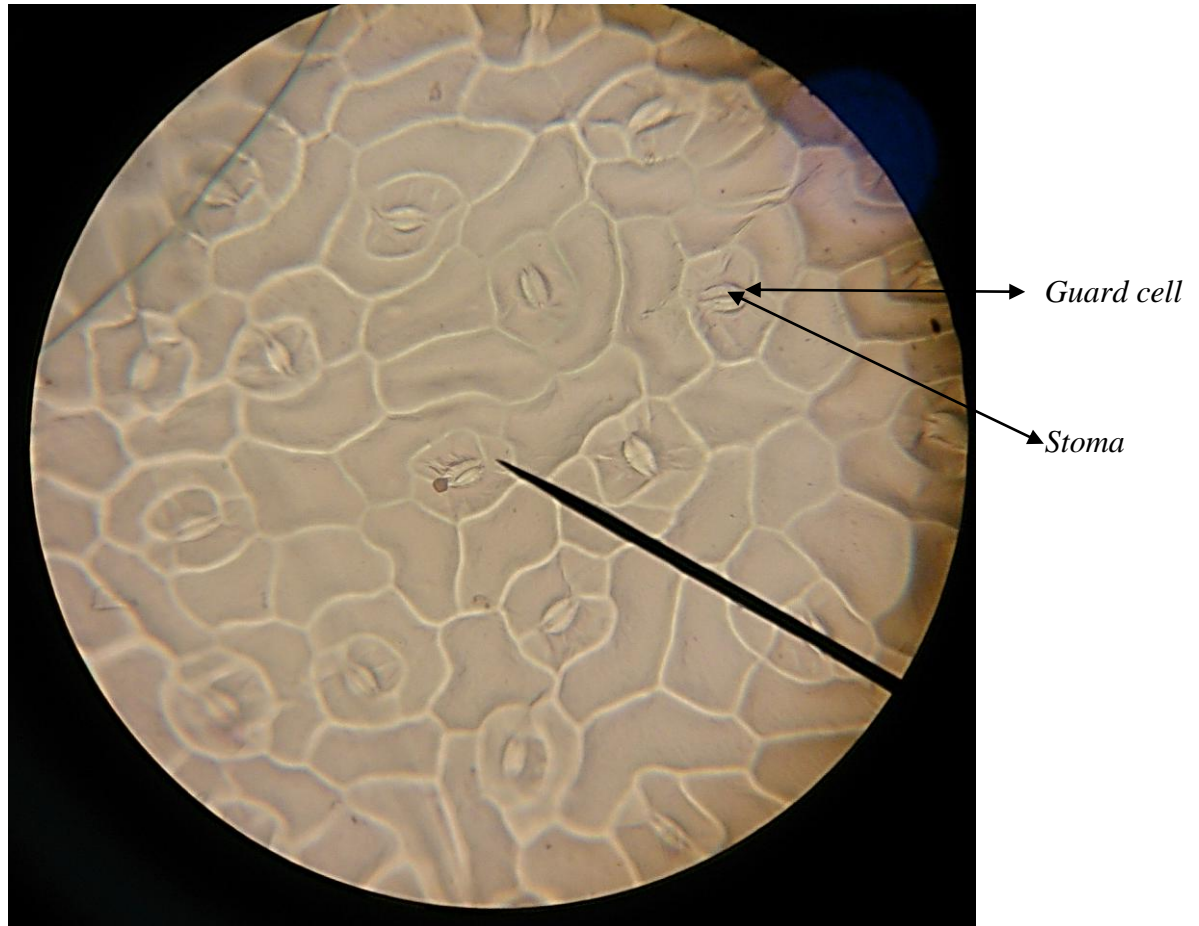


Plate 79: Abaxial or upper surface of Crowder pea $\times 400$

The adaxial or the upper epidermal of *Crowderpea* revealed the presence of stomatal index as 22.58% U.E, irregular epidermal cell shape, paracytic stomata type. The cell wall thickness were moderately thick and the epidermal contours were curved (Plate 79).

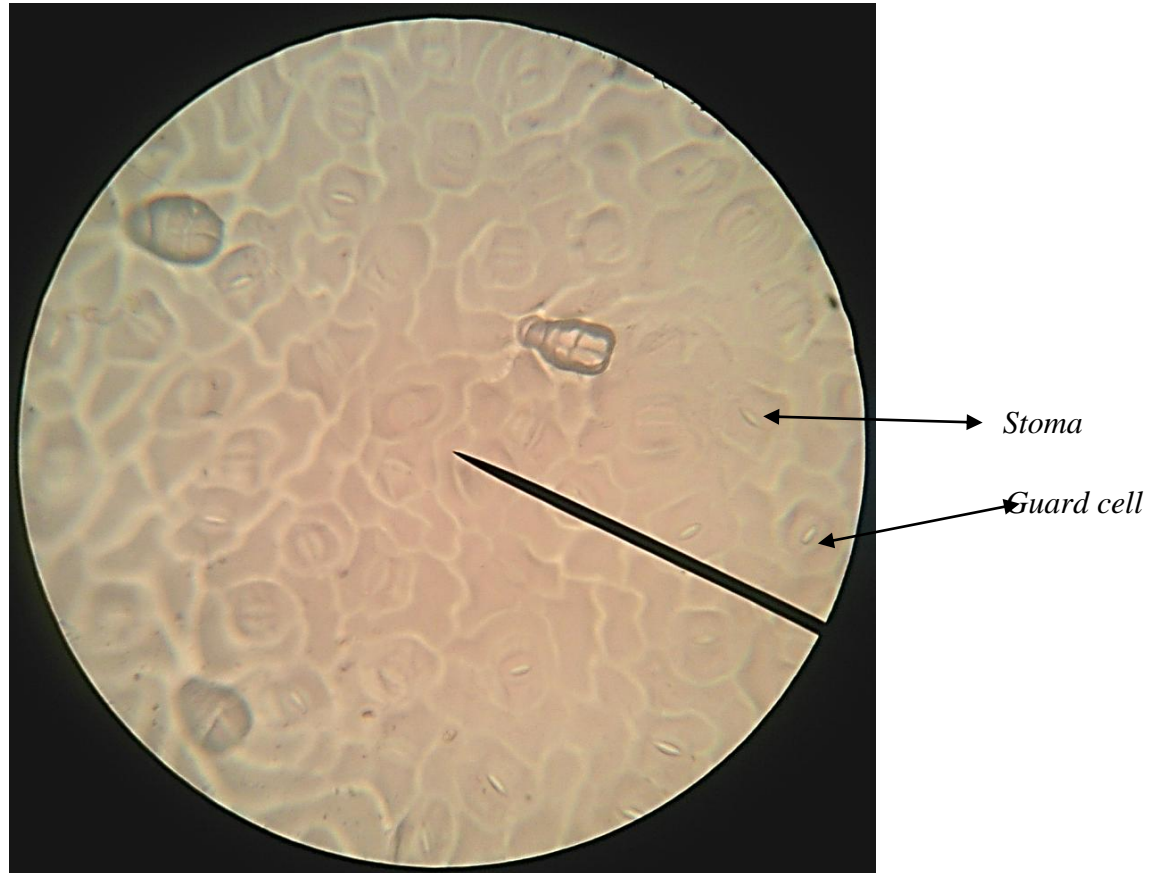


Plate 80: Adaxial or lower surface of *Crowderpea* $\times 400$

4.3 Cytological Characters.

The result of the cytological (mitotic) study on 'Ifebrown' showed that there were multiple nuclei in the cell. This showed that it grew faster and also highly meristematic and underwent faster cell replication. Chromosomes of this variety came out mostly from telophase and prophase stages. Metaphase was difficult resulting to poor counting of the chromosomes. Number of chromosomes varied because it is a hybrid; thus $2n = 24, 2n = 26$ (Plate 81).

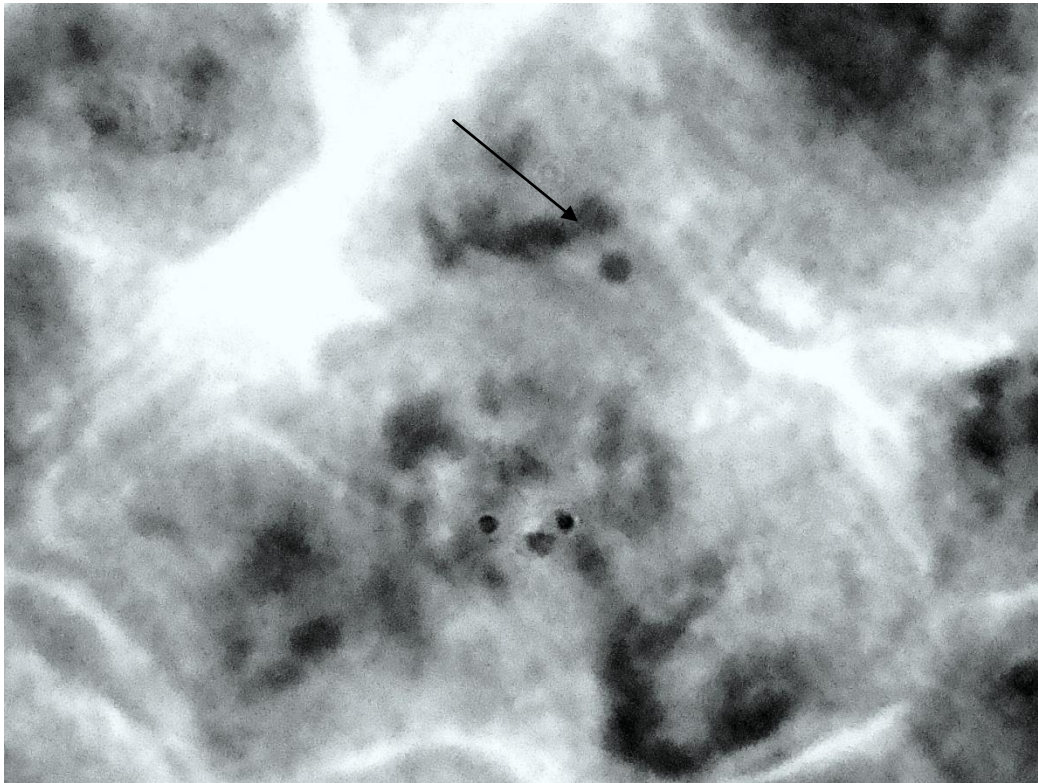


Plate 81: Diploid chromosome of 'Ifebrown' variety. $\times 1000$

The result of the mitotic study on 'Oloka' showed the chromosomes at the late metaphase stage. It had diffuse centromeres making the variety to have variation in their chromosomenumbers. Chromosome number observed was $2n = 22$, and $2n = 24$. This showed that hybrid occurred in the variety (Plate82).

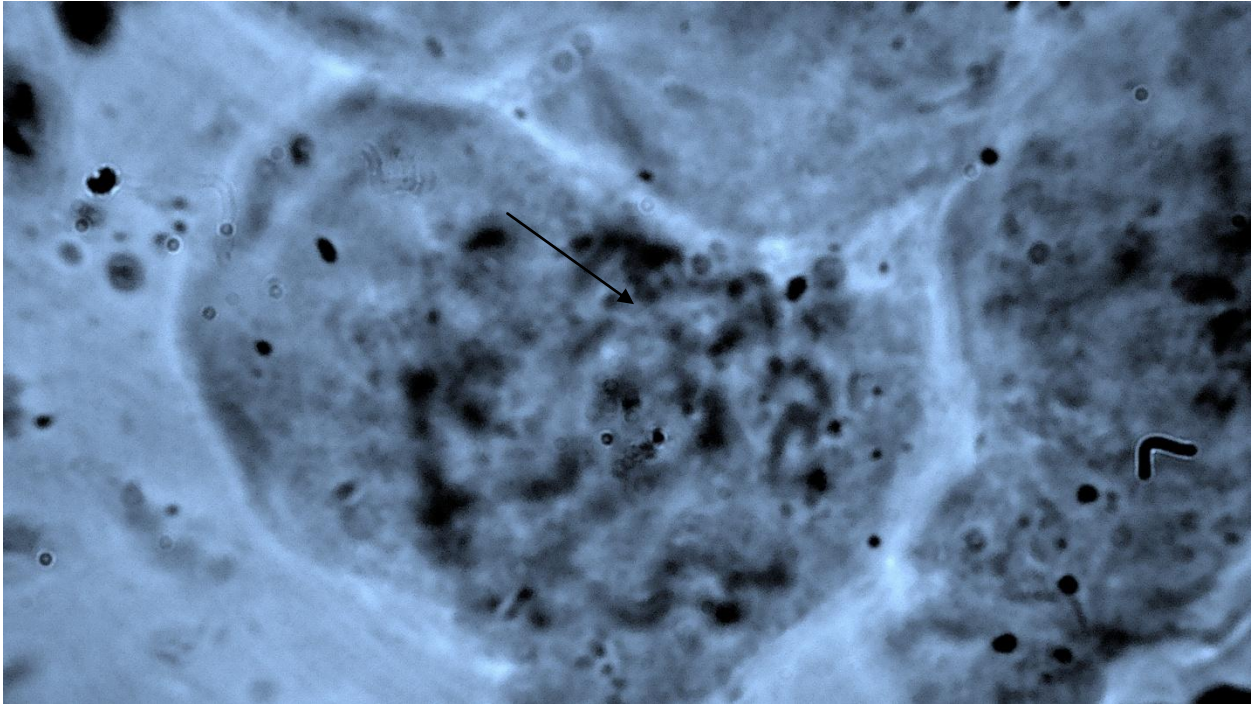


Plate 82: Diploid chromosome of 'Oloka' variety. $\times 1000$

The result of the mitotic study on Iron beans revealed the chromosomes at the early metaphase stage. The chromosomes were in bivalent form with a clear centromeres and a pair of sister

chromatid. Chromosome number was $2n = 22$ and $2n = 24$ because it can occur in hybrid form or different ploidy level (Plate 83).



Plate 83: Diploid chromosome of Iron beans $\times 1000$

The result of the mitotic study on 'Sokoto guzo' showed the chromosome number as $2n = 22$. The picture showed a complete diffuse nucleus (Plate 84).

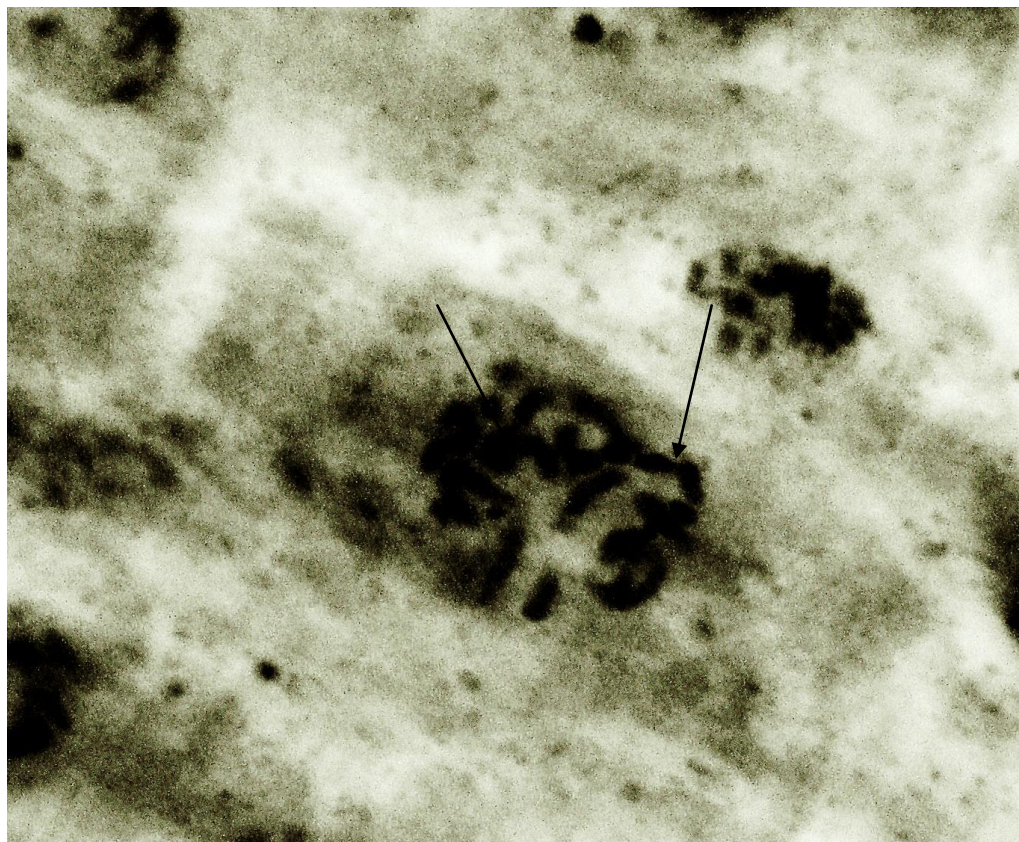


Plate 84: Diploid chromosome of 'Sokoto guzo' $\times 1000$

The result of the mitotic study on 'Kafanji' variety showed constricted chromosome appearing in late prophase (Plate 85).



Plate 85: Diploid chromosome of 'Kafanji' variety $\times 1000$

The result of the mitotic study of Crowder peas showed the number of chromosomes to be $2n=24$ and $2n=26$ respectively. The chromosome was seen at the late prophase stage. It has few numbers of actively dividing cells when compared to other varieties of the species (Plate 86).

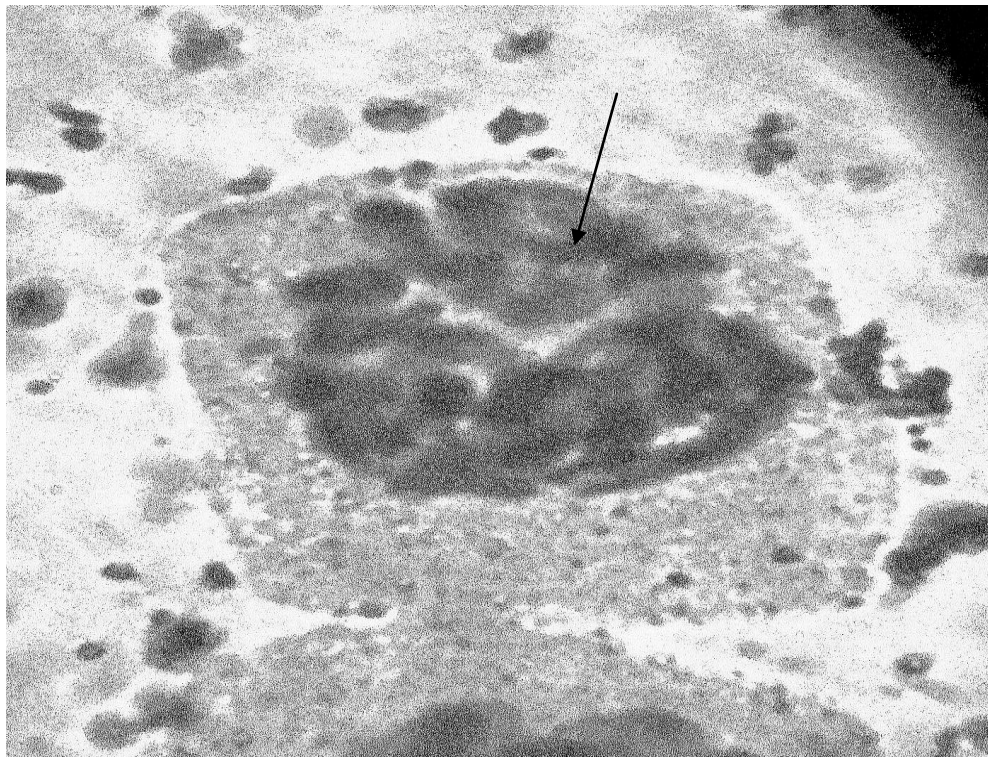


Plate 86: Diploid chromosome of Crowder pea $\times 1000$

The result of the mitotic study of 'Potiskum' variety appeared at the telophase stage, the chromatids were seen darkly interwoven with the spindle fibre and the chromosomes were seen arranged around the South and North poles of the nucleus, with chromosome number of $2n = 22$ (Plate 87).

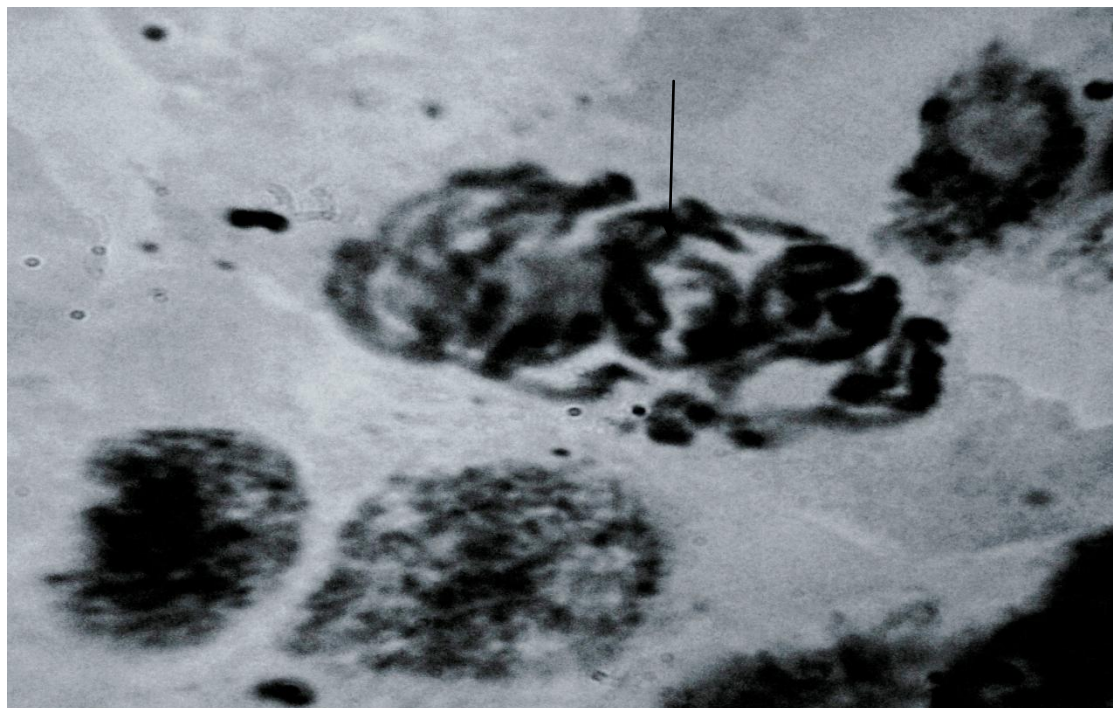


Plate 87: Diploid chromosome of 'Potiskum'×1000

4.4: Phytochemical and Proximate Characters

Qualitative Phytochemical Composition of the Leaf, Stem, Root and Seed of *Vigna unguiculata* Varieties

The stem, leaves, root and seed of Var."Ifebrown" on qualitative analysis shows the presence

tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 6)

Table 6: Qualitative Result of Phytochemical Analysis of Var. "Ifebrown"

Constituents	Stems	Leaves	Root	Seeds
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponin	+	+	+	+
Alkaloids	+	+	+	+
Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var. Crowderpea on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 7)

Table 7: Qualitative Result of Phytochemical Analysis of Var. Crowder pea.

Constituents	Stems	Leaves	Roots	Seeds
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var. "Oloka" on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 8).

Table 8: Qualitative Result of Phytochemical Analysis of Var. Oloka.

Constituents	Stems	Leaves	Roots	Seeds
Tannin	+	+	+	+
Flavnoids	+	+	+	+
Saponins	+	+	+	+

Alkaloids	+	+	+	+
Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var.”Kafanji” on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 9).

Table 9: Qualitative Result of Phytochemical Analysis of Var. ‘Kafanji’.

Constituents	Stems	Leaves	Roots	Seeds
Tannin	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var.”Potiskum” on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 10).

Table 10: Qualitative Result of Phytochemical Analysis of Var. Potiskum

Constituents	Stems	Leaves	Roots	Seeds
Tannin	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+

Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var.Ironbeans on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 11)

Table 11: Qualitative Result of Phytochemical Analysis of Var.Iron beans.

Constituents	Stems	Leaves	Roots	Seeds
Tannin	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Sterol	+	+	+	+
Phenol	+	+	+	+

The stem, leaves, root and seed of Var.'Sokoto guzo' on qualitative analysis shows the presence of tannins, flavonoids, saponin, alkaloids, sterol and phenol (Table 12)

Table 12: Qualitative Result of Phytochemical Analysis of Var.'Sokoto guzo'

Constituents	Stems	Leaves	Roots	Seeds
Tannin	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+

Sterol	+	+	+	+
Phenol	+	+	+	+

Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of *Vigna unguiculata* Varieties

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of 'Kafanji' variety revealed that the percentage of tannin was highest in the leaf (1.18 ± 0.028) and lowest in the seed (0.73 ± 0.014). The percentage of saponin was highest in the leaf (1.63 ± 0.035) and lowest in the seed (0.89 ± 0.007). Percentage of flavonoid was highest in the leaf (1.08 ± 0.021) and lowest in the seed (0.74 ± 0.007). The percentage of alkaloid was highest in the stem (1.92 ± 0.028) and lowest in the seed (1.05 ± 0.014). The percentage of sterol was highest in the leaf (0.28 ± 0.000) and lowest in the seed (0.06 ± 0.000), while the percentage of phenol was highest in the leaf (0.16 ± 0.000) and lowest in the seed (0.08 ± 0.001). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 13).

Table 13: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of 'Kafanji' Beans Variety

Part of Plant	% Composition*					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.18 ± 0.028^a	1.63 ± 0.035^a	1.08 ± 0.021^a	1.53 ± 0.014^b	0.28 ± 0.000^a	0.16 ± 0.000^a
Stem	1.09 ± 0.000^b	1.36 ± 0.028^b	1.07 ± 0.021^a	1.92 ± 0.028^a	0.19 ± 0.007^b	0.14 ± 0.014^b
Root	0.91 ± 0.014^c	1.27 ± 0.014^c	0.94 ± 0.021^b	1.42 ± 0.000^c	0.17 ± 0.021^b	0.11 ± 0.000^c
Seed	0.73 ± 0.014^d	0.89 ± 0.007^d	0.74 ± 0.007^c	1.05 ± 0.014^d	0.06 ± 0.000^c	0.08 ± 0.001^d
p-value	**	**	**	**	**	**

Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of Crowderpea (black beans) variety revealed that the percentage of tannin was highest in the stem (1.64 ± 0.000) and lowest in the seed (0.81 ± 0.014). The percentage of saponin was highest in the stem (1.65 ± 0.000) and lowest in the seed (0.94 ± 0.007). The percentage of flavonoid was highest in the root (1.18 ± 0.014) and lowest in the seed (0.67 ± 0.021). The percentage of alkaloid was highest in the stem (2.07 ± 0.007) and lowest in the seed (1.16 ± 0.014). The percentage of sterol was highest in the stem (0.18 ± 0.000) and lowest in the seed (0.06 ± 0.001), while the percentage of phenol was highest in the stem (0.23 ± 0.000) and lowest in the seed (0.08 ± 0.001). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 14).

Table 14: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Crowderpea (Black Beans) Variety

% Composition*						
Part of Plant	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.15 ± 0.064^b	1.06 ± 0.000^c	1.05 ± 0.000^c	1.36 ± 0.014^c	0.11 ± 0.014^b	0.15 ± 0.000^c
Stem	1.64 ± 0.000^a	1.65 ± 0.000^a	1.14 ± 0.000^b	2.07 ± 0.007^a	0.18 ± 0.000^a	0.23 ± 0.000^a
Root	1.56 ± 0.007^a	1.43 ± 0.000^b	1.18 ± 0.014^a	1.81 ± 0.014^b	0.12 ± 0.000^b	0.17 ± 0.000^b
Seed	0.81 ± 0.014^c	0.94 ± 0.007^d	0.67 ± 0.021^d	1.16 ± 0.014^d	0.06 ± 0.001^c	0.08 ± 0.001^d
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of 'Ifebrown' variety revealed that the percentage of tannin was highest in the stem (1.46 ± 0.000) and lowest in the seed (0.69 ± 0.000). The percentage of saponin was highest in the stem (1.49 ± 0.000) and lowest in the seed (1.04 ± 0.021). The percentage of flavonoid was highest in the stem (1.25 ± 0.000) and lowest in the seed (0.73 ± 0.000). The percentage of alkaloid was highest in the stem (3.19 ± 0.007) and lowest in the seed (1.24 ± 0.007), the percentage of sterol was highest in the stem (0.28 ± 0.007) and lowest in the seed (0.05 ± 0.002), while the percentage of phenol was highest in the stem (0.22 ± 0.028) and lowest in the seed (0.08 ± 0.003). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 15).

Table 15: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of 'Ifebrown' Beans Variety

% Composition*						
Part of Plant	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.24 ± 0.000^c	1.34 ± 0.000^b	0.92 ± 0.000^c	1.78 ± 0.007^c	0.13 ± 0.007^c	0.13 ± 0.000^c
Stem	1.46 ± 0.000^a	1.49 ± 0.000^a	1.25 ± 0.000^a	3.19 ± 0.007^a	0.28 ± 0.007^a	0.22 ± 0.028^a
Root	1.31 ± 0.014^b	1.09 ± 0.007^c	1.09 ± 0.007^b	2.49 ± 0.014^b	0.16 ± 0.007^b	0.18 ± 0.007^b
Seed	0.69 ± 0.000^d	1.04 ± 0.021^d	0.73 ± 0.000^d	1.24 ± 0.007^d	0.05 ± 0.002^d	0.08 ± 0.003^d
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of ‘Oloka’ variety revealed that the percentage of tannin was highest in the stem (1.47 ± 0.021) and lowest in the seed (0.85 ± 0.014). The percentage of saponin was highest in the stem (1.91 ± 0.014) and lowest in the seed (1.05 ± 0.021). The percentage of flavonoid was highest in the stem (1.48 ± 0.000) and lowest in the seed (0.81 ± 0.014). The percentage of alkaloid was highest in the stem (2.79 ± 0.014) and lowest in the seed (1.25 ± 0.000). The percentage of sterol was highest in the stem (0.25 ± 0.014) and lowest in the seed (0.10 ± 0.001), while the percentage of phenol was highest in the stem (0.37 ± 0.021) and lowest in the seed (0.12 ± 0.000). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 16)

Table 16: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of ‘Oloka’ Beans Variety

Part of Plant	% Composition*					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.14 ± 0.007^c	1.27 ± 0.021^c	1.31 ± 0.021^c	1.20 ± 0.000^d	0.15 ± 0.000^b	0.16 ± 0.000^c
Stem	1.47 ± 0.021^a	1.91 ± 0.014^a	1.48 ± 0.000^a	2.79 ± 0.014^a	0.25 ± 0.014^a	0.37 ± 0.021^a
Root	1.28 ± 0.000^b	1.49 ± 0.014^b	1.42 ± 0.000^b	2.47 ± 0.021^b	0.22 ± 0.035^a	0.20 ± 0.000^b
Seed	0.85 ± 0.014^d	1.05 ± 0.021^d	0.81 ± 0.014^d	1.25 ± 0.000^c	0.10 ± 0.001^c	0.12 ± 0.000^d
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of ‘Sokoto guzo’ variety revealed that the percentage of tannin was highest in the leaf (1.18 ± 0.000) and lowest in the seed (0.84 ± 0.000). The percentage of saponin was highest in the leaf (1.57 ± 0.050) and lowest in the root (0.95 ± 0.000). The percentage of flavonoid was highest in the leaf (1.67 ± 0.000) and lowest in the seed (0.67 ± 0.021). The percentage of alkaloid was highest in the leaf (1.79 ± 0.014) and lowest in the seed (1.19 ± 0.014), the percentage of sterol was highest in the leaf (0.34 ± 0.021) and lowest in the seed (0.07 ± 0.003), while the percentage of phenol was highest in the leaf (0.19 ± 0.014) and lowest in the root (0.08 ± 0.007). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 17).

Table 17: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of ‘Sokoto guzo’ Beans Variety

Part of Plant	% Composition*					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.18 ± 0.000^a	1.57 ± 0.050^a	1.67 ± 0.000^a	1.79 ± 0.014^a	0.34 ± 0.021^a	0.19 ± 0.014^a
Stem	1.07 ± 0.007^b	1.31 ± 0.021^b	1.35 ± 0.000^b	1.52 ± 0.000^b	0.29 ± 0.021^a	0.09 ± 0.000^b
Root	0.86 ± 0.000^c	0.95 ± 0.000^d	1.06 ± 0.000^c	1.31 ± 0.014^c	0.22 ± 0.028^b	0.08 ± 0.007^b
Seed	0.84 ± 0.000^d	1.06 ± 0.014^c	0.67 ± 0.021^d	1.19 ± 0.014^d	0.07 ± 0.003^c	0.09 ± 0.001^b
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the quantitative phytochemical composition of the leaf, stem, root and seed of 'Potiskum' variety revealed that the percentage of tannin was highest in the leaf (1.25 ± 0.007) and root (1.25 ± 0.014) and lowest in the seed (0.74 ± 0.021). The percentage of saponin was highest in the leaf (1.39 ± 0.014) and lowest in the root (0.92 ± 0.000). The percentage of flavonoid was highest in the leaf (1.31 ± 0.021) and lowest in the seed (0.64 ± 0.014). The percentage of alkaloid was highest in the leaf (1.34 ± 0.014) and lowest in the seed (1.04 ± 0.000), the percentage of sterol was highest in the leaf (0.24 ± 0.014) and lowest in the seed (0.04 ± 0.001), while the percentage of phenol was highest in the leaf (0.14 ± 0.000) and lowest in the seed (0.09 ± 0.001). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 18).

Table 18: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of 'Potiskum' Beans Variety

Part of Plant	% Composition*					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.25 ± 0.007^a	1.39 ± 0.014^a	1.31 ± 0.021^a	1.34 ± 0.014^a	0.24 ± 0.014^a	0.14 ± 0.000^a
Stem	1.23 ± 0.014^a	1.16 ± 0.000^b	1.19 ± 0.014^b	1.28 ± 0.000^b	0.19 ± 0.000^b	0.11 ± 0.014^b
Root	1.25 ± 0.014^a	0.92 ± 0.000^c	1.05 ± 0.000^c	1.22 ± 0.035^c	0.16 ± 0.014^c	0.09 ± 0.003^b
Seed	0.74 ± 0.021^b	0.93 ± 0.014^c	0.64 ± 0.014^d	1.04 ± 0.000^d	0.04 ± 0.001^d	0.09 ± 0.001^b
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the phytochemical composition of the leaf, stem, root and seed of Iron bean variety revealed that the percentage of tannin was highest in the leaf (1.45 ± 0.014) and seed (0.85 ± 0.007). The percentage of saponin was highest in the leaf (1.27 ± 0.028) and lowest in the seed (0.82 ± 0.028). The percentage of flavonoid was highest in the leaf (1.13 ± 0.000) and lowest in the seed (0.76 ± 0.028). The percentage of alkaloid was highest in the leaf (1.42 ± 0.021) and lowest in the seed (1.10 ± 0.028). The percentage of sterol was highest in the leaf (0.20 ± 0.000) and lowest in the seed (0.04 ± 0.002^d), while the percentage of phenol was highest in the leaf (0.15 ± 0.000) and lowest in the root (0.09 ± 0.001) and seed (0.09 ± 0.001). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf, stem, root and seed ($p < 0.05$) (Table 19).

Table 19: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Iron Bean Variety

Part of Plant	% Composition					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Leaf	1.45 ± 0.014^a	1.27 ± 0.028^a	1.13 ± 0.000^a	1.42 ± 0.021^a	0.20 ± 0.000^a	0.15 ± 0.000^a
Stem	1.17 ± 0.014^b	1.19 ± 0.021^b	0.93 ± 0.014^b	1.32 ± 0.000^b	0.17 ± 0.007^b	0.12 ± 0.014^b
Root	0.88 ± 0.035^c	1.06 ± 0.007^c	0.90 ± 0.007^b	1.19 ± 0.014^c	0.13 ± 0.007^c	0.09 ± 0.001^c
Seed	0.85 ± 0.007^c	0.82 ± 0.028^d	0.76 ± 0.028^c	1.10 ± 0.028^d	0.04 ± 0.002^d	0.09 ± 0.001^c
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different** $p < 0.05$

Comparative analysis of the phytochemical composition of the leaf of seven varieties of *Vigna unguiculata* ('Kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans) indicated that the percentage of tannin was highest in Iron beans (1.45 ± 0.014) and lowest in Oloka (1.14 ± 0.007). The percentage of saponin was highest in 'Kafanji' (1.63 ± 0.035) and lowest in Crowderpea (1.06 ± 0.000). The percentage of flavonoid was highest in 'Sokoto guzo' (1.67 ± 0.000) and lowest in 'Ifebrown' (0.92 ± 0.000). The percentage of alkaloid was highest in 'Sokoto guzo' (1.79 ± 0.014) and lowest in Oloka (1.20 ± 0.000). The percentage of sterol was highest in 'Sokoto guzo' (0.34 ± 0.021) and lowest in Crowderpea (0.11 ± 0.014), while the percentage of phenol was highest in 'Sokoto guzo' (0.19 ± 0.014) and lowest in 'Ifebrown' (0.13 ± 0.000). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the leaf of 'kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans ($p < 0.05$) (Table 20).

Table 20: Comparative Phytochemical Analysis of the Leaf of Seven Varieties of *Vigna unguiculata*

Variety	% Composition*					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Kafanji	1.18 ± 0.028^b	1.63 ± 0.035^a	1.08 ± 0.021^d	1.53 ± 0.014^b	0.28 ± 0.000^b	0.16 ± 0.000^b
Crowderpea	1.15 ± 0.064^c	1.06 ± 0.000^d	1.05 ± 0.000^d	1.36 ± 0.014^d	0.11 ± 0.014^f	0.15 ± 0.000^b
Ifebrown	1.24 ± 0.000^b	1.34 ± 0.000^b	0.92 ± 0.000^e	1.78 ± 0.007^a	0.13 ± 0.000^e	0.13 ± 0.000^d
Oloka	1.14 ± 0.007^c	1.27 ± 0.021^c	1.31 ± 0.021^b	1.20 ± 0.000^e	0.15 ± 0.000^e	0.16 ± 0.000^b
Sokoto guzo	1.18 ± 0.000^b	1.57 ± 0.050^a	1.67 ± 0.000^a	1.79 ± 0.014^a	0.34 ± 0.021^a	0.19 ± 0.014^a
Potiskum	1.25 ± 0.007^b	1.39 ± 0.014^b	1.31 ± 0.021^b	1.34 ± 0.014^d	0.24 ± 0.014^c	0.14 ± 0.000^c
Iron Beans	1.45 ± 0.014^a	1.27 ± 0.028^c	1.13 ± 0.000^c	1.42 ± 0.021^c	0.20 ± 0.000^d	0.15 ± 0.000^b
p-value	**	**	**	**	**	**

Comparative analysis of the phytochemical composition of the stem of seven varieties of *Vigna unguiculata* ('kafanji', Crowderpea, 'Ifebrown', and 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans) indicated that the percentage of tannin was highest in Crowderpea (1.64 ± 0.000) and lowest in 'Sokoto guzo' (1.07 ± 0.007). The percentage of saponin was highest in 'Oloka' (1.91 ± 0.014) and lowest in 'Potiskum' (1.16 ± 0.000). The percentage of flavonoid was highest in oloka (1.48 ± 0.000) and lowest in iron beans (0.93 ± 0.014). The percentage of alkaloid was highest in 'Ifebrown' (3.19 ± 0.007) and lowest in 'Potiskum' (1.28 ± 0.000). The percentage of sterol was highest in 'Sokoto guzo' (0.29 ± 0.021) and lowest in Iron beans (0.17 ± 0.007), while the percentage of phenol was highest in 'Oloka' (0.37 ± 0.021) and lowest in 'Sokoto guzo' (0.09 ± 0.000). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the stem of 'kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans ($p < 0.05$) (Table 21).

Table 21: Comparative Phytochemical Analysis of the Stem of Seven Varieties of *Vigna unguiculata*

Variety	% Composition					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Kafanji	1.09 ± 0.000^e	1.36 ± 0.028^d	1.07 ± 0.021^f	1.92 ± 0.028^d	0.19 ± 0.007^c	0.14 ± 0.014^c
Crowderpea	1.64 ± 0.000^a	1.65 ± 0.000^b	1.14 ± 0.000^b	2.07 ± 0.007^c	0.18 ± 0.000^c	0.23 ± 0.000^b
Ifebrown	1.46 ± 0.000^b	1.49 ± 0.000^c	1.25 ± 0.000^c	3.19 ± 0.007^a	0.28 ± 0.007^a	0.22 ± 0.028^b
Oloka	1.47 ± 0.021^b	1.91 ± 0.014^a	1.48 ± 0.000^a	2.79 ± 0.014^b	0.25 ± 0.014^b	0.37 ± 0.021^a
Sokoto Guzo	1.07 ± 0.007^e	1.31 ± 0.021^e	1.35 ± 0.000^b	1.52 ± 0.000^e	0.29 ± 0.021^a	0.09 ± 0.000^d
Potiskum	1.23 ± 0.014^c	1.16 ± 0.000^f	1.19 ± 0.014^d	1.28 ± 0.000^g	0.19 ± 0.000^c	0.11 ± 0.014^c
Iron Beans	1.17 ± 0.014^d	1.19 ± 0.021^f	0.93 ± 0.014^g	1.32 ± 0.000^f	0.17 ± 0.007^c	0.12 ± 0.014^c
p-value	**	**	**	**	**	**

Comparative analysis of the phytochemical composition of the root of seven varieties of *Vigna unguiculata* ('kafanji', Crowderpea, 'Ifebrown', and 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans) indicated that the percentage of tannin was highest in Crowderpea (1.56 ± 0.007) and lowest in 'Sokoto guzo' (0.86 ± 0.000). The percentage of saponin was highest in 'Oloka' (1.49 ± 0.014) and lowest in 'Potiskum' (0.92 ± 0.000). Percentage of flavonoid was highest in Oloka (1.42 ± 0.000) and lowest in Iron beans (0.90 ± 0.007). The percentage of alkaloid was highest in ifebrown (2.49 ± 0.014) and lowest in iron beans (1.19 ± 0.014). The percentage of sterol was highest in 'Oloka' (0.22 ± 0.035) and lowest in Crowderpea (0.12 ± 0.000), while the percentage of phenol was highest in Oloka (0.20 ± 0.000) and lowest in 'Sokoto guzo' (0.08 ± 0.007). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol and phenol between the root of 'kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans ($p < 0.05$) (Table 22).

Table 22: Comparative Phytochemical Analysis of the Root of Seven Varieties of *Vigna unguiculata*

Variety	% Composition					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Kafanji	0.91 ± 0.014^d	1.27 ± 0.014^c	0.94 ± 0.021^e	1.42 ± 0.000^c	0.17 ± 0.021^b	0.11 ± 0.000^c
Crowderpea	1.56 ± 0.007^a	1.43 ± 0.000^b	1.18 ± 0.014^b	1.81 ± 0.014^b	0.12 ± 0.000^b	0.17 ± 0.000^b
Ifebrown	1.31 ± 0.014^b	1.09 ± 0.007^d	1.09 ± 0.007^c	2.49 ± 0.014^a	0.16 ± 0.007^b	0.18 ± 0.007^b
Oloka	1.28 ± 0.000^b	1.49 ± 0.014^a	1.42 ± 0.000^a	2.47 ± 0.021^a	0.22 ± 0.035^a	0.20 ± 0.000^a
Sokoto Guzo	0.86 ± 0.000^e	0.95 ± 0.000^f	1.06 ± 0.000^d	1.31 ± 0.014^d	0.22 ± 0.028^a	0.08 ± 0.007^e
Potiskum	1.25 ± 0.014^c	0.92 ± 0.000^g	1.05 ± 0.000^d	1.22 ± 0.035^e	0.16 ± 0.014^b	0.09 ± 0.003^d
Iron Beans	0.88 ± 0.035^e	1.06 ± 0.007^e	0.90 ± 0.007^f	1.19 ± 0.014^c	0.13 ± 0.007^b	0.09 ± 0.001^d
p-value	**	**	**	**	**	**

Comparative analysis of the phytochemical composition of the seed of seven varieties of *Vigna unguiculata* ('kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', and 'Potiskum' and Iron beans) indicated that the percentage of tannin was highest in 'Oloka' (0.85 ± 0.014) and lowest in 'Ifebrown' (0.69 ± 0.000). Percentage of saponin was highest in 'Sokoto guzo' (1.06 ± 0.014) and lowest in Iron beans (0.82 ± 0.028). The percentage of flavonoid was highest in 'Oloka' (0.81 ± 0.014) and lowest in 'Potiskum' (0.64 ± 0.014). The percentage of alkaloid was highest in Oloka (1.25 ± 0.000) and lowest in 'Potiskum' (1.04 ± 0.000). The percentage of sterol was highest in oloka (0.10 ± 0.001) and lowest in 'Potiskum' (0.04 ± 0.001) and Iron beans (0.04 ± 0.002). The percentage of phenol was highest in 'Oloka' (0.12 ± 0.000) and lowest in 'kafanji' (0.08 ± 0.001) and Crowderpea (0.08 ± 0.001) (Table 23).

Table 23: Comparative Phytochemical Analysis of the Seed of Seven Varieties of *Vigna unguiculata*

Variety	% Composition					
	Tannin	Saponin	Flavonoid	Alkaloid	Sterol	Phenol
Kafanji	0.73 ± 0.014^c	0.89 ± 0.007^c	0.74 ± 0.007^b	1.05 ± 0.014^d	0.06 ± 0.000^c	0.08 ± 0.001^c
Crowderpea	0.81 ± 0.014^b	0.94 ± 0.007^b	0.67 ± 0.021^c	1.16 ± 0.014^b	0.06 ± 0.001^c	0.08 ± 0.001^c
Ifebrown	0.69 ± 0.000^d	1.04 ± 0.021^a	0.73 ± 0.000^b	1.24 ± 0.007^a	0.05 ± 0.002^d	0.08 ± 0.003^c
Oloka	0.85 ± 0.014^a	1.05 ± 0.021^a	0.81 ± 0.014^a	1.25 ± 0.000^a	0.10 ± 0.001^a	0.12 ± 0.000^a
Sokoto Guzo	0.84 ± 0.000^a	1.06 ± 0.014^a	0.67 ± 0.021^c	1.19 ± 0.014^b	0.07 ± 0.003^b	0.09 ± 0.001^b
Potiskum	0.74 ± 0.021^c	0.93 ± 0.014^b	0.64 ± 0.014^c	1.04 ± 0.000^d	0.04 ± 0.001^d	0.09 ± 0.001^b
Iron Beans	0.85 ± 0.007^a	0.82 ± 0.028^d	0.76 ± 0.028^b	1.10 ± 0.028^c	0.04 ± 0.002^d	0.09 ± 0.001^b
p-value	**	**	**	**	**	**

The percentage of phytate acid was highest in ‘Oloka’ (1.74 ± 0.021) and lowest in Iron beans (1.46 ± 0.014). The percentage of trypsin was highest in ‘Oloka’ (16.75 ± 0.014) and lowest in iron beans (11.49 ± 0.014). The percentage of starchyose was highest in ‘Oloka’ (1.90 ± 0.000) and lowest in ‘Ifebrown’ (1.62 ± 0.028), the percentage of raffinose was highest in ‘Sokoto guzo’ (1.35 ± 0.000) and lowest in ‘Potiskum’ (1.18 ± 0.000), while the percentage of oxalate was highest in ‘Oloka’ (2.74 ± 0.021) and lowest in iron beans (1.67 ± 0.050). The analysis of variance showed a significant difference in the percentage of tannin, saponin, flavonoid, alkaloid, sterol, phenol, phytate, trypsin, starchyose, raffinose and oxalate between the seed of ‘kafanji’, Crowderpea, ‘Ifebrown’, ‘Oloka’, ‘Sokoto guzo’, ‘Potiskum’ and Iron beans ($p < 0.05$) (Table 24).

Table 24: Comparative analysis of phytate acid, Trpsin, Starchyose, Raffinose and Oxalate constituents of the seven varieties of *Vigna unguiculata*

Variety	% Composition				
	Phytate Acid	Trypsin	Starchyose	Raffinose	Oxalate
Kafanji	1.61 ± 0.014^b	12.75 ± 0.014^e	1.78 ± 0.000^c	1.24 ± 0.000^d	1.85 ± 0.000^d
Crowdrpea	1.53 ± 0.007^c	13.87 ± 0.042^c	1.84 ± 0.021^b	1.28 ± 0.02^c	1.94 ± 0.000^c
Ifebrown	1.50 ± 0.028^c	11.62 ± 0.028^f	1.62 ± 0.028^d	1.22 ± 0.028^b	1.94 ± 0.021^c
Oloka	1.74 ± 0.021^a	16.75 ± 0.014^a	1.90 ± 0.000^a	1.29 ± 0.000^b	2.74 ± 0.021^a
Sokoto Guzo	1.65 ± 0.000^b	15.32 ± 0.028^b	1.85 ± 0.000^b	1.35 ± 0.000^a	2.62 ± 0.028^b
Potiskum	1.61 ± 0.014^b	13.61 ± 0.014^d	1.63 ± 0.035^d	1.18 ± 0.000^e	1.82 ± 0.028^d
Iron Beans	1.46 ± 0.014^d	11.49 ± 0.014^g	1.67 ± 0.021^d	1.22 ± 0.028^d	1.67 ± 0.050^e
p-value	**	**	**	**	**

Proximate Composition of the Leaf, Stem, Root and Seed of seven varieties of *Vigna unguiculata*.

Result of the proximate composition of the leaf, stem, root and seed of 'Kafanji' variety revealed that percentage of moisture was highest in the seed (11.37 ± 0.021) and lowest in the stem (8.98 ± 0.000). The percentage of dry matter was highest in the stem (91.02 ± 0.000) and lowest in the seed (88.65 ± 0.000), the percentage of ash was highest in the leaf (15.33 ± 0.134) and lowest in the seed (3.47 ± 0.028). The percentage of crude fibre was highest in the stem (13.78 ± 0.028) and lowest in the seed (3.73 ± 0.014). The percentage of ether extract was highest in the seed (3.17 ± 0.014) and lowest in the root (0.65 ± 0.000), while the percentage of crude protein was highest in the seed (19.77 ± 0.042) and lowest in the root (7.94 ± 0.028). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 25).

Table 25: Proximate Composition of the Leaf, Stem, Root and Seed of Kafanji Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	9.44 ± 0.028^b	90.56 ± 0.028^b	15.33 ± 0.134^a	12.81 ± 0.071^b	1.25 ± 0.021^b	14.84 ± 0.085^b
Stem	8.98 ± 0.000^c	91.02 ± 0.000^a	12.76 ± 0.148^b	13.78 ± 0.028^a	0.82 ± 0.057^c	9.58 ± 0.028^c
Root	9.68 ± 0.255^b	90.32 ± 0.255^b	12.88 ± 0.028^b	12.23 ± 0.099^c	0.65 ± 0.000^d	7.94 ± 0.028^d
Seed	11.37 ± 0.021^a	88.65 ± 0.000^c	3.47 ± 0.028^c	3.73 ± 0.014^d	3.17 ± 0.014^a	19.77 ± 0.042^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the proximate composition of the leaf, stem, root and seed of black beans (Crowder peas) variety revealed that percentage of moisture was highest in the seed (10.28 ± 0.000) and lowest in the stem (6.87 ± 0.050). The percentage of dry matter was highest in the stem (93.14 ± 0.050) and lowest in the seed (89.72 ± 0.000). The percentage of ash was highest in the leaf (13.62 ± 0.028) and lowest in the seed (3.78 ± 0.000). The percentage of crude fibre was highest in the root (14.76 ± 0.057) and lowest in the seed (3.67 ± 0.021). The percentage of ether extract was highest in the seed (2.94 ± 0.000) and lowest in the root (0.45 ± 0.000), while the percentage of crude protein was highest in the seed (18.58 ± 0.177) and lowest in the root (8.75 ± 0.014). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 26).

Table 26: Proximate Composition of the Leaf, Stem, Root and Seed of Crowderpea Variety
% Composition*

Part of Plant	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	8.76 ± 0.000^b	91.24 ± 0.000^b	13.62 ± 0.028^a	12.75 ± 0.014^b	1.39 ± 0.014^b	14.75 ± 0.014^b
Stem	6.87 ± 0.050^c	93.14 ± 0.050^a	10.74 ± 0.021^c	12.82 ± 0.028^b	0.57 ± 0.014^c	8.94 ± 0.028^c
Root	6.95 ± 0.297^c	93.05 ± 0.297^a	11.71 ± 0.127^b	14.76 ± 0.057^a	0.45 ± 0.000^d	8.75 ± 0.014^c
Seed	10.28 ± 0.000^a	89.72 ± 0.000^c	3.78 ± 0.000^d	3.67 ± 0.021^c	2.94 ± 0.000^a	18.58 ± 0.177^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the proximate composition of the leaf, stem, root and seed of 'Ifebrown' variety revealed that percentage of moisture was highest in the seed (10.62 ± 0.000) and lowest in the root (7.72 ± 0.170). The percentage of dry matter was highest in the root (92.28 ± 0.170) and lowest in the seed (89.38 ± 0.000). The percentage of ash was highest in the stem (16.78 ± 0.028) and lowest in the seed (3.64 ± 0.000). The percentage of crude fibre was highest in the root (15.27 ± 0.042) and lowest in the seed (3.89 ± 0.042). The percentage of ether extract was highest in the seed (2.67 ± 0.050) and lowest in the root (0.77 ± 0.014), while the percentage of crude protein was highest in the seed (19.52 ± 0.028) and lowest in the stem (7.72 ± 0.120). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 27)

Table 27: Proximate Composition of the Leaf, Stem, Root and Seed of 'Ifebrown' Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	8.58 ± 0.177^b	91.43 ± 0.177^b	15.76 ± 0.057^b	13.77 ± 0.042^d	1.84 ± 0.021^b	13.82 ± 0.028^b
Stem	7.88 ± 0.057^c	92.12 ± 0.057^a	16.78 ± 0.028^a	14.68 ± 0.113^b	1.06 ± 0.000^c	7.72 ± 0.120^d
Root	7.72 ± 0.170^c	92.28 ± 0.170^a	13.46 ± 0.035^c	15.27 ± 0.042^a	0.77 ± 0.014^d	8.47 ± 0.021^c
Seed	10.62 ± 0.000^a	89.38 ± 0.000^c	3.64 ± 0.000^d	3.89 ± 0.042^c	2.67 ± 0.050^a	19.52 ± 0.028^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the proximate composition of the leaf, stem, root and seed of 'Oloka' variety revealed that the percentage of moisture was highest in the seed (10.74 ± 0.021) and lowest in the root (7.87 ± 0.042). The percentage of dry matter was highest in the stem (92.13 ± 0.042) and lowest in the seed (89.27 ± 0.021). The percentage of ash was highest in the stem (14.91 ± 0.014) and lowest in the seed (4.13 ± 0.000), the percentage of crude fibre was highest in the stem (14.32 ± 0.028) and lowest in the seed (3.79 ± 0.014). The percentage of ether extract was highest in the seed (2.92 ± 0.000) and lowest in the root (0.78 ± 0.028), while the percentage of crude protein was highest in the seed (21.78 ± 0.028) and lowest in the root (8.96 ± 0.000). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 28)

Table 28: Proximate Composition of the Leaf, Stem, Root and Seed of 'Oloka' Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	9.16 ± 0.000^b	90.84 ± 0.000^c	14.78 ± 0.028^a	12.94 ± 0.000^c	1.77 ± 0.021^b	15.81 ± 0.042^b
Stem	7.87 ± 0.042^d	92.13 ± 0.042^a	14.91 ± 0.014^a	14.32 ± 0.028^a	0.85 ± 0.000^c	9.84 ± 0.000^c
Root	8.75 ± 0.014^c	91.25 ± 0.014^b	12.32 ± 0.113^b	13.82 ± 0.028^b	0.78 ± 0.028^d	8.96 ± 0.000^d
Seed	10.74 ± 0.021^a	89.27 ± 0.021^d	4.13 ± 0.000^c	3.79 ± 0.014^d	2.92 ± 0.000^a	21.78 ± 0.028^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the proximate composition of the leaf, stem, root and seed of ‘Sokoto guzo’ variety revealed that the percentage of moisture was highest in the seed (11.34 ± 0.085) and lowest in the stem (7.87 ± 0.042). The percentage of dry matter was highest in the stem (92.08 ± 0.028) and lowest in the seed (88.66 ± 0.085). Percentage of ash was highest in the leaf (14.56 ± 0.057) and lowest in the seed (4.06 ± 0.000). The percentage of crude fibre was highest in the root (14.71 ± 0.014) and lowest in the seed (3.88 ± 0.028). The percentage of ether extract was highest in the seed (2.47 ± 0.021) and lowest in the stem (0.78 ± 0.000), while the percentage of crude protein was highest in the seed (19.81 ± 0.042) and lowest in the root (9.17 ± 0.014). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 29).

Table 29: Proximate Composition of the Leaf, Stem, Root and Seed of ‘Sokoto guzo’ Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	8.93 ± 0.014^b	91.07 ± 0.014^b	14.56 ± 0.057^a	13.62 ± 0.028^c	1.63 ± 0.014^b	14.87 ± 0.042^b
Stem	7.87 ± 0.042^c	92.08 ± 0.028^a	13.85 ± 0.007^b	13.87 ± 0.042^b	0.78 ± 0.000^d	9.32 ± 0.120^c
Root	8.94 ± 0.028^b	91.06 ± 0.028^b	11.38 ± 0.028^c	14.71 ± 0.014^a	0.85 ± 0.00^c	9.17 ± 0.014^c
Seed	11.34 ± 0.085^a	88.66 ± 0.085^c	4.06 ± 0.000^d	3.88 ± 0.028^d	2.47 ± 0.021^a	19.81 ± 0.042^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

Result of the proximate composition of the leaf, stem, root and seed of 'Potiskum' variety revealed that the percentage of moisture was highest in the seed (11.49 ± 0.014) and lowest in the stem (7.90 ± 0.000). The percentage of dry matter was highest in the stem (92.10 ± 0.000) and lowest in the seed (88.51 ± 0.014). The percentage of ash was highest in the stem (14.76 ± 0.057) and lowest in the seed (3.68 ± 0.000), the percentage of crude fibre was highest in the stem (14.31 ± 0.014) and lowest in the seed (3.68 ± 0.035). The percentage of ether extract was highest in the seed (2.84 ± 0.000) and lowest in the root (0.86 ± 0.000), while the percentage of crude protein was highest in the seed (21.38 ± 0.106) and lowest in the stem (8.24 ± 0.000). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 30).

Table 30: Proximate Composition of the Leaf, Stem, Root and Seed of 'Potiskum' Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	9.14 ± 0.000^b	90.86 ± 0.000^c	13.82 ± 0.028^b	13.78 ± 0.028^b	1.29 ± 0.014^b	14.81 ± 0.014^b
Stem	7.90 ± 0.000^d	92.10 ± 0.000^a	14.76 ± 0.057^a	14.31 ± 0.014^a	0.94 ± 0.000^c	8.24 ± 0.000^d
Root	8.88 ± 0.057^c	91.12 ± 0.057^b	11.65 ± 0.000^c	12.53 ± 0.106^c	0.86 ± 0.000^d	9.76 ± 0.057^c
Seed	11.49 ± 0.014^a	88.51 ± 0.014^d	3.68 ± 0.000^d	3.68 ± 0.035^d	2.84 ± 0.000^a	21.38 ± 0.106^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Result of the proximate composition of the leaf, stem, root and seed of iron beans variety revealed that the percentage of moisture was highest in the seed (10.41 ± 0.156) and lowest in the stem (8.62 ± 0.028). The percentage of dry matter was highest in the stem (91.38 ± 0.028) and lowest in the seed (89.59 ± 0.156). Percentage of ash was highest in the leaf (14.79 ± 0.014) and lowest in the seed (3.84 ± 0.000). The percentage of crude fibre was highest in the stem (13.57 ± 0.042) and lowest in the seed (3.78 ± 0.028). The percentage of ether extract was highest in the seed (3.12 ± 0.000) and lowest in the root (0.76 ± 0.000), while the percentage of crude protein was highest in the seed (19.57 ± 0.042) and lowest in the root (7.71 ± 0.127). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf, stem, root and seed ($p < 0.05$) (Table 31).

Table 31: Proximate Composition of the Leaf, Stem, Root and Seed of Iron Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Leaf	9.33 ± 0.099^b	90.67 ± 0.099^b	14.79 ± 0.014^a	12.91 ± 0.014^b	1.09 ± 0.007^b	15.24 ± 0.000^b
Stem	8.62 ± 0.028^c	91.38 ± 0.028^a	13.82 ± 0.028^b	13.57 ± 0.042^a	0.83 ± 0.014^c	8.83 ± 0.099^c
Root	9.25 ± 0.014^b	90.75 ± 0.014^b	12.32 ± 0.028^c	12.79 ± 0.014^c	0.76 ± 0.000^d	7.71 ± 0.127^d
Seed	10.41 ± 0.156^a	89.59 ± 0.156^c	3.84 ± 0.000^d	3.78 ± 0.028^d	3.12 ± 0.000^a	19.57 ± 0.042^a
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Comparative analysis of the proximate composition of the leaf of seven varieties of *Vigna unguiculata* ('kafanji', crowderpea, and 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans) indicated that the percentage of moisture was highest in 'kafanji' (9.44 ± 0.028) and lowest in 'Ifebrown' (8.58 ± 0.177). The percentage of dry matter was highest in 'Ifebrown' (91.43 ± 0.177) and lowest in kafanji (90.56 ± 0.028). Percentage of ash was highest in 'Ifebrown' (15.76 ± 0.057) and lowest in Crowderpea (13.62 ± 0.028). The percentage of crude fibre was highest in potiskum (13.78 ± 0.028) and lowest in crowderpea (12.75 ± 0.014). The percentage of ether extract was highest in ifebrown (1.84 ± 0.021) and lowest in iron beans (1.09 ± 0.007), while the percentage of crude protein was highest in 'Oloka' (15.81 ± 0.042) and lowest in 'Ifebrown' (13.82 ± 0.028). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the leaf of 'kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans ($p < 0.05$) (Table 32)

Table 32: Comparative Proximate Analysis of the Leaf of Seven Varieties of *Vigna unguiculata*

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	9.44 ± 0.028^a	90.56 ± 0.028^d	15.33 ± 0.134^b	12.81 ± 0.071^d	1.25 ± 0.021^f	14.84 ± 0.085^c
Crowderpea	8.76 ± 0.000^c	91.24 ± 0.000^b	13.62 ± 0.028^f	12.75 ± 0.014^d	1.39 ± 0.014^d	14.75 ± 0.014^d
Ifebrown	8.58 ± 0.177^d	91.43 ± 0.177^a	15.76 ± 0.057^a	13.77 ± 0.042^a	1.84 ± 0.021^a	13.82 ± 0.028^e
Oloka	9.16 ± 0.000^b	90.84 ± 0.000^c	14.78 ± 0.028^c	12.94 ± 0.000^c	1.77 ± 0.021^b	15.81 ± 0.042^a
Sokoto guzo	8.93 ± 0.014^c	91.07 ± 0.014^b	14.56 ± 0.057^d	13.62 ± 0.028^b	1.63 ± 0.014^c	14.87 ± 0.042^c
Potiskum	9.14 ± 0.000^b	90.86 ± 0.000^c	13.82 ± 0.028^e	13.78 ± 0.028^a	1.29 ± 0.014^e	14.81 ± 0.014^c
Iron Beans	9.33 ± 0.099^a	90.67 ± 0.099^c	14.79 ± 0.014^c	12.91 ± 0.014^c	1.09 ± 0.007^g	15.24 ± 0.000^b
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different ** $p < 0.05$

Comparative analysis of the proximate composition of the stem of seven varieties of *Vigna unguiculata* ('kafanji', Crowderpea (black beans), 'Ifebrown', 'Oloka', 'Sokoto guzo', and 'potiskum' and Iron beans) indicated that the percentage of moisture was highest in 'kafanji' (8.98 ± 0.000) and lowest in crowderpea (6.87 ± 0.050). The percentage of dry matter was highest in black beans (93.14 ± 0.050) and lowest in 'kafanji' (91.02 ± 0.000). The percentage of ash was highest in 'Ifebrown' (16.78 ± 0.028) and lowest in black beans (10.74 ± 0.021). The percentage of crude fibre was highest in ifebrown (14.68 ± 0.113) and lowest in black beans (12.82 ± 0.028). The percentage of ether extract was highest in ifebrown (1.06 ± 0.000) and lowest in black beans (0.57 ± 0.014), while the percentage of crude protein was highest in 'Oloka' (9.84 ± 0.000) and lowest in 'Ifebrown' (7.72 ± 0.120). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the stem of 'kafanji', black beans, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'potiskum' and Iron beans ($p < 0.05$) (Table 33).

Table 33: Comparative Proximate Analysis of the Stem of Seven Varieties of *Vigna unguiculata*

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	8.98 ± 0.000^a	91.02 ± 0.000^d	12.76 ± 0.148^e	13.78 ± 0.028^c	0.82 ± 0.057^c	9.58 ± 0.028^b
Crowderpea	6.87 ± 0.050^d	93.14 ± 0.050^a	10.74 ± 0.021^f	12.82 ± 0.028^e	0.57 ± 0.014^e	8.94 ± 0.028^d
Ifebrown	7.88 ± 0.057^c	92.12 ± 0.057^b	16.78 ± 0.028^a	14.68 ± 0.113^a	1.06 ± 0.000^a	7.72 ± 0.120^f
Oloka	7.87 ± 0.042^c	92.13 ± 0.042^b	14.91 ± 0.014^b	14.32 ± 0.028^b	0.85 ± 0.000^c	9.84 ± 0.000^a
Sokoto guzo	7.87 ± 0.042^c	92.08 ± 0.028^b	13.85 ± 0.007^d	13.87 ± 0.042^c	0.78 ± 0.000^d	9.32 ± 0.120^c
Potiskum	7.90 ± 0.000^c	92.10 ± 0.000^b	14.76 ± 0.057^c	14.31 ± 0.014^b	0.94 ± 0.000^b	8.24 ± 0.000^e
Iron Beans	8.62 ± 0.028^b	91.38 ± 0.028^c	13.82 ± 0.028^d	13.57 ± 0.042^d	0.83 ± 0.014^c	8.83 ± 0.099^d
p-value	**	**	**	**	**	**

columns followed by the same letter are not significantly different ** $p < 0.05$

Comparative analysis of the proximate composition of the root of seven varieties of *V. unguiculata* ('kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'potiskum' and Iron beans) indicated that the percentage of moisture was highest in 'kafanji' (9.68 ± 0.255) and lowest in black beans (6.95 ± 0.297). The percentage of dry matter was highest in crowderpea (93.05 ± 0.297) and lowest in 'kafanji' (90.32 ± 0.255). The percentage of ash was highest in 'Ifebrown' (13.46 ± 0.035) and lowest in 'Sokoto guzo' (11.38 ± 0.028), the percentage of crude fibre was highest in 'Ifebrown' (15.27 ± 0.042) and lowest in 'kafanji' (12.23 ± 0.099), the percentage of ether extract was highest in 'potiskum' (0.86 ± 0.000) and lowest in Crowderpea (0.45 ± 0.000), while the percentage of crude protein was highest in potiskum (9.76 ± 0.057) and lowest in 'kafanji' (7.94 ± 0.028). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the root of 'kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'Potiskum' and Iron beans ($p < 0.05$) (Table 34).

Table 34: Comparative Proximate Analysis of the Root of Seven Varieties of *Vigna unguiculata*

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	9.68 ± 0.255^a	90.32 ± 0.255^c	12.88 ± 0.028^b	12.23 ± 0.099^f	0.65 ± 0.000^d	7.94 ± 0.028^f
Crowderpea	6.95 ± 0.297^e	93.05 ± 0.297^a	11.71 ± 0.127^d	14.76 ± 0.057^b	0.45 ± 0.000^e	8.75 ± 0.014^d
Ifebrown	7.72 ± 0.170^d	92.28 ± 0.170^b	13.46 ± 0.035^a	15.27 ± 0.042^a	0.77 ± 0.014^c	8.47 ± 0.021^e
Oloka	8.75 ± 0.014^c	91.25 ± 0.014^c	12.32 ± 0.113^c	13.82 ± 0.028^c	0.78 ± 0.028^b	8.96 ± 0.000^c
Sokoto guzo	8.94 ± 0.028^c	91.06 ± 0.028^c	11.38 ± 0.028^c	14.71 ± 0.014^b	0.85 ± 0.000^a	9.17 ± 0.014^b
Potiskum	8.88 ± 0.057^c	91.12 ± 0.057^c	11.65 ± 0.000^d	12.53 ± 0.106^e	0.86 ± 0.000^a	9.76 ± 0.057^a
Iron Beans	9.25 ± 0.014^b	90.75 ± 0.014^d	12.32 ± 0.028^c	12.79 ± 0.014^d	0.76 ± 0.000^c	7.71 ± 0.127^g
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different ** $p < 0.05$

Comparative analysis of the proximate composition of the seed of seven varieties of *Vigna unguiculata* ('kafanji', Crowderpea, 'Ifebrown', 'Oloka', 'Sokoto guzo', 'potiskum' and Iron beans) indicated that the percentage of moisture was highest in 'potiskum' (11.49 ± 0.014) and lowest in Crowderpea (10.28 ± 0.000). The percentage of dry matter was highest in Crowderpea (89.72 ± 0.000) and lowest in 'kafanji' (88.65 ± 0.000). The percentage of ash was highest in 'Oloka' (4.13 ± 0.000) and lowest in 'kafanji' (3.47 ± 0.028). The percentage of crude fibre was highest in 'Ifebrown' (3.89 ± 0.042) and lowest in Crowderpea (3.67 ± 0.021). The percentage of ether extract was highest in 'kafanji' (3.17 ± 0.014) and lowest in 'Sokoto guzo' (2.47 ± 0.021), while the percentage of crude protein was highest in 'Oloka' (21.78 ± 0.028) and lowest in Crowderpea (18.58 ± 0.177). The analysis of variance showed a significant difference in the percentage of moisture, dry matter, ash, crude fibre, ether extract and crude protein between the seed of 'kafanji', crowderpea, 'ifebrown', 'oloka', 'sokoto guzo', 'potiskum' and iron beans ($p < 0.05$) (Table 35).

Table 35: Comparative Proximate Analysis of the Seed of Seven Varieties of Beans

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	11.37 ± 0.021^a	88.65 ± 0.000^c	3.47 ± 0.028^g	3.73 ± 0.014^b	3.17 ± 0.014^a	19.77 ± 0.042^c
Crowderpea	10.28 ± 0.000^c	89.72 ± 0.000^a	3.78 ± 0.000^d	3.67 ± 0.021^c	2.94 ± 0.000^b	18.58 ± 0.177^e
Ifebrown	10.62 ± 0.000^b	89.38 ± 0.000^b	3.64 ± 0.000^f	3.89 ± 0.042^a	2.67 ± 0.050^d	19.52 ± 0.028^d
Oloka	10.74 ± 0.021^b	89.27 ± 0.021^b	4.13 ± 0.000^a	3.79 ± 0.014^b	2.92 ± 0.000^b	21.78 ± 0.028^a
Sokoto Guzo	11.34 ± 0.085^a	88.66 ± 0.085^c	4.06 ± 0.000^b	3.88 ± 0.028^a	2.47 ± 0.021^e	19.81 ± 0.042^c
Potiskum	11.49 ± 0.014^a	88.51 ± 0.014^c	3.68 ± 0.000^c	3.68 ± 0.035^c	2.84 ± 0.000^c	21.38 ± 0.106^b
Iron Beans	10.41 ± 0.156^c	89.59 ± 0.156^a	3.84 ± 0.000^c	3.78 ± 0.028^b	3.12 ± 0.000^b	19.57 ± 0.042^d
p-value	**	**	**	**	**	**

Columns followed by the same letter are not significantly different ** $p < 0.05$

CHAPTER FIVE

5.1 Discussion

Morphology aids in the classification of plants, this is as well as cytology, phytochemical and anatomy. Morphological data of plants are easily observable and obtainable and thus are used frequently in plant identification (Radford and Cadell, 1986). The external morphological evidence provides the basic language for plant characterization, identification, classification and relationships. (Sharma, 1993), noted morphological characters as vegetative, phenological and floral characters, as well as seeds and fruit morphology; thus morphological features of plants are those external diagnostic features of plants (Radford and Cadell, 1986). The study on the physical form and the external structure of the seven varieties of *Vigna unguiculata* available in Anambra State, revealed significant differences among the varieties in the leaf, stem, pod and petiole length. (Figs 1-5). Phenotypic variations observed in the seed sizes, colour, seed texture and hilum colour were evident among these seven varieties. All the variables used were important in determining their variations but none of them could identify any genotype as a duplicate of the other. High variability among cowpea genotypes based on phenotypic characters has been reported by some researchers (Aremu *et al.*, 2007 and Adewal *et al.*, 2010). Leaf type showed trifoliate and a sub-globose shape. Ojomo, (1977), Kohle, (1970), Fery, (1985) Oluwatosin, (2002) studied the inheritance of the leaf shape and reported this as a qualitative trait. Also Oluwatosin, (2002) and Ojomo, (1977) study on cowpea concluded that hastate leaf shape was dominant to the common ovate or sub globose leaf shape, but this was disagreed in this study of the seven varieties because the leaf shape observed was subglobose. However, (Saunders *et al.*, 1960) also reported that the hastate leaf shape was incompletely dominant over the ovate or sub globose leaf shapes. There are wide ranges among plant genotype in shape, size and number of leaves produced by plants. Dorchester (1945) emphasized that leaflet shape could be used as an adequate morphological marker to distinguish soybean cultivars and other leguminous plants. Cowpea has a compound leaf with three leaflets. A true cowpea leaf consists of two asymmetrical lateral leaflets and one symmetrical terminal leaflet with longer petiole than that of the lateral ones. Leaf appearance in plants is an important process involved in canopy

development (Crawford *et al.*, 1997). Some of the morphological observations in this study supported the earlier reports of Agbogidi and Ofuoku (2005) that plants respond differently to environmental factors based on their genetic make up and their adaptation capability. Also Morphological characteristics of plants are commonly used in the description of varietal differences. Botanists like Hutchinson (1973) described morphology as a major criterion for plant classification over many centuries. He used woody and herbaceous characters as his major classifications. Coode (1967) used fruit characteristics in the delimitation of species of the genus *Valerianella*. The variability in the stem length (Fig 4), pod weight (Fig 5), pod length (Table 3), pod width, Canonical discriminant function (Fig.6), studied strengthens the importance of variability in the study. Crawford *et al.*, (1997) reported that the variability of leaf appearance is an important process involved in canopy development. Seed colours and pod weight is a good taxonomic tool. This was issued in (1912) study in the US cultivated forms. Three groups in his study was separated according to seed and pod characteristics which was ranked at all the possible taxonomic levels. Wesphal (1974) used this cultigroup method using pod and seed weight. Based on this report, the pod weight of 'Ifebrown' was highest and lowest in 'kafanji' varieties this can be added to other variables as a taxonomic tool. The distinguishing morphological features observed in this study were of systematic value because they were seasonably constant in the taxa (*Vigna unguiculata*), signifying the stability and differences in their morphological characteristics. Olowokudejo (1990) made similar observations in his study on the morphology of *Capsicum* species, likewise (Aziagba, *et al.*, 2015) study on the Macromorphological observations in *Capsicum* Varieties. Morphological variations among species however is inevitable, thus the variations within the varieties in the sizes of leaf as shown by previous authors have variability within the same plant due to light intensity acting on the leaves, thus affecting the carbohydrate balance which in turn affects the length, shapes and width of leaves (Campey *et al.*, 2000., Wilson *et al.*, 1998) as a result of environmental and geographical factors and localities caused by varying light intensities. Thus Morphological studies used in this research assessed the reliability of the characters of the seven varieties of *Vigna unguiculata* in the systematic consideration and has proven this to be of immense interest in taxonomy (Okwulehie and Okoli, 1999). Vegetative and phenotypic morphology observed strengthens the intra and interspecific relationship in the taxa. Genotypes based on phenotypic characters have been reported by some authors (Ariyo, 2007; Adewale *et al.*, 2010). Specific variation which

differentiates genotypes with respect to some phenotypic characters may have ensued from natural and environmental mutations of the phenotypic traits. Morphological plasticity as reported by Karuri *et al.*, 2010 cited by Oliveri *et al.*, 2003 stated that the variation in the morphological characters of genotypes which are phenotypically similar is due to genetic differentiation especially in the presence of varying conditions and parallel evolution. The significant performance of some cowpea lines (for some phenotypic traits example number of seeds in the pods, hilum colours, seed colours, pod weight, seed texture and pod length) indicates that cowpea breeding and improvement is advancing in Nigeria. Hilum colour, seed texture, seed size and seed colour are the most distinct morphological characters of the seven varieties that differentiate the varieties from each other.

Stomata are homologous structures and of universal occurrence in plants. Stomata characters have been very useful in taxonomic delimitation. This is apart from their functional relevance in evapotranspiration. This was observed in the stomatal structures of the seven varieties of *Vigna unguiculata* studied. Comparative studies by Patial and Patial (1987) used these stomatal characters in the taxonomic consideration at the sub-generic level. Result of the epidermal features of the studied taxa revealed some diagnostic characteristics that could be used for taxonomic decision. A feature that may separate the varieties and the accessions from one another has been reported by the earlier works of Okwulehie and Okoli (1999); Edeoga and Emeka (2000). They both used comparative morphology of different species in establishing relationship among various taxa. The cell wall and cell shape as well as the stomatal type revealed correlations among the studied taxa. Some of the varieties possess paracytic on both the abaxial and adaxial surfaces and only Var. 'Ifebrown' possess anisocytic stomata on the abaxial surfaces, while paracytic were more dominant in the varieties. Stace (1965) suggested that environmental condition, such as humidity play a significant role in determining the pattern of anticlinal walls. The appearance of more stomata on the abaxial surface was probably an adaptation to water loss (Mbagwu *et al.*, 2008). This is also reported in the findings of Metcalfe and Chalk (1979); Mbagwu and Edeoga (2006) who both observed that stomata are usually more on the lower epidermis usually in *Amarantus* and *Vigna* respectively. The shape of epidermal cells, types and arrangement of stomata and size are important systematic parameters. The description on the structure and development conforms to Metcalfe and Chalk (1950) who worked on the epidermal morphology and stomatal types in dicotyledons. The epidermal

characteristics shared by the seven varieties explain why they are in the same genus *Vigna*. In general, macro and micromorphological features observed on the leaves are consistent with those of Metcalfe and Chalk (1950) and Philipson (1963) for the description of leaf anatomy of Leguminosae (Fabaceae). The different micro-morphological characteristics of the varieties of *Vigna unguiculata* examined can be a reliable tool in the systematic delineation of the taxa. The shape of epidermal cells, types and arrangement of stomata, epidermal cell wall contours and stomatal index are also an important systematic parameters (Gill and Keratela, 1982; Edeoga, 1991).

Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for elucidation of phylogenetic relationships. Anatomical data have not only been useful at the higher levels but in certain instances have been successfully used even at the specific level. Literature searches revealed the scientific importance and implications of anatomical features in different groups of plants. They include Dioscoreaceae, where certain anatomical features were used in the characterization of *Dioscorea alata* (L.) and *Dioscorea smilacifolia* (L.) (Edeoga, 2002). In Costaceae, differences in features of vegetative anatomy suggested a separate specific status for *Costus afer* and *Costus lucanusianus* as opposed to the conspecific treatment given to them by previous researchers (Edeoga and Okoli, 1997). In Leguminosae-Caesalpinoideae, the nature of unicellular and multicellular trichomes are described in certain species of *Senna Tourn* ex Mill and *Senna hirsuta* was reported to be diagnostic in acquisition of these two types of trichomes. (Edeoga and Osawe, 1996). Other studies of interest relating to anatomy of different angiospermous groups could be found in Curcubitaceae (Okoli, 1987), Dicotyledons as a whole (Metcalfe and Chalk, 1950), Leguminosae-Papilionoideae (Mbagwu and Edeoga, 2006). In the transverse section of the leaf of the seven varieties, a layer of epidermis was observed on both upper and lower blade. The starch grains in plates 39-45 were visible. The midrib revealed similar structures in all the varieties studied. This was also reported in the anatomical study on the species of *Solanum* (Furlan *et al.*, 1999). Collenchyma types were the same, angular in the varieties studied. Parenchyma cells are large (Plate 40) intercellular spaces and bundle sheath extensions were revealed in Plates 39, 40, 41 and 45. These anatomical features probably suggested a close affinity and the features they share together as being under one genus. Vascular structures of stem correspond with the pattern referred to in dicotyledonous plants by Cutler (1978) and

Metcalf and Chalk (1950). Transverse sections of the stem of some of the varieties of *Vigna unguiculata* contain pores that were angular in shape while others have round shape, arranged in radial multiple and diffuse porous (plates 46-52). These differences also suggested moderate affinity in some of the varieties. Apotracheal parenchyma with diffuse aggregate, (plates 47 and 51) terminal type (plate 50) reticulate (plate 46) and banded in plates 48 and 49) were also observed (plates 46, 47 and 52). Paratracheal parenchyma was all banded. Paratracheal parenchyma was exhibited in advanced form whereas apotracheal parenchyma is a primitive condition (Esua, 1977, Roy, 2006). This suggests that Kafanji and Oloka varieties may be primitive. Ray sizes were more than half the width of pores (plates 47 and 48), aggregate and storied (plates 50, 52, 49, 51 and 48). The variation in the position of the rays and pore sizes and numbers strengthens the reliability of anatomical characters in systematic botany as stated by Ayensu (1970) in *Dioscorea rotundata*, *Dioscorea cayenensis* Lam., Mbagwu and Edeoga (2006) in *Vigna* species, Edeoga (2002) in *Dioscorea* species. Protoxylems (plates 49, 50, 51 and 52) and large pith on the stem of the varieties were typical of a dicotyledonous stem. The cambium initials were observed in the primary vascular bundle (22-28 in number) between the xylem and phloem at the basal part of the stem.

The transverse section of the petiole of the seven varieties, revealed epidermal cells as uniseriate branched trichomes covered by a thickened cuticle. Angular collenchyma (plates 53, 54, 55, 58 and 59) were in 2-3 layers. Parenchyma cells exhibited intercellular spaces (plates 54, 55 and 58). Vascular bundles were visible having 10-14 numbers. Cells were irregular in shape. The results showed the intraspecific relationship between them. Type, size, shape, stella patterns, vascular bundles, rays, parenchyma, epidermal and phloem cells are some of the basic anatomical characters of well established taxonomic value. (Sharma, 1993 and Pandey, and Pandey, 2007). These results were consistent with the description given by Heneidak and Shaheen (2007) in their investigation of the petioles of some papilionoid species. In this connection, Shaheen (2006, 2007) reported the usefulness of anatomy of stem-leaf transitional zone in the identification of some mimosoid and caesalpinoid species. This study also reveals the importance of stem-leaf transitional zone in the identification of *Vigna* species.

The transverse section of the roots of the seven varieties has one layer of epidermal cells though (plate 60) has lenticels, because the root segments of cowpea are not thick. (Cutler, 1978). Xylem vessel size and the numbers were of various dimensions. This was the most discriminating traits

of legumes. In the seven varieties, there was presence of moderate xylem passage per root, indicating that they were capable of absorbing water moderately and well equipped for drought episodes. This was reported in the findings of Cutler, (1978) and Esau, (1965) on the reasons for variations in cells as a result of age of the plant or the environment. The anatomical features shared in common by the varieties suggested close affinity in the taxa. This was also reported in the findings of Aziagba *et al.*, (2014) of homodominant characters in the root of *Capsicum* species. Anatomical parameters have played an important role in plant taxonomy (Metcalf and Chalk, 1957). However, they have been largely unexploited in taxonomic studies because of their restricted value for distinguishing species or taxa of infraspecific rank. The differences between species are usually quantitative rather than qualitative and demand statistical analysis of a large number of samples. Anatomical characters have proved to be more useful for delimitation of higher taxonomic ranks, such as genera and families. Anatomical parameters have been used in solving significant taxonomic problems within different taxonomic groups. The findings in this study were able to provide these important taxonomic parameters that can help in the systematic study of these taxa.

The chromosomes of cowpea are very small, but this study has shown that though very small they can be subjected to detailed cytological observations. The cytological study was done using the root tips pre-treated with 8-hydroxyquinoline and fixed in Carnoy's fluid made the observation of chromosomes of the varieties possible not excluding some disorders. Various stages of mitosis were recorded to determine mitotic activity in dividing cells. Some of the chromosomes were seen at the telophase stage. Lag phases were observed as a result of the chromosomes which failed to arrive at the spindle at the same time as the others. The diploid chromosome number was observed to be 22 at the prophase. This finding was also reported by Jones (1978) that chromosomal features are being regarded as decision making characters in the study of phylogenetic affinities and evolutionary development, and as indications of appropriate classifications of several plant groups. He also observed that chromosome number indicates the occurrence of polyploidy and reflects differences in the basic chromosome numbers among plants which may be reflected in their treatment in flora. This is also to state that at genomic level, plant diversity correlates with a high degree of variation in overall genome sizes, ploidy level and chromosome number (Leitch 2008). At late metaphase, some of the chromosomes of the dividing cells in the root tips were observed to lie on the equator of the spindle as an indistinguishable

mass. At early anaphase, the chromosomes appear to move pole-wards, with many of them showing V- shape configuration. These results were obtained at various cell divisions which occurred at various intervals of harvest. Magri-Allegra & Zannone (1965), using mitotic root tip investigations, observed the incidence of bridges, laggards, centric and acentric rings in chromosomes of plant treated with some mutagen doses. Chromosome breakages and translocations were commonly observed, and the rates of occurrence varied with treatment agent and dose. The results were confirmed in a similar study in *Vicia faba* reported by Savage (1968). Those cells which carry unusually large number of chromosome aberrations produce a few or no progenies thus observed in this study. Consequently, future growth is from cells least damaged genetically. The phenomenon has been recorded in *Tradescantia paludosa* by Sax (1951) and similar examples have also been recorded in crops by other workers especially Bora *et al.*, (1961) on *Arachis hypogea* and *Plantago ovate*. The gross level of chromosome defects and alteration was observed generally in their work likewise in this study of seven varieties of *Vigna unguiculata*. Numerous types of chromosomal adaptation and ploidy alterations that were suspected in this study can be an outcome from the aberrations in the ubiquitous mitotic processes, including whole genome duplications and chromosome rearrangements. Increasingly; these processes are found to provide underlying mechanism for plant speciation, particularly in response to environmental change (Bennett and Leitch, 2003). Preliminary qualitative phytochemical analysis made for the leaf, stems, seed and root parts of seven varieties of *V. unguiculata* revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, phenols, phytic acid crude protein and carbohydrate. These secondary metabolites are resorted to have many biological and therapeutic properties (Okwu, 2005), so this species are used by man in different ways according to his needs, particularly as food and medicine. Major part of the traditional healing involves the use of plant extracts containing active constituents (Ahmad, *et al.*, 1998). Natural products are a source of synthetic and herbal medicine (Singh and Singh, 2001). They are non toxic, having no side effects and easily available at affordable prices (Britto and Mahesh, 2007). Fabaceae is cultivated as a nutraceutical in all over the world. The phytochemical constituents are known to have antimicrobial activity (Ebana *et al.*, 1995). The phytochemical constituents of seven varieties of *Vigna unguiculata* ('Ifebrown', 'Kafanji', Crowderpea, 'Potiskum', 'Iron beans', 'Sokoto guzo' and 'Oloka') available in Anambra State Nigeria were shown in Tables 16-29. It was noted that

the chemical composition of herbal extracts can vary widely with the plant variety (Wang *et al.*, 2004). The chemical characters are more important when they show a high degree of correlation with other features. The plants classified by taste, colour, smell etc. were the practice of ancient people. The taxonomical studies in relation to chemistry involve the study of the distribution of chemical compounds in related families of plants. The compounds exist in individual parts of plants such as bark, wood, leaves, roots etc. With the development of improved techniques for studying biological molecules, especially proteins and nucleic acids, the knowledge in phytochemistry has greatly increased which have been used to improve classification of plants. Man classified plants as edible and inedible which is based on their chemical differences. The presence of these typical metabolites has been reported in many plants to be responsible for pharmacological activities of medicinal plants (Gutierrez-lugo *et al.*, 2002, Okoli *et al.*, 2007, Mothana *et al.*, 2006). Chemical data provide information in situations where other forms of data are insufficient (Singh, 2000). This was the reason for comparing the phytochemical composition in the parts of the plants. The distribution of serological characteristics of seed proteins with primulaceae is studied and the results obtained are in accordance with the proposed subdivisions of the family made on morphological basis (John, 1978).

Comparison was made on the phytochemical compositions in different parts of the seven varieties, in the root; Tannin content was highest in Ifebrown than in other varieties. In the stem comparison, it was highest in the stem of Crowderpea (1.64 ± 0.000). In the leaf comparison it was highest in the potiskum leaf (1.45 ± 0.014) and in the seed of oloka (0.85 ± 0.014). Aziagba *et al.*, (2014) also revealed the presence of tannin as the highest phytochemical in the seed of *Capsicum* species var. Atarugu. Tannin occurs naturally in plants and acts as a defensive mechanism against pathogens, herbivores and hostile environmental conditions (Heming, 1989). Harborne, (1973) in his study on plants chemical revealed that the phytochemical investigation of plants involves authentication and extraction of the plant material, separation and isolation of the plant constituents, characterization of the isolated compounds, investigation of the biosynthetic pathways to particular compounds and quantitative evaluations. The presence of alkaloids and Tannins in all the varieties is an indication of close relationship among the varieties. Alkaloids, tannins, flavonoids and phenolic compounds have been reported by Edeoga *et al.*, (2003) as biotic compounds in plants. Saponin comparison in the parts studied revealed that Oloka variety has the highest saponin in the root, (1.31 ± 0.014), in the stem, (1.91 ± 0.014).

The leaf of Kafanji varieties has the highest saponin constituents (1.63 ± 0.035) and Sokoto guzo contains the highest saponin in the seed (1.06 ± 0.014). This finding was also reported by Ezeabara *et al.*, (2014) study on the composition of saponin in various percentages in parts of *Citrus* species with the root having the highest constituents. They also suggested that this might be as a result of the need to protect plants against soil pathogen attack. Saponin has a wide range of pharmacological and medicinal activities. Saponin content was highest in Kafanji leaf (1.63 ± 0.035). Some of the general properties of saponins include formation of forms in aqueous solution, hemolytic activity and cholesterol binding properties and bitterness (Sodipo *et al.*, 2000). They are also responsible for imparting a bitter taste and astringency properties to raw *Vigna unguiculata* (Iwe, 2003); and they are found in most dicotyledonous plants which is a tool in the systematic study making emphasis on their quantitative compositions. The total content of phenols was 0.37 ± 0.021 observed in the stem of Oloka. The phenolic components of higher plants may act as antioxidants or as agents of other mechanism contributing to anticarcinogenic or cardio-protective actions. (Okwu, 2004, 2005). Generally nontoxic phenolic compounds are believed to play vital roles in the development of adverse flavours, colour reactions and odours of oil seed protein sources (Iwe, 2003). The leaf phenolics of the leaves of the 11 species Fouquieriaceae studied reveal that there is no variation among the species in the phenolic distribution. All species contained ellagic acid, isoquercitrin, rutin, caffeic acid and scopolin (Scogin, 1978) this study revealed variations in the phenolic compositions of the seven varieties studied.

Flavonoids comparison revealed that 'Oloka' variety contains the highest flavonoids in the root (1.42 ± 0.000), stem (1.48 ± 0.000) and in the seed (0.81 ± 0.014) this revealed that Oloka varieties is a good medicinal resource. Flavonoid are phytochemical compounds which are widely distributed in all the vascular plants. They are plant pigments without nitrogen; they are powerful anti oxidants and work with carotenes to protect the plants from free radicals. Sokoto guzo leaf possesses the highest flavonoids. (Okigbo *et al.*, 2009) reported that Flavonoids are 15 – carbon plant secondary metabolites generally distributed throughout the plant kingdom. Quantitative evaluation of this chemical will assist to ascertain the identification of an unknown plant when other parameters has failed. (John, 1978). The taxonomy of *Primula* species has been in dispute due to the high morphologic variability and several hybridizations. Three morphological features of the trichome, size and dimensional ratio of stalk, neck and gland head studied revealed the

three trichome elements to be typical for each species of *Primula* – *Primula auricula*, *Primula daonensis* and *Primula hirsute* while the morphological characters treat the 3 species differently. Flavonoids studies were different in the three species because three different flavanoid profiles were obtained. Phytochemical investigations of the flavonoid composition of leaves are taxonomic markers. Thus both the morphological and taxonomic markers support the separation of the three species (Fico *et al.*, 2007). *Vigna unguiculata* varieties showed similar morphological characteristic in their phylotaxy but the phytochemical investigation in their parts have helped to strengthen their intraspecific and interspecific relationship as member of the same genus. The main average showed no real significant difference in the composition of their phytochemical.

The nutritional value of cowpea is in the composition of its grain. The grain is rich in protein up to around 30% and other nutrients and anti-nutrients in some varieties. Moisture content was highest in Var. 'Kafanji' (8.98 ± 0.000). Dry matter was highest in Var. Crowderpea (93.14 ± 0.050). Ash content in the stem of Var. 'Ifebrown' was highest (16.78 ± 0.028). Crude fibre was the highest in the stem of Var. 'Ifebrown' (14.68 ± 0.113). Ether extract content was highest in Var. 'Ifebrown' beans (1.06 ± 0.000). Crude protein composition in the stem was highest in Var. 'Oloka' variety (9.84 ± 0.0002). This reveals that all parts of cowpea are used for food. The leaves, green pods, green peas and dry grains dishes are consumed differently either as food or in the preparation of drugs. Root comparison revealed that moisture in Kafanji variety was the highest (9.68 ± 0.255). Dry matter content was highest in Crowther peas (93.05 ± 0.297). Ash content in Kafanji was highest (12.88 ± 0.0028). Crude fibre was highest in Ifebrown variety (15.27 ± 0.042). Ether extract content was highest in Potiskum variety (0.86 ± 0.000). Crude Protein was revealed as the highest composition of proximate in Potiskum variety. These parts are nutritious providing protein, vitamins and mineral especially micronutrient. The grains are rich in Amino acids, lysine and tryptosin making it better than cereals making them a good supplement for cereal and other root and tuber based diets.

The proximate analysis carried out on the seed part of *Vigna unguiculata* varieties, revealed that Crude protein was highest in Oloka (21.78 ± 0.028). Phytic acid (1.74 ± 0.021) was found in greater quantity in Oloka variety. Trypsin (16.75 ± 0.014) and starchyose (1.90 ± 0.000) in Oloka was also higher than in others. Sokotoguzo has the highest raffinose (1.35 ± 0.000) and the highest oxalate in the variety was in oloka variety. (2.74 ± 0.021). The values obtained from the analysis for each of the parts extracts revealed there were significant difference in the composition of both the

phytochemical and the proximate composition of the seven varieties of *Vigna unguiculata* ($p \leq 0.05$). The values obtained for each extract were within values reported for cowpea by Duke (1981) and Longe (1980). Protein content was highest in the seed of Oloka variety (21.78 ± 0.028). This was also within the report by Okwu and Orji, (2007) on the phytochemical and Nutritional composition of *Glycine max* and *Vigna unguiculata* though the percentages varies which may be attributed to both the environment and chemicals used for preparation.

The seeds of *Vigna unguiculata* have high content of protein and lipids (Table 33). The highest quantity of crude protein was contained in Var. Oloka. This was also reported by Ihekoronye and Ngoddy (1985), Enwere, (1998) and Iwe, (2003). *Vigna unguiculata* is a good source of Lysine, adequate in tryptophan but deficient in methione and cystine (Enwere, 1998). Consequently, its protein, which is rich in lysine but poor in methione and cystine, can be used to complement cereal proteins, which have low lysine but high methionine and cystine (Bressani, 1975). This implies that the quality of *Vigna unguiculata* proteins can be improved by the addition of methione and cystine sources. Dehulling decrease the amount of phytic acid in the grain of cowpea. It is recommended for cowpea before cooking, where people are using them in great amounts as in developing countries, Moreover when such legumes are used for baby foods, dehulling helps to prevent mineral deficiency. Cowpea grain contains trypsin, a digestive enzyme which breaks down proteins in the small intestine, secreted by the pancreas as trypsinogen. It is initially formed as a larger, inactive molecule in the pancreas and is transported to the small intestine, where it is activated to digest food molecules. This protease is also a regulator of many other digestive proteases. A lack of its production in the pancreas is a component of the disorder cystic fibrosis. Analysis of 18 pea and five bean varieties for trypsin inhibitor activity gave values ranging from 0.15-4.62 TIU/mg (Griffiths, 1984). It can be concluded that dehulled defatted cowpea has considerably lower levels for trypsin inhibitor activity than other peas. The level of trypsin inhibitor activity in cowpea protein isolates (CPIA and CPIB) were 4293 and 4290 TIU/g. It was shown that the anti-nutritional factors of cowpeas can be reduced and nutritional quality improved by plant breeding, dehulling, heat treatment or supplementation of diets with enzymes (Balail and N.G, 2014).

Vigna unguiculata contained carbohydrates. The carbohydrate includes starch, sugar, lignin and cellulose and other minor carbohydrates comprising pectin substances, arabinogalactans and oxylogucans (Iwe, 2003). Starchyose, sucrose and raffinose are permanent sugar found in some

legumes example *Glycinemax*. The highest values of crude fiber are 14.68, 13.78, 15.27 and 3.89 which is the lowest value in seed. Fiber is indispensable component of a healthy and balanced food. This is because fiber has a physiological effect on the gastrointestinal function of promoting the reduction of intracolonic pressure, which is beneficial in diverticular disease such as cancer of the colon (Akobundu, 1999). Fiber also has a biochemical effect on the absorption and reabsorption of cholesterol and bile acids (Okwu and Emenike, 2007). The fiber content in these seeds helps in the excretion of bile acids and prevents the reabsorption of bile acids and consequently the absorption of dietary fat cholesterol. This in turn lowers the cholesterol pool and prevents the formations of plaque whose components are cholesterol, some fats and protein (Akobundu, 1999; Okwu and Emenike, 2007). Dry weight values vary in all the varieties. *Vigna unguiculata* provides great benefits as nutritious food crops. Their high protein content can be useful for the peasants of rural communities who cannot afford protein rich food like meats and eggs. The plants provide numerous medicinal benefits and the presence of phytochemicals (natural flavonoids) impart upon them antioxidants, anti-inflammatory and emulsifying properties. The phytochemical analysis indicated that the seven varieties are rich in phytochemical in their parts at different percentages. These findings in the phytochemical constituents can be used as a tool in the systematic study of taxa. The compositions of the phytochemicals in different parts of the seven varieties of *Vigna unguiculata* studied show the affinity relationship in the genus. This was supported by the findings by Omondi (2015) on the use of phytochemical composition of 50 selected plants to show classification relationship amongst ten selected families, his findings revealed saponin as the most abundant in all plants, followed by alkaloids then flavonoids, steroids terpenes. He further revealed that plant families Poaceae, Solanaceae and Rutaceae have phytochemical composition mean of 60.0 indicating they are closely related. Apocynaceae, Asteraceae and Malvaceae families had phytochemical composition mean of 53.5, 50.3 and 50.2. The three means do not significantly differ, indicating the three families are related. Lamiaceae, Fabaceae and Acanthaceae had phytochemical composition mean of 46.8, 43.5 and 40.2. The means of these three plant families do not significantly differ, indicating that the plant families are also related. The Araceae family had a mean of 3.07 which differs from all the other means, indicating that this family based on its phytochemical composition, is not closely related to any of the nine plant families.

Phytochemical and proximate compositions observed in this study suggest their classification as food and drug and also indicated that the seven varieties are phylogenetically related. These

Findings in addition to other parameters in this study a tool in authenticating the identification and classification of plant varieties making reference to their mean values and how they significantly differ.

5.2 Conclusion

The importance of morphological features cannot be overemphasized, however, they must be supplemented with additional characters in order to guarantee the level of sensitivity needed to correctly identify and delimit species into particular taxa, this study used Morphological, Anatomical, Cytological, Phytochemical and Proximate studies in interpreting the inter and intraspecific relationship in the seven varieties of *Vigna unguiculata* available in Anambra State. The seed size, hilum colour, seed texture, seed colour, leaf, stem, pod length, pod width, pod weight, leaf sizes were very much significant after this study. It was concluded that variability helps in the identification of cowpea varieties. Characters like plant height, number; size, leaf base and shape were also helpful in the characterization of cowpea. Most of the morphological characteristics observed were similar and consistent indicating that the seven varieties are phylogenetically related. Var.'Kafanji', Var.'Oloka' and Var.'Sokotoguzo' are more closely related, this close affinity was also same in Var.'Potiskum', Var.'Iron beans', Crowderpea and Var.'Ifebrown'. Though the anatomical studies showed some similarities and variations, the similarities were more accurate, while variations were tangential or negligible to merit their separation into species, one may accept that such differences can only separate plants into varieties or cultivars of a species. On account of these, the seven varieties of cowpea *Vigna unguiculata* have close relationship because they are ancestrally related. The intraspecific relationships among these varieties are moderate. The number of chromosomes possessed by the varieties was not consistent, this may be attributed to the time of harvest and because they are hybrids or polyploidy nature. The percentage phytochemical and proximate constituents of these varieties expose the possible utilisation of any of the members as food and drug. This is so because there seems to be evidence of no real significant differences in the composition of the phytochemical and proximate compositions among these varieties. P Anova at $P < 0.05$ in the

phytochemical and proximate studies revealed similarity in chemical compositions which also indicate close affinity within the varieties and a tool in the systematic study of the varieties. Therefore breeding programme is necessary to develop varieties and hybrids that will make *Vigna unguiculata* more dependable, sustainable and a profitable food crops for the peasant farmers and better product for industrial utilization. This is meant to stimulate interest in the improvement and manipulation of this crop for better performance either for the agricultural and pharmaceutical industries especially in the systematic study of the taxa since the identification and genomic status of this plant have been provided. Therefore I suggest an application of these findings in future taxonomic treatment of the genus *Vigna*.

5.3 Contribution of this Research to Knowledge

This research has contributed in establishing a clearer understanding of the intra and interspecific relationship in the varieties of *Vigna unguiculata* (L.) Walp. This study used information from several taxonomic evidences to study the identification and characterization of seven varieties of *Vigna unguiculata* available in Anambra State. Morphological study on the seven varieties of *Vigna unguiculata* revealed the importance of variability of characters in the identification and characterization of *V. unguiculata* thereby stabilizing the identification of the plant using morphological characteristics.

The anatomical study revealed information that is of taxonomic importance as new ideas on the identification and characterization of the seven varieties was established. This study was one of the detailed anatomical studies on *V. unguiculata* parts. Cells which make up the parts of the studied specimen and their locations were revealed. Variations and similarities on the parts of the plant extracts used were established. Variations were not consistent but similarities were more accurate which form a tool for their identification.

The cytological study showed inconsistency on the mitotic study. The number of chromosomes possessed by each variety was revealed at different stages. Consequently a basic number confirmed for this genus was $2n=22$ for diploids, but the diploid chromosomes obtained in this research were $2n=22$, $2n=24$ and 26 respectively. This indicates their polyploid nature.

The phytochemical study examined were within the same mean average indicating they are phylogenetically related this is because the means were not significantly different and therefore should remain in the same genus and also revealed, was their use in ethnomedicine and their

nutritive values. Var.'Oloka', Var.'Sokoto guzo', Var.'Ifebrown' and Var.'Potiskum' are more nutritional and medicinal than other varieties. Var.'Oloka' variety was the most preferable for both nutritional and medicinal use; this is because its parts constitute the highest percentage of phytochemical and proximate than others. Var.'Ifebrown' and Var.'Potiskum' constitute a high percentage of crude fibre in their parts and therefore recommended for older people. Var.'Oloka' was recommended for growing children because of its high proteinous content. Var.'Kafanji', Var.'Oloka' and Var.'Sokoto guzo' are more closely related taxonomically and likewise Var.'Potiskum', Var.' Iron beans', Var.'Crowderpea and Var.' Ifebrown' are more closely related. Prior to this, a taxonomic key (Dichotomous key) was constructed for the identification of *Vigna unguiculata* available in Anambra State using all the information from various parameters used in this study. This study also suggested a new species ('specific epithet') name for Crowderpea (*Vigna bibianensis*) and Sokoto guzo (*Vigna zigbanensis*)

In summary, this study contributed the following to knowledge;

An order was created in the taxonomy of varieties of *V.unguiculata*

Dignostic characters from morphology, anatomy, cytology, phytochemical and proximate studies were provided from the study.

Chromosomes were visible at various stages for each variety confirming their ploidy nature.

New name was suggested for species 'specific epithet' as *Vigna bibianensis* and *Vigna zigbanensis*.

Finally a taxonomic key was constructed for easy identification of the varieties.

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5.4 Summary

Characters of two varieties of *Vigna unguiculata* var.'Sokoto guzo' and var.'Potiskum'

1. Morphological characters

i. Seed colour was white

ii. Seed shape was kidney

iii. Hilum colour of var.Sokoto guzo was black while var.Potiskum was pink

2. Anatomical characters

- i. Apotrachial parenchyma was terminal in the stem of Var.Sokoto guzo and banded in Var.Potiskum.
- ii. Paratrachial parenchyma in the root of var.Sokoto guzo was banded; broad and conspicuous in var. Potiskum.
- iii. Pores/vessels were round in the both varieties.

3. Cytological characters

- i. At the early metaphase stage chromosome count $2n = 22$ for var.Sokoto guzo.
- ii. Chromosome count of var.Potiskum was $2n=22$ at telophase stage darkly interwoven with the spindle.

Prominent distinctive characteristics observed in Ifebrown.

1. Morphological characters

- i. Seed colour was brown
- ii. Seed shape was rhomboid
- iii. Hilum colour was brown
- iv. Seed size was large

2. Anatomical characters

- i. Pores/vessels round in shape.
- ii. Vessels arrangement exclusively solitary in stem and arranged in clusters in the root.
- iii. Apotrachial parenchyma was reticulate in stem and banded in root
- iv. Stomata type in abaxial was anisocytic while it was paracytic in adaxial surface.

3. Cytological characters

Chromosome count was $2n=24$, $2n=26$ observed at telophase and prophase stage .This is as a result of faster replication of the cell.

Outstanding characteristic features observed in crowder pea (black beans).

1. Morphological character

- i. Seed size was medium
- ii. Seed colour was black
- iii. Seed shape was kidney
- iv. Hilum colour was brown

2. Anatomical characters

- i. Pores/vessels was scattered and angular
- ii. Arrangement of vessels was radial multiple.
- iii. Apotrachial parenchyma was diffuse and aggregate while paratrachial was broad and conspicuous in the root
- iv. Rays has traumatic canals.

3. Chromosome count was $2n=24$ observed at late prophase stage.

Striking distinguishing characters of var Oloka

1. Morphological characters

- i. Seed colour was white mixed brown (multiple colours)
- ii. Seed shape kidney
- iii. Hilum colour was black
- iv. Seed size was medium size

2. Anatomical characters

- i. Pore/vessels angular in shape
- ii. Vessels arrangement multiple radial in stem while in root exclusively solitary
- iii. Paratrachial in root scanty and vasicentric
- iv. Apotrachial was reticulate in root, diffuse aggregate in stem.

Cytological characters

Chromosome count was $2n=22$ at late metaphase.

The Oloka and Kafanji varieties of *Vigna unguiculata* was observed as most primitive varieties.

Ifebrown variety was presumed to be a hybrid.

Var.Crowderpea was suggested to be one of the species of *vigna* to have another specific epithet.

Reasons;

- I.This is because the variety crowderpea also called southernpea has other colours of the seed, like black, cream, light brown, brown spots of black.
- ii. The variety is named after the colour of the seed.
- iii. It has distinct characters as was revealed in this research.

The name *Vigna bibianensis* was proposed for this species

Sokoto guzo variety was also considered for a new name

Reasons:

- I.The variety is named after a town where it is cultivated
- ii. This name is not well known like others named after a place
- iii. The variety is called by many names like in Ibo land Agwa akara

The name *Vigna zigbanensis* was suggested for var.Sokoto guzo

Bracketed Dichotomous key for *Vigna unguiculata* consumed in Anambra State

1. Seed white or multiple colour.....2
1. Seed black or brown3

2. Hilum black or pink.....white varieties
2. Hilum white or brown.....Ifebrown, Kafanji, Crowderpea

3. Seed texture rough.....6
3. Seed texture smoothKafanji

4. Seed shape (kidney).....go to 2
4. Seed shape (Rhomboid)Ifebrown

5. Seed size large or meduim.....8
5. Seed size small.....10

6. Vessels arrangement in multiple radial in stem and exclusively solitary in root.....Oloka
6. Vessels arrangement exclusively solitary in stem and clusters in rootIfebrown

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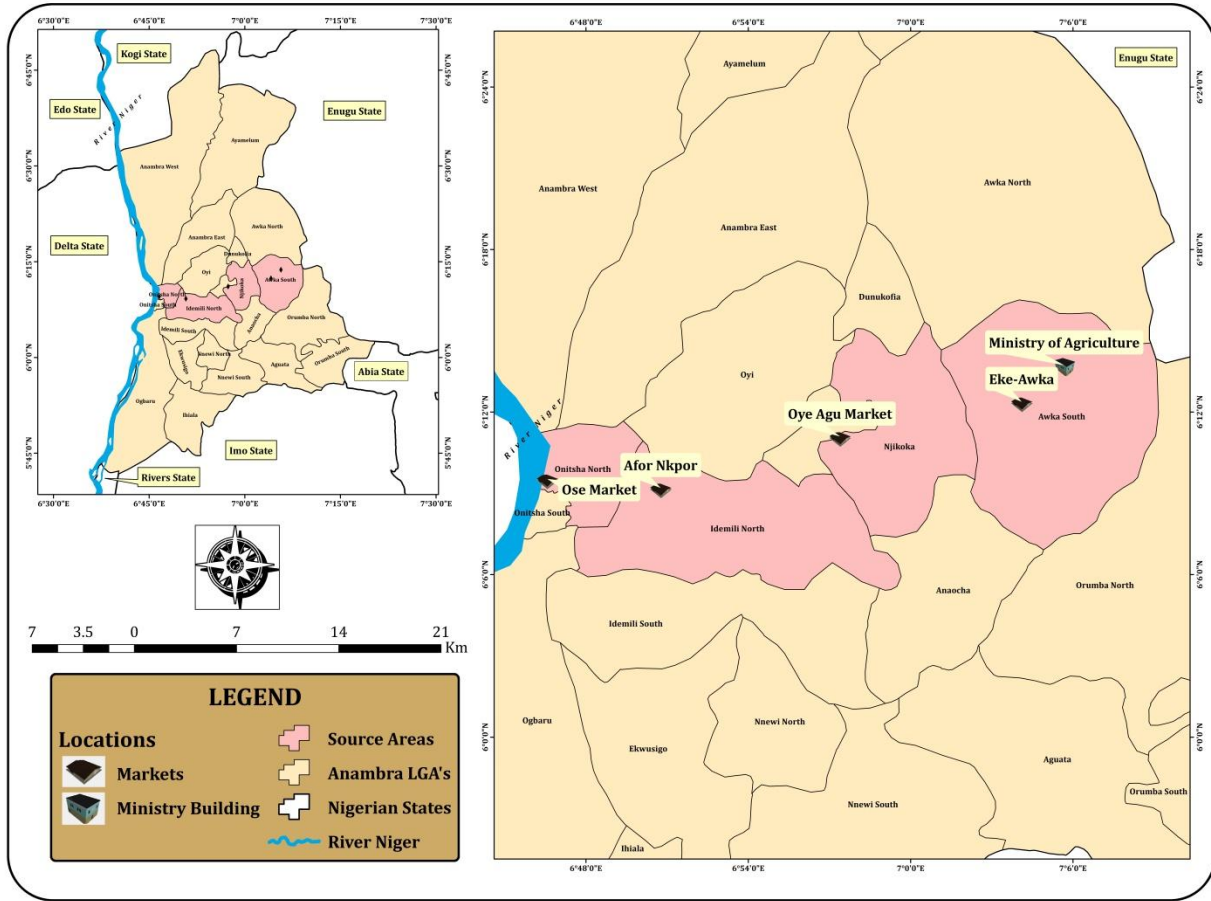
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APPENDIX 1:Map of Anambra State Nigeria showing locations of collection of specimens used for the research



Appendix 2: Proximate Composition of the Leaf, Stem, Root and Seed of Kafanji Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Leaf</i>	9.44±0.028 ^b	90.56±0.028 ^b	15.33±0.134 ^a	12.81±0.071 ^b	1.25±0.021 ^b	14.84±0.085 ^b
<i>Stem</i>	8.98±0.000 ^c	91.02±0.000 ^a	12.76±0.148 ^b	13.78±0.028 ^a	0.82±0.057 ^c	9.58±0.028 ^c
<i>Root</i>	9.68±0.255 ^b	90.32±0.255 ^b	12.88±0.028 ^b	12.23±0.099 ^c	0.65±0.000 ^d	7.94±0.028 ^d
<i>Seed</i>	11.37±0.021 ^a	88.65±0.000 ^c	3.47±0.028 ^c	3.73±0.014 ^d	3.17±0.014 ^a	19.77±0.042 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

APPENDIX 3: Proximate Composition of the Leaf, Stem, Root and Seed of Crowderpea Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Leaf</i>	8.76±0.000 ^b	91.24±0.000 ^b	13.62±0.028 ^a	12.75±0.014 ^b	1.39±0.014 ^b	14.75±0.014 ^b
<i>Stem</i>	6.87±0.050 ^c	93.14±0.050 ^a	10.74±0.021 ^c	12.82±0.028 ^b	0.57±0.014 ^c	8.94±0.028 ^c
<i>Root</i>	6.95±0.297 ^c	93.05±0.297 ^a	11.71±0.127 ^b	14.76±0.057 ^a	0.45±0.000 ^d	8.75±0.014 ^c
<i>Seed</i>	10.28±0.000 ^a	89.72±0.000 ^c	3.78±0.000 ^d	3.67±0.021 ^c	2.94±0.000 ^a	18.58±0.177 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 4: Proximate Composition of the Leaf, Stem, Root and Seed of Ifebrown Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
<i>Leaf</i>	8.58±0.177 ^b	91.43±0.177 ^b	15.76±0.057 ^b	13.77±0.042 ^d	1.84±0.021 ^b	13.82±0.028 ^b
<i>Stem</i>	7.88±0.057 ^c	92.12±0.057 ^a	16.78±0.028 ^a	14.68±0.113 ^b	1.06±0.000 ^c	7.72±0.120 ^d
<i>Root</i>	7.72±0.170 ^c	92.28±0.170 ^a	13.46±0.035 ^c	15.27±0.042 ^a	0.77±0.014 ^d	8.47±0.021 ^c
<i>Seed</i>	10.62±0.000 ^a	89.38±0.000 ^c	3.64±0.000 ^d	3.89±0.042 ^c	2.67±0.050 ^a	19.52±0.028 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 5: Proximate Composition of the Leaf, Stem, Root and Seed of Oloka Beans Variety

Part of Plant	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
<i>Leaf</i>	9.16±0.000 ^b	90.84±0.000 ^c	14.78±0.028 ^a	12.94±0.000 ^c	1.77±0.021 ^b	15.81±0.042 ^b
<i>Stem</i>	7.87±0.042 ^d	92.13±0.042 ^a	14.91±0.014 ^a	14.32±0.028 ^a	0.85±0.000 ^c	9.84±0.000 ^c
<i>Root</i>	8.75±0.014 ^c	91.25±0.014 ^b	12.32±0.113 ^b	13.82±0.028 ^b	0.78±0.028 ^d	8.96±0.000 ^d
<i>Seed</i>	10.74±0.021 ^a	89.27±0.021 ^d	4.13±0.000 ^c	3.79±0.014 ^d	2.92±0.000 ^a	21.78±0.028 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

APPENDIX 6: Proximate Composition of the Leaf, Stem, Root and Seed of Sokoto Guzo Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Leaf</i>	8.93±0.014 ^b	91.07±0.014 ^b	14.56±0.057 ^a	13.62±0.028 ^c	1.63±0.014 ^b	14.87±0.042 ^b
<i>Stem</i>	7.87±0.042 ^c	92.08±0.028 ^a	13.85±0.007 ^b	13.87±0.042 ^b	0.78±0.000 ^d	9.32±0.120 ^c
<i>Root</i>	8.94±0.028 ^b	91.06±0.028 ^b	11.38±0.028 ^c	14.71±0.014 ^a	0.85±0.00 ^c	9.17±0.014 ^c
<i>Seed</i>	11.34±0.085 ^a	88.66±0.085 ^c	4.06±0.000 ^d	3.88±0.028 ^d	2.47±0.021 ^a	19.81±0.042 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 7: Proximate Composition of the Leaf, Stem, Root and Seed of Potiskum Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Leaf</i>	9.14±0.000 ^b	90.86±0.000 ^c	13.82±0.028 ^b	13.78±0.028 ^b	1.29±0.014 ^b	14.81±0.014 ^b
<i>Stem</i>	7.90±0.000 ^d	92.10±0.000 ^a	14.76±0.057 ^a	14.31±0.014 ^a	0.94±0.000 ^c	8.24±0.000 ^d
<i>Root</i>	8.88±0.057 ^c	91.12±0.057 ^b	11.65±0.000 ^c	12.53±0.106 ^c	0.86±0.000 ^d	9.76±0.057 ^c
<i>Seed</i>	11.49±0.014 ^a	88.51±0.014 ^d	3.68±0.000 ^d	3.68±0.035 ^d	2.84±0.000 ^a	21.38±0.106 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 8: Proximate Composition of the Leaf, Stem, Root and Seed of Iron Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Leaf</i>	9.33±0.099 ^b	90.67±0.099 ^b	14.79±0.014 ^a	12.91±0.014 ^b	1.09±0.007 ^b	15.24±0.000 ^b
<i>Stem</i>	8.62±0.028 ^c	91.38±0.028 ^a	13.82±0.028 ^b	13.57±0.042 ^a	0.83±0.014 ^c	8.83±0.099 ^c
<i>Root</i>	9.25±0.014 ^b	90.75±0.014 ^b	12.32±0.028 ^c	12.79±0.014 ^c	0.76±0.000 ^d	7.71±0.127 ^d
<i>Seed</i>	10.41±0.156 ^a	89.59±0.156 ^c	3.84±0.000 ^d	3.78±0.028 ^d	3.12±0.000 ^a	19.57±0.042 ^a
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 9: Comparative Proximate Analysis of the Leaf of Seven Varieties of Beans

<i>Variety</i>	<i>% Composition*</i>					
	<i>Moisture</i>	<i>Dry Matter</i>	<i>Ash</i>	<i>Crude Fibre</i>	<i>Ether Extract</i>	<i>Crude Protein</i>
<i>Kafanji</i>	9.44±0.028 ^a	90.56±0.028 ^d	15.33±0.134 ^b	12.81±0.071 ^d	1.25±0.021 ^f	14.84±0.085 ^c
<i>Black Beans</i>	8.76±0.000 ^c	91.24±0.000 ^b	13.62±0.028 ^f	12.75±0.014 ^d	1.39±0.014 ^d	14.75±0.014 ^d
<i>Ifebrown</i>	8.58±0.177 ^d	91.43±0.177 ^a	15.76±0.057 ^a	13.77±0.042 ^a	1.84±0.021 ^a	13.82±0.028 ^e
<i>Oloka</i>	9.16±0.000 ^b	90.84±0.000 ^c	14.78±0.028 ^c	12.94±0.000 ^c	1.77±0.021 ^b	15.81±0.042 ^a
<i>Sokoto</i>	8.93±0.014 ^c	91.07±0.014 ^b	14.56±0.057 ^d	13.62±0.028 ^b	1.63±0.014 ^c	14.87±0.042 ^c
<i>Guzo</i>						
<i>Potiskum</i>	9.14±0.000 ^b	90.86±0.000 ^c	13.82±0.028 ^e	13.78±0.028 ^a	1.29±0.014 ^e	14.81±0.014 ^c
<i>Iron Beans</i>	9.33±0.099 ^a	90.67±0.099 ^c	14.79±0.014 ^c	12.91±0.014 ^c	1.09±0.007 ^g	15.24±0.000 ^b
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 10: Comparative Proximate Analysis of the Stem of Seven Varieties of Beans

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	8.98±0.000 ^a	91.02±0.000 ^d	12.76±0.148 ^e	13.78±0.028 ^c	0.82±0.057 ^c	9.58±0.028 ^b
Black Beans	6.87±0.050 ^d	93.14±0.050 ^a	10.74±0.021 ^f	12.82±0.028 ^e	0.57±0.014 ^e	8.94±0.028 ^d
Ifebrown	7.88±0.057 ^c	92.12±0.057 ^b	16.78±0.028 ^a	14.68±0.113 ^a	1.06±0.000 ^a	7.72±0.120 ^f
oloka	7.87±0.042 ^c	92.13±0.042 ^b	14.91±0.014 ^b	14.32±0.028 ^b	0.85±0.000 ^c	9.84±0.000 ^a
Sokoto Guzo	7.87±0.042 ^c	92.08±0.028 ^b	13.85±0.007 ^d	13.87±0.042 ^c	0.78±0.000 ^d	9.32±0.120 ^c
Potiskum	7.90±0.000 ^c	92.10±0.000 ^b	14.76±0.057 ^c	14.31±0.014 ^b	0.94±0.000 ^b	8.24±0.000 ^e
Iron Beans	8.62±0.028 ^b	91.38±0.028 ^c	13.82±0.028 ^d	13.57±0.042 ^d	0.83±0.014 ^c	8.83±0.099 ^d
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 11: Comparative Proximate Analysis of the Root of Seven Varieties of Beans

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	9.68±0.255 ^a	90.32±0.255 ^e	12.88±0.028 ^b	12.23±0.099 ^f	0.65±0.000 ^d	7.94±0.028 ^f
Black Beans	6.95±0.297 ^e	93.05±0.297 ^a	11.71±0.127 ^d	14.76±0.057 ^b	0.45±0.000 ^e	8.75±0.014 ^d
Ifebrown	7.72±0.170 ^d	92.28±0.170 ^b	13.46±0.035 ^a	15.27±0.042 ^a	0.77±0.014 ^c	8.47±0.021 ^e
Oloka	8.75±0.014 ^c	91.25±0.014 ^c	12.32±0.113 ^c	13.82±0.028 ^c	0.78±0.028 ^b	8.96±0.000 ^c
Sokoto Guzo	8.94±0.028 ^c	91.06±0.028 ^c	11.38±0.028 ^e	14.71±0.014 ^b	0.85±0.000 ^a	9.17±0.014 ^b
Potiskum	8.88±0.057 ^c	91.12±0.057 ^c	11.65±0.000 ^d	12.53±0.106 ^e	0.86±0.000 ^a	9.76±0.057 ^a
Iron Beans	9.25±0.014 ^b	90.75±0.014 ^d	12.32±0.028 ^c	12.79±0.014 ^d	0.76±0.000 ^c	7.71±0.127 ^g
p-value	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Table 12: Comparative Proximate Analysis of the Seed of Seven Varieties of Beans

Variety	% Composition*					
	Moisture	Dry Matter	Ash	Crude Fibre	Ether Extract	Crude Protein
Kafanji	11.37±0.021 ^a	88.65±0.000 ^c	3.47±0.028 ^g	3.73±0.014 ^b	3.17±0.014 ^a	19.77±0.042 ^c
Black Beans	10.28±0.000 ^c	89.72±0.000 ^a	3.78±0.000 ^d	3.67±0.021 ^c	2.94±0.000 ^b	18.58±0.177 ^e
Ifebrown	10.62±0.000 ^b	89.38±0.000 ^b	3.64±0.000 ^f	3.89±0.042 ^a	2.67±0.050 ^d	19.52±0.028 ^d
Oloka	10.4±0.021 ^b	89.27±0.021 ^b	4.13±0.000 ^a	3.79±0.014 ^b	2.92±0.000 ^b	21.78±0.028 ^a
Sokoto	11.34±0.085 ^a	88.66±0.085 ^c	4.06±0.000 ^b	3.88±0.028 ^a	2.47±0.021 ^e	19.81±0.042 ^c
Guzo						
Potiskum	11.49±0.014 ^a	88.51±0.014 ^c	3.68±0.000 ^e	3.68±0.035 ^c	2.84±0.000 ^c	21.38±0.106 ^b
Iron Beans	10.41±0.156 ^c	89.59±0.156 ^a	3.84±0.000 ^c	3.78±0.028 ^b	3.12±0.000 ^b	19.57±0.042 ^d
p-value	**	**	**	**	**	**

Columns followed by the same letter are not significantly different

**p<0.05

Appendix 13: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Kafanji Beans Variety

Part of Plant	% Composition*					
	Tannin	Saponin	Flavenoid	Alkaloid	Sterol	Phenol
Leaf	1.18±0.028 ^a	1.63±0.035 ^a	1.08±0.021 ^a	1.53±0.014 ^b	0.28±0.000 ^a	0.16±0.000 ^a
Stem	1.09±0.000 ^b	1.36±0.028 ^b	1.07±0.021 ^a	1.92±0.028 ^a	0.19±0.007 ^b	0.14±0.014 ^b
Root	0.91±0.014 ^c	1.27±0.014 ^c	0.94±0.021 ^b	1.42±0.000 ^c	0.17±0.021 ^b	0.11±0.000 ^c
Seed	0.73±0.014 ^d	0.89±0.007 ^d	0.74±0.007 ^c	1.05±0.014 ^d	0.06±0.000 ^c	0.08±0.001 ^d
p-value	**	**	**	**	**	**

Columns followed by the same letter are not significantly different

**p<0.05

Appendix 14: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Black

Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavenoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Leaf</i>	1.15±0.064 ^b	1.06±0.000 ^c	1.05±0.000 ^c	1.36±0.014 ^c	0.11±0.014 ^b	0.15±0.000 ^c
<i>Stem</i>	1.64±0.000 ^a	1.65±0.000 ^a	1.14±0.000 ^b	2.07±0.007 ^a	0.18±0.000 ^a	0.23±0.000 ^a
<i>Root</i>	1.56±0.007 ^a	1.43±0.000 ^b	1.18±0.014 ^a	1.81±0.014 ^b	0.12±0.000 ^b	0.17±0.000 ^b
<i>Seed</i>	0.81±0.014 ^c	0.94±0.007 ^d	0.67±0.021 ^d	1.16±0.014 ^d	0.06±0.001 ^c	0.08±0.001 ^d
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

**p<0.05

Appendix 15: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Ifebrown Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavonoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Leaf</i>	1.24±0.000 ^c	1.34±0.000 ^b	0.92±0.000 ^c	1.78±0.007 ^c	0.13±0.007 ^c	0.13±0.000 ^c
<i>Stem</i>	1.46±0.000 ^a	1.49±0.000 ^a	1.25±0.000 ^a	3.19±0.007 ^a	0.28±0.007 ^a	0.22±0.028 ^a
<i>Root</i>	1.31±0.014 ^b	1.09±0.007 ^c	1.09±0.007 ^b	2.49±0.014 ^b	0.16±0.007 ^b	0.18±0.007 ^b
<i>Seed</i>	0.69±0.000 ^d	1.04±0.021 ^d	0.73±0.000 ^d	1.24±0.007 ^d	0.05±0.002 ^d	0.08±0.003 ^d
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

**p<0.05

Appendix 16: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Oloka Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavonoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Leaf</i>	1.14±0.007 ^c	1.27±0.021 ^c	1.31±0.021 ^c	1.20±0.000 ^d	0.15±0.000 ^b	0.16±0.000 ^c
<i>Stem</i>	1.47±0.021 ^a	1.91±0.014 ^a	1.48±0.000 ^a	2.79±0.014 ^a	0.25±0.014 ^a	0.37±0.021 ^a
<i>Root</i>	1.28±0.000 ^b	1.49±0.014 ^b	1.42±0.000 ^b	2.47±0.021 ^b	0.22±0.035 ^a	0.20±0.000 ^b
<i>Seed</i>	0.85±0.014 ^d	1.05±0.021 ^d	0.81±0.014 ^d	1.25±0.000 ^c	0.10±0.001 ^c	0.12±0.000 ^d
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 17: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Sokoto Guzo Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavonoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Leaf</i>	1.18±0.000 ^a	1.57±0.050 ^a	1.67±0.000 ^a	1.79±0.014 ^a	0.34±0.021 ^a	0.19±0.014 ^a
<i>Stem</i>	1.07±0.007 ^b	1.31±0.021 ^b	1.35±0.000 ^b	1.52±0.000 ^b	0.29±0.021 ^a	0.09±0.000 ^b
<i>Root</i>	0.86±0.000 ^c	0.95±0.000 ^d	1.06±0.000 ^c	1.31±0.014 ^c	0.22±0.028 ^b	0.08±0.007 ^b
<i>Seed</i>	0.84±0.000 ^d	1.06±0.014 ^c	0.67±0.021 ^d	1.19±0.014 ^d	0.07±0.003 ^c	0.09±0.001 ^b
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

** $p < 0.05$

Appendix 18: Quantitative Phytochemical Composition of the Leaf, Stem, Root and Seed of Potiskum Beans Variety

<i>Part of Plant</i>	<i>% Composition*</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavonoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Leaf</i>	1.25±0.007 ^a	1.39±0.014 ^a	1.31±0.021 ^a	1.34±0.014 ^a	0.24±0.014 ^a	0.14±0.000 ^a
<i>Stem</i>	1.23±0.014 ^a	1.16±0.000 ^b	1.19±0.014 ^b	1.28±0.000 ^b	0.19±0.000 ^b	0.11±0.014 ^b
<i>Root</i>	1.25±0.014 ^a	0.92±0.000 ^c	1.05±0.000 ^c	1.22±0.035 ^c	0.16±0.014 ^c	0.09±0.003 ^b
<i>Seed</i>	0.74±0.021 ^b	0.93±0.014 ^c	0.64±0.014 ^d	1.04±0.000 ^d	0.04±0.001 ^d	0.09±0.001 ^b
<i>p-value</i>	**	**	**	**	**	**

*Columns followed by the same letter are not significantly different

***p*<0.05

APPENDIX 19: Comparative Phytochemical Analysis of the Stem of Seven Varieties of Beans

<i>Variety</i>	<i>% Composition</i>					
	<i>Tannin</i>	<i>Saponin</i>	<i>Flavonoid</i>	<i>Alkaloid</i>	<i>Sterol</i>	<i>Phenol</i>
<i>Kafanji</i>	1.09±0.000 ^e	1.36±0.028 ^d	1.07±0.021 ^f	1.92±0.028 ^d	0.19±0.007 ^c	0.14±0.014 ^c
<i>Black Beans</i>	1.64±0.000 ^a	1.65±0.000 ^b	1.14±0.000 ^b	2.07±0.007 ^c	0.18±0.000 ^c	0.23±0.000 ^b
<i>Ifebrown</i>	1.46±0.000 ^b	1.49±0.000 ^c	1.25±0.000 ^c	3.19±0.007 ^a	0.28±0.007 ^a	0.22±0.028 ^b
<i>Oloka</i>	1.47±0.021 ^b	1.91±0.014 ^a	1.48±0.000 ^a	2.79±0.014 ^b	0.25±0.014 ^b	0.37±0.021 ^a
<i>Sokoto Guzo</i>	1.07±0.007 ^e	1.31±0.021 ^e	1.35±0.000 ^b	1.52±0.000 ^e	0.29±0.021 ^a	0.09±0.000 ^d
<i>Potiskum</i>	1.23±0.014 ^c	1.16±0.000 ^f	1.19±0.014 ^d	1.28±0.000 ^g	0.19±0.000 ^c	0.11±0.014 ^c
<i>Iron Beans</i>	1.17±0.014 ^d	1.19±0.021 ^f	0.93±0.014 ^g	1.32±0.000 ^f	0.17±0.007 ^c	0.12±0.014 ^c
<i>p-value</i>	**	**	**	**	**	**

Appendix 20: Comparative Phytochemical Analysis of the Root of Seven Varieties of Beans

Variety	% Composition					
	Tannin	Saponin	Flavenoid	Alkaloid	Sterol	Phenol
Kafanji	0.91±0.014 ^d	1.27±0.014 ^c	0.94±0.021 ^e	1.42±0.000 ^c	0.17±0.021 ^b	0.11±0.000 ^c
Black Beans	1.56±0.007 ^a	1.43±0.000 ^b	1.18±0.014 ^b	1.81±0.014 ^b	0.12±0.000 ^b	0.17±0.000 ^b
Ifebrown	1.31±0.014 ^b	1.09±0.007 ^d	1.09±0.007 ^c	2.49±0.014 ^a	0.16±0.007 ^b	0.18±0.007 ^b
Aloka	1.28±0.000 ^b	1.49±0.014 ^a	1.42±0.000 ^a	2.47±0.021 ^a	0.22±0.035 ^a	0.20±0.000 ^a
Sokoto Guzo	0.86±0.000 ^e	0.95±0.000 ^f	1.06±0.000 ^d	1.31±0.014 ^d	0.22±0.028 ^a	0.08±0.007 ^e
Potiskum	1.25±0.014 ^c	0.92±0.000 ^g	1.05±0.000 ^d	1.22±0.035 ^e	0.16±0.014 ^b	0.09±0.003 ^d
Iron Beans	0.88±0.035 ^e	1.06±0.007 ^e	0.90±0.007 ^f	1.19±0.014 ^e	0.13±0.007 ^b	0.09±0.001 ^d
<i>p-value</i>	**	**	**	**	**	**

APPENDIX 21: Comparative Phytochemical Analysis of the Seed of Seven Varieties of Beans

Variety	% Composition					
	Tannin	Saponin	Flavenoid	Alkaloid	Sterol	Phenol
Kafanji	0.73±0.014 ^c	0.89±0.007 ^c	0.74±0.007 ^b	1.05±0.014 ^d	0.06±0.000 ^c	0.08±0.001 ^c
Black Beans	0.81±0.014 ^b	0.94±0.007 ^b	0.67±0.021 ^c	1.16±0.014 ^b	0.06±0.001 ^c	0.08±0.001 ^c
Ifebrown	0.69±0.000 ^d	1.04±0.021 ^a	0.73±0.000 ^b	1.24±0.007 ^a	0.05±0.002 ^d	0.08±0.003 ^c
Oloka	0.85±0.014 ^a	1.05±0.021 ^a	0.81±0.014 ^a	1.25±0.000 ^a	0.10±0.001 ^a	0.12±0.000 ^a
Sokoto Guzo	0.84±0.000 ^a	1.06±0.014 ^a	0.67±0.021 ^c	1.19±0.014 ^b	0.07±0.003 ^b	0.09±0.001 ^b
Potiskum	0.74±0.021 ^c	0.93±0.014 ^b	0.64±0.014 ^c	1.04±0.000 ^d	0.04±0.001 ^d	0.09±0.001 ^b
Iron Beans	0.85±0.007 ^a	0.82±0.028 ^d	0.76±0.028 ^b	1.10±0.028 ^c	0.04±0.002 ^d	0.09±0.001 ^b
<i>p-value</i>	**	**	**	**	**	**