CHAPTER 1

INTRODUCTION

Colocasia esculenta (L.) Schott belongs to the genus *Colocasia* Schott, of the family Araceae and is widely cultivated for its edible corms as a staple food. Mostly tropical or subtropical plants, the aroids grow mainly in moist or shady habitats. Araceae is one of the most diverse monocotyledonous families, comprising nine subfamilies, 106 genera, and 3300 species (Croat, 1979; French *et al*., 1995; Mayo *et al*., 1997; Govaerts and Frodin, 2002; Keating, 2003a, 2004; Herrera *et al*., 2008). It is most diverse in the tropics, with the Pacific slopes of the northern Andes home to the greatest number of species (Croat, 1992b; Vargas *et al*., 2004; Mora *et al*., 2006; Herrera *et al*., 2008). Growth forms of Araceae include herbs, vines, hemiepiphytes, epiphytes, lithophytes, rheophytes, and helophytes (Croat, 1990, 1992a; Mayo *et al*., 1997; Bown, 2000; Herrera *et al*., 2008). *Colocasia esculenta* is commonly known as cocoyam, dasheen, eddoe, elephant's-ear and taro. It is a perennial herb with tuberous stems and stolons found in marshy places, streams, rivers and ponds (Swapna *et al*., 2011). In addition, in deep water it may develop floating leaves; inflorescence axillary, differentiated into basal green convolute tube and upper, expanded, yellow limbs. It grows to a height of 1-2 metres, the plant consists of central corm (lying just below the soil surface) from which leaves grow upward, and roots grow downwards, while cormels, daughter corms and runners (stolons) grow laterally. The root system is fibrous and lies mainly in the top one metre of soil (Onwueme, 1978). The corms are the economically important part of the plant and these, like the leaves, show considerable variation due to growing conditions with the most elongated corms being characteristic of wet, paddy type conditions. Each corm consists of three parts: the skin, cortex and core. The cortex and the core consist of parenchyma tissue with fibres within the core. The skin may be smooth or fibrous and the root system is superficial and fibrous (Lebot, 2009).

Aroideae, Calloideae, Gymnostachyoideae, Lasioideae, Lemnoideae, Monsteriodeae, and Orontioideae, Pothoideae and Zamioculcoideae are nine subfamilies of Araceae (Keating, 2003b, 2004). The major edible aroids are classified in two tribes and five genera; Colocasiodeae (*Alocasia*, *Colocasia* and *Xanthosoma*) and Lasioideae (*Cyrtosperma* and *Amorphophallus*). Therefore, *Colocasia* is one of the five genera (*Alocasia*, *Amorphophallus*, *Colocasia*, *Cyrtosperma* and *Xanthosoma*) of economic importance in the family Araceae (Plucknett, 1983; Gomez-Beloz and Rivero, 2006). In Nigeria, *Colocasia* and *Xanthosoma* which are among the edible genera of this family, form important food crops, whose leaves could be eaten as vegetable, and corms and cormels eaten as sources of staple carbohydrate (Osuji, 2013). In addition, both genera are commonly known as cocoyam. Unfortunately, there has been much confusion over the use of the term cocoyam, which has been applied to two widely grown species, *C. esculenta* (L.) Schott and *Xanthosoma sagittifolium* (L.) Schott (Wilson, 1984; Manner, 2011; Manner and Taylor, 2011). Morton (1972) suggested that cocoyam be used as a group term for *Xanthosoma* species. The common name, cocoyam was divided into new cocoyam or tannia (*X. mafaffa* syn *X. sagittifolium*.) and the old cocoyam or taro (*C. esculenta*) (Manner, 2011). *C. esculenta* is the third most important source of staple carbohydrate after yam and cassava (Udealor and Ezulike, 2011; Ugwuaja and Chiejina, 2011). It is an important source of calories to many Nigerians. Like other members of the family, the plant contains an irritant which causes intense discomfort to the lips, mouth and throat (Brown, 2000). This acridity is caused in part by microscopic needle-like raphides of calcium oxalate monohydrate and in part by another chemical, probably a protease (Bradbury and Roger, 1998). The acridity helps to naturally deter herbivores from eating it. It must be processed by cooking, soaking or fermenting, sometimes along with an acid (lime or tamarind) before being eaten (Ramanatha *et al.*, 2010).

Colocasia Schott, a small genus of family Araceae, comprising about 13 species, is widely distributed in tropical and subtropical Asia (Mayo *et al.*, 1997). *C. esculenta* is considered as a single polymorphic species (Purseglove, 1972; Plucknett, 1983). The specific epithet, *esculenta*, means "edible" in Latin. It is native to tropical Polynesia and Southeastern Asia (Wagner *et al*., 1999). Kolchaar (2006) reported that various lines of ethnobotanical evidence suggested that *C. esculenta* originated from south central Asia, probably in India of the Malay Peninsula. Wild forms occur in various parts of south eastern Asia (Purseglove, 1972). From its centre of origin, it spread east ward to the rest of South–East Asia and to China, Japan, Philippines, and the Pacific Islands (Onwueme, 1994). From Asia it spread west ward to Arabia and the Mediterranean region. It arrived on the east coast of Africa over 2,000 years ago. It was taken by voyagers, first across the continent of Africa, and later on slave trade to the Caribbean. Today *C. esculenta* (Lin.) Schott is pantropical in its distribution and cultivation. The largest area of cultivation is in West Africa, which therefore account for the greatest quantity of production. Significant quantities of taro are also grown in the Caribbean and virtually in all humid and sub-humid parts of Asia (Purseglove, 1972; Anukworji *et al*., 2012). It is most probably, the oldest crop on earth with a history going back more than 10,000 years. Evidence of taro use during the early and mid-Holocene period has been found in the form of taro starch traces on tools used to process starchy food at Kuk Swamp in the Western Highlands of New Guinea (Fullager *et al*., 2006).

Colocasia esculenta is a popular tuber crop in Southeastern Nigeria and is extensively grown in Anambra State by mainly women. Planting period is from March to June (rainy season); and harvesting, from November/December (dry season). Onwueme (1999) reported that root formation and rapid growth occur directly after planting with corm formation being at three months. This plant does not produce flowers frequently; hence its cultivation is principally by vegetative propagation. However, Onwueme (1999) stated that it can flower or set seed easily, if artificial gibberellic acid is

applied. It is usually propagated vegetatively using suckers, head-setts (the top 2 cm of the main corm together with the first 30 cm of the petiole), small corms or corm pieces (Onwueme, 1999; Lebot, 2009). Cormels are also used for vegetative propagation of *C. esculenta*.

Colocasia esculenta is a large perennial herbaceous plant up to 2 m in height. Leaves are large (20–85 cm long and 20–60 cm wide), peltate (petiole attached to the leaf near the center rather than margin), entire, ovate to saggitate with leaf tips pointed and rounded basal lobes. Petioles are up to 2 m in length, rise up in whorls from the apex of the corm, variable in colour from light greenish yellow to dark red depending on cultivar, and not necessarily uniform, variegated petioles occur. Inflorescence is a spadix (a simple fleshy spike) surrounded by a bract-like spathe. The spathe consists of two unequal parts: the lower green, up to 5 cm; the upper part deciduous, yellow, up to 35 cm and distally rolled. Roots are mainly on the surface, fibrous, and adventitious. The corm is large underground starchy stem, oblong or globular in shape with diameters up to 20 cm and weighing up to 1 kg or more. Colours cover a range from grey to purple to red and yellow. Corm colour is also not necessarily uniform throughout (Manner and Taylor, 2011).

Osuji (2013) stated that only one species of *C. esculenta* exists in Nigeria. Various ethnic groups in Nigeria have different names for *C. esculenta*; and this attested to its nationwide distribution and use. It is known as ede/akaso/ uli in Ibo; guaza in Hausa; koko in Yoruba; mkpon in Efik, and ikereburu in Ijo (Aiyeloja and Bello, 2006; Nyananyo, 2006; Okujagu, 2008; USDA, 2013). Unfortunately, since the last decade, *C. esculenta* is in the verge of extinction in Southeastern Nigeria, probably due to its high susceptibility to bacterial leaf blight disease. This disease causes serious damage to the plant and has concequently resulted in its scarcity and high cost. Moreover, Onwueme (1999) reported that production of *C. esculenta* has one serious pest, the taro beetle, and one serious disease, taro leaf blight. The taro beetle (*Papuana* sp.) attacks the developing corms leaving holes which spoil the crop

and allow rot organisms to enter. Research is taking place into finding a biological control. Taro leaf blight (*Phytophthera colocasiae*) can destroy a leaf lamina in 10-20 days with the disease able to cause losses of 30-50%. An integrated control programme combined with breeding resistant varieties offer the most promising ways to control the problem (Onwueme, 1999). Various workers have reported that leaf blight caused by *Phytophthera colocasiae* is the commonest and the most important foliar disease responsible for devastating cocoyam in several countries (Misra, 1993; Mathews, 1998; Brunt *et al*., 2001; Ugwuaja and Chiejina, 2011). Moreover, due to devastating nature of *C. esculenta* leaf blight disease and its imminent danger to food security in Nigeria, serious research is currently undertaken in Southeastern, Nigeria to combat the situation.

Plant taxonomy is basically considered as the science of identifying, naming and classifying plants. Plants are man's prime companions in this universe, being the source of food and energy, shelter and clothing, drugs and beverages, oxygen and aesthetic environment, and as such they have been the dominant component of this taxonomic activity through the ages (Singh, 2004). Plants are classified based on similarities and differences provided by taxonomic information. Taxonomic evidence can be gathered from a wide variety of sources, from all parts of plant during all stages of its development. Information from morphology, anatomy, histochemical localization of calcium oxalate, phytochemistry, proximate analysis and cytology has been reported useful in plant taxonomy. Morphology is the study of forms and features of different plant organs such as roots, stems, leaves, seeds and fruits (Dutta, 2004). Morphological data are made up of both external and internal characters. Evidence from the external characters provides the basic language for plant characterization, identification, classification and relationship. External morphological characters are characters that are visible without the aid of a microscope and may be diagnostic or differential. Diagnostic characters are characters that are exclusive in a group leading to speedy identification of

that group while differential characters are characters that are shared among other groups. Morphological characters are the strongest tools used in taxonomic classification of plants. However, knowledge and application of other available taxonomic tools is crucial. Anatomy is the gross internal structure of a plant organ, as seen in a section. Anatomical features are widely used in systematics for identification and for placing anomalous group in satisfactory position in classification and for indicating patterns of relationship that may be obscured by superficial convergence in morphological characters (Sharma, 1993). Stern (1978) stated that comparative anatomy has helped and will continue to help botanists in their efforts to establish genetically sound systems of classification. Some of the basic evidently anatomical characters of well-established taxonomic value are the type, size, shape, wall sculpture and pattern of wood cells, stellar patterns, types of vascular bundles, rays, ground tissue and parenchyma, epidermal and mesophyll tissue, stomata, trichomes, sclereids, nodes, and phloem cells (Pandey, 1981; Sharma, 1993). Phytochemistry is the chemical contents of plants; and those with taxonomic significance are normally considered in plant classification. Chemical evidence has, in fact been used ever since man first began to classify plants as edible and inedible, obviously based on their chemical differences. Chemical taxonomy is based on the investigation of the distribution of chemical compounds or groups of biosystematically related plants. Moreover, chemotaxonomic studies include the investigations of the pattern of the compounds existing in plants, and in all the individual parts of the plants, such as bark, wood, leaves, and roots. Calcium oxalate is a secondary metabolite, which has been reported widely in plants (Osuji and Ndukwu, 2005). The calcium oxalate accumulation is linked to the detoxification of calcium (Ca^{2+}) in the plant (Martin *et al*., 2012). Plants accumulate oxalate in the range of 3%-80% (w/w) of their dry weight (Libert and Franceschi, 1987), via a biomineralization process in a variety of shapes (Prychid *et al*., 2008). Almost all members of the aroid family contain minute crystals of calcium oxalate, distributed throughout their tissues, which may be implicated in the irritating quality found in many Araceae species (Okeke *et al.*, 2009).

Cytology is the study of cells. Chromosome size, shape, number and behaviour at meiosis are generally considered taxonomic significant. Cytotaxonomy can be an effective tool when a species with many varieties are considered and it can allow a more accurate knowledge of the relationships (Dewey, 1984; Gianfranco *et al*., 2008).

Speciation is a general term for a number of processes which involve the production of new species (Singh, 2004). New species may develop through the mechanism of abrupt speciation or gradual speciation. Gradual speciation is a more common phenomenon in nature. It may involve phyletic evolution when one species might evolve into something different from its ancestor over a period of time (phyletic speciation). Alternatively, a population belonging to a single species might differentiate into two evolutionary lines through divergent evolution (additive speciation). The plausibility of speciation is enhanced by population subdivision. Simultaneous emergence of more than two new species from a subdivided population is highly probable.

Osuji (2013) reported that *C. esculenta* and *Xanthosoma mafaffa* have few cultivars each. *Xanthosoma sagittifolium* is a synonym of *Xanthosoma mafaffa,* a popular species of *Xanthosoma* in Nigeria. The cultivars vary in their vegetative features and in the way they are processed or consumed based on acridity and taste. Acridity in plants is believed to be connected with the occurrence of calcium oxalate. Calcium oxalate is the most abundant insoluble mineral found in plants and it is common among many plant families (Doege, 2003). They are widespread in flowering plants, including both dicotyledons and monocotyledons. Calcium oxalate crystals may form in any organ or tissue within plants. They occur in roots, stems, leaves, flowers, fruits and seeds (Franceschi and Horner, 1980) and within epidermal (Zindler-Frank, 1975), ground (Horner and Whitmoyer, 1972), and vascular (Wang *et al*., 1994) tissues. Unlike phytoliths, which vary considerably in size and shape across families, calcium oxalate crystals are generally restricted to five basic morphological types. They include: needle shaped raphides,

rectangular or pencil shaped styloids, mace head shaped aggregates called druses, block shaped aggregates called crystal sand, variously shaped prisms (Horner and Wagner 1995; Saadi and Mondal, 2011). Ergastic substances in plants are known to be objects of defensive mechanism (Uno *et al*., 2001). While most of them are very limited in their occurrence, some have proved to be of wide distribution among plants. Individual plant species typically display quite specific anatomical, morphological and developmental patherns of crystal accumulation, reflecting genetic regulation of crystal formation (Kausch and Horner, 1982). Crystal morphology and distribution are usually similar within specific taxa and differ among divergent taxa to the extent that they provide key characters for systematics.

The history of classification of *Colocasia* is fairly long. The classification of various edible aroids has been a source of interest to botanists for more than two centuries ago. Gomez-Beloz and Rivero (2006) reported that some of the first attempts were done in 1753 by Linnaeus; 1832 and 1856 by Schott and 1939 by Hill. In more recent studies, a debate continues whether there are two distinct species of the aroid, namely *Colocasia antiquorum* (L.) Schott and *C. esculenta* (L.) Schott or one polymorphic species, either *C. antiquorum* or *C. esculenta* with several varieties, as a result of variance within *Colocasia* (Plucknett, 1983; Onyilagha *et al*., 1987).

Currently, *C. esculenta* is considered as the major species with two varieties, *Colocasia esculenta* (L.) Schott var. *antiquorum* (Schott) Hubbard & Rehder; synonym: *Colocasia esculenta* var. *globulifera* Engl. & Krause and *Colocasia esculenta* (L.) Schott var. *esculenta* (Brooks, 2001; Nyananyo, 2006). Parvin *et al.* (2008) reported seven perennial varieties of *C. esculenta* namely, Panikachu, Mankachu, Kalokachu, Ashukachu, Ghatmankachu or Moulvikachu, Goalpatakachu and Bankachu collected from different places of Bangladesh. Moreover, several cultivars/varieties have been reported in Nigeria. They include ‘Akiri’, ‘Akonoke’, ‘Cocoindia’, ‘Ede Ghana’, ‘Ede ofe’, ‘Ede ofe green’, ‘Ede ofe purple’, ‘Nadu’, ‘Nworoko’, ‘Odogolo’, ‘Kochuom’, ‘Nwine’, ‘Ugwuta’ (‘Coco India’), ‘Ukpong’

(Onyilagha *et al*., 1987; Ekanem and Osuji, 2006; Anikwe *et al*., 2007; Ugwuoke *et al.*, 2008; Ogbonna and Nweze, 2012; Bede *et al.* 2013; Ugbajah, 2013; Orji *et al.*, 2014; Ogbonna *et al.*, 2015; Orji and Ogbonna, 2015; Olatunji and Nwakor, 2015; Olatunji *et al.*, 2015; Osuji and Nwala, 2015); 'Nachi' and 'Ugwuta' (Ugwuaja and Chiejina, 2011); 'Congoma', 'Nkenge', 'Panya' and 'Uyo local' (Bassey *et al.*, 2016). Ugwuoke *et al.* (2008) reported the two cocoyam varieties as *Colocasia esculenta* var. 'Ede ofe' and *C. esculenta* var. 'Ugwuta' which they collected from the experimental farm of the Department of Crop Science, University of Nigeria, Nsukka, Enugu State. Onyilagha *et al*. (1987) reported that the qualities possessed by 'nwine' and 'ukpong' cultivars which include pink tuber skin, long or club shaped cormels and non-irritation in the throat when boiled and eaten, clearly distinguished them from those of Ede-ofe and Kochuom.

The names of varieties of *C. esculenta* in Nigeria are mainly based on the locality of the varieties and the excess of the local names imposed a taxonomic confusion. This has led to difficulty in their identification and description; which necessitates a taxonomical elucidation of varieties of this species. Obviously, there is variability in the morphology of these varieties which could lead to emergence of new species as a result of long duration of the cultivation of this crop. Moreover, there is a dearth of current information on the taxonomic investigation of varieties of *C. esculenta*; which implied that thorough taxonomic research has not been done on this plant. As a result, a taxonomic study on varieties of *C. esculenta* becomes a necessity, hence this study.

Aim and Objectives

The aim of this work was to taxonomically elucidate five varieties of *C. esculenta* and the objectives were to:

1. determine the morphological characters of varieties of *C. esculenta*

2. study the anatomical characters of these varieties of *C. esculenta*
3. examine the histochemical characters of the varieties
4. evaluate the phytochemical characters
5. assess the proximate constituents of the varieties
6. investigate the cytological status of these varieties of *C. esculenta*
7. compare the differences and similarities among the observed characteristics of the varieties

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Geographic Distribution

Eleven species of *Colocasia* are currently recognized in China (Yang *et al*., 2003). In addition, some species, such as *C. esculenta*, are widely cultivated in tropical, subtropical and temperate area as an important food crop. *C. esculenta* is most widely known as taro, eddoe, dasheen and cocoyam. It originated from Southeast Asia and is reported to be one of the first crops cultivated by humans (Doku, 1981; Brown, 2000). It is widely distributed in Africa, Australia, Europe, Northern America and Southern America (USDA, 2013). It spreads from India eastward to Japan and the Pacific (including Hawaii, New Zealand, New Guinea and Polynesia). It moved westward from the Mediterranean across Africa and into Europe.

Safo-Kantaka (2004) reported the botanical description, growth and development, ecology, propagation and planting, diseases and pests, harvesting and handling after harvest of *C. esculenta*.

2.2 Botanical Description

Colocasia esculenta is an erect perennial herb up to 2 m tall, but mostly grown as an annual; root sytem adventitious, fibrous and shallow; storage stem (corm) massive (up to 4 kg), cylindrical or spherical, up to 30 cm × 15 cm, marked by a number of rings, usually brown, with lateral buds giving rise to cormels, suckers or stolons. The leaves are arranged spirally but in a rosette, simple and peltate. The petiole is up to 1 m long, with distinct sheath. The blade is cordate, up to 85 cm × 60 cm, with rounded lobes at base, entire, thick, glabrous, with 3 main veins. The inflorescence is a spadix tipped by a sterile appendage, surrounded by a spathe and supported by a peduncle much shorter than petiole. The flowers are unisexual, small, without perianth; male flowers in upper part of spadix, with stamens entirely fused; female flowers at base of spadix, with superior, 1-celled ovary having an almost sessile

stigma. The male and female flowers are separated by a band of sterile flowers. The fruit is a many-seeded berry, densely packed and forming a fruiting head. The seeds are ovoid to ellipsoid, less than 2 mm long, with copious endosperm.

2.3 Growth and Development

Planting is done usually in the beginning of the rainy season. After planting, growth of new roots and leaves starts after 2 weeks, the growth of suckers after 2 months. Growth of the corms also starts after about 2 months but in flooded taro after 3–5 months. There is a continuous turnover of leaves. After 4–5 months leaf area and mass reach their maximum, thereafter leaf stalks become shorter and leaf blades smaller and fewer. Most clones rarely flower and many do not flower at all. However, flowering can be induced by treatment with gibberellic acid. Picking of the leaves may start when the plants have about 6 leaves, 3 months after planting. Intensive leaf harvesting may reduce corm size and yield, and number of suckers. Corms are ready for harvesting 8–10 months after planting for rainfed taro, and 9–12 months for wetland taro, although the corms reach their maximum weight a few months later.

2.4 Ecology

Taro does best in tropical lowland in areas where annual rainfall exceeds 2000 mm. It is well adapted to high temperatures and relative humidity. Most types respond well to fairly stable temperature regimes of 21–27°C. Taro is rather tolerant to shade and for that reason suitable as intercrop under coconut, cocoa or coffee. Eddoe types are more resistant to drought and low temperatures than dasheen types, and the former are grown successfully as far north as Korea and Japan. Taro can withstand highly reduced soil conditions. It is mainly found in marshy areas and on river banks in savanna areas. It can be grown under dryland and flooded conditions, each requiring adapted cultivars. Cultivars adapted to wet soil conditions withstand flooding without damage provided the water is not

stagnant. Flooded cultivation is more intensive and requires greater attention than dryland cultivation. Under flooded conditions, the water level should not rise to a depth of more than 5–8 cm; with this method it takes longer to mature in comparison to dryland taro, but yields are higher. Eddoe types prefer well-drained loamy soils, and dasheen types grow best where the soil is heavy and has high moisture-holding capacity. A pH of 5.5–6.5 is optimal. Some cultivars tolerate high soil salinity.

2.5 Propagation and Planting

Taro is propagated vegetatively. It is sometimes difficult to keep planting material in a healthy condition during the dry season or periods of drought. Essentially 4 types of planting material are used: side suckers growing from the main corm, small unmarketable cormels (60–150 g), corm pieces, and setts or 'huli', that is, the apical 1–2 cm of the main corm with 15–20 cm of the leaf stalks attached. In Ghana, planting is mainly by use of either young suckers or mature setts cut from harvested corms. Planting material must be taken from healthy plants. Cormels are planted at a depth of 5–7.5 cm. Planting on ridges facilitates harvesting. For upland cultivation in the Philippines, furrows 30 cm deep and 80 cm apart are prepared. In flooded culture fields are ploughed, puddled and carefully levelled. Planting is done after draining the field or into 2–5 cm of standing water. The planting distance is 50–80 cm in the row and 70–120 cm between rows. For breeding purposes taro can be propagated by seed.

2.6 Diseases and Pests

Taro blight (*Phytophthora colocasiae*) is a major wetland taro disease, causing purple to brown circular water-soaked lesions. It is the most devastating taro disease, particularly in the Pacific region, where it has caused considerable losses. For example, in the Solomon Islands, the disease resulted in the crop being partially replaced by sweet potato. This disease is partially controlled by use of copper- or phosphor-based fungicides, but spraying is tedious and costly. Increasing plant spacing or

intercropping reduces losses. Resistance has been found in germplasm collections. Several species of *Pythium* cause taro soft rot, with wilting and chlorosis of leaves. Control is possible by use of healthy planting material, crop rotation and treating planting materials with fungicide. Sclerotium rot caused by *Sclerotium rolfsii* is characterized by stunting of the plant, rotting of corm and formation of many spherical sclerotia in the corm. Control is by soil drenching. In both flooded and upland taro, dark brown spots that appear in older leaves are caused by *Cladosporium colocasicola* and *Phyllosticta colocasiae*. Dasheen mosaic virus (DsMV) and other viruses have been reported but are seldom serious. In the Pacific region the alomae virus disease causes serious damage. Symptoms start with a feathery mosaic on the leaves followed by crinkling and formation of outgrowths on the surface. Finally the entire plant becomes stunted and dies. Alomae disease is caused by the combined infestation by the taro large bacilliform virus (TLBV) and the taro small bacilliform virus (TSBV). Presence of TLBV only results in a milder form of the disease called 'bobone'. The viruses are transferred by a grasshopper and a mealy bug, respectively, but not by mechanical contact. In Hawai'i, two diseases of unknown causative agents characterized by small, hardened portions in the lower third of the corm ('hard rot') and a soft rubbery corm which is low in starch content and exudes water when squeezed ('lohloli') are threatening. Attack by root-knot nematodes (*Meloidogyne* spp.) can result in considerable crop loss. Control is by treating planting material with water at 40°C for 50 minutes, by the use of disease-free material or by soil fumigation.

Insect pest on taro may cause serious damage. Damage by *Hercothrips indicus* (synonym: *Heliothrips indicus*) thrips is shown as a silvery discoloration of the leaves and can result in severe leaf shedding. Adults of taro beetles (*Papuana* spp., example, *Papuana huebneri* and *Papuana woodlarkiana*) tunnel in the corm up to the growing point. Young plants wilt and die but older plants usually recover. This pest is reported in the Pacific and South-East Asia, but not in Africa. It can be controlled by applying insecticide in the planting holes. Severe losses have been suffered by a number of countries growing

the crop (Polynesia, Hawai'i, the Caroline Islands and Samoa), as a result of leafhoppers. Biological control and insecticide dusting are effective control measures. Larvae of the sweet potato hawk moth (*Agrius convolvuli*) defoliating the plant reduce corm quality.

2.7 Harvesting

The harvest of the leaves may start 2 months after planting. Unfolding or young expanded leaves are preferred. From then on, continuous harvesting is assured provided sufficient water is available. Harvesting immature corms may start from 5 months onwards. Maturity of the crop depends on the cultivar and method of cultivation (upland or flooded). Dasheen types take about 8–10 months to mature while eddoe types mature in 5–7 months. Irrigated taro matures a few months later. Dasheen types may be ratooned, lifting the main tuber and leaving the small ones for successive harvests. Maturity indicators are yellowing of the leaves and a slight lifting of tubers. Commercial growers in Ghana allow the crop to remain over one year when prices are not favourable.

2.8 Handling after Harvest

Leaves can only be stored for a few days. Corms can be stored under ambient condition for up to 6 weeks, provided they have not been bruised during harvesting. However, they often keep well for only 2 weeks. Corms of dasheen types can be stored at 10°C for up to 6 months. To avoid post-harvest problems, harvesting is best done when the corms are dry. For the fresh market, the corms are usually washed and the roots and fibres are discarded. In Ghana, the entire top parts are removed, whereas in Asia, 30–45 cm of the leaf stalks are usually left attached. Pieces of the corm may be dried and stored as chips. Storing planting material at about 2°C results in a delay of growth of 40–60 days; storage at 11–13°C and a relative humidity of 85–90% improves later growth. Corms for planting are normally left in the ground and are harvested when needed.

2.9 Taxonomy of *Colocasia esculenta* (L.) Schott

Kingdom: Plantae

Order: Alismatales

Family: Araceae

Subfamily: Aroideae

Tribe: Colocasiodeae

Genus: *Colocasia* Schott

Species: *Colocasia esculenta* (L.) Schott

Varieties: 1. *Colocasia esculenta* (L.) (Schott) var. *antiquorum* Hubbard & Rehder (eddoe), Synonym: *Colocasia esculenta* var. *globulifera* Engl. & Krause

2. *Colocasia esculenta* (L.) Schott var. *esculenta* (dasheen)

Common names:

English: Cocoyam, dasheen, eddoe, elephant's-ear, taro.

French: colocasie

Spanish: alcocaz / colocasia / malanga / tayoba

Swedish: taro

India: arum

German: Taro / Zehrwurzel

Transcribed Korean: toran

South Africa (Zulu): madumbe

Malay: daun keladi

Portuguese (Brazil): inhame / inhame-branco / inhame-da-África / inhame-da-costa / taioba-de-São-Tomé.

(Brooks, 2001; Dutta, 2004; Nyananyo, 2006; Ugwuaja and Chiejina, 2011; Halligudi, 2013; USDA, 2013).

The plethora of common names for *C. esculenta* in different languages attests to its worldwide distribution and use (Gomez-Beloz and Rivero, 2006). Linnaeus first described this edible aroid in 1753 as two distinct species, *Arum colocasia* and *A. esculentum*. In 1832, Schott established the genus *Colocasia* and renamed the two as *Colocasia antiquorum* and *C. esculenta*, respectively. In 1856, Schott reconsidered and used one name, *Colocasia antiquorum*, to describe a single polymorphic species and reduced other *Colocasia* that had been described as separate species to varieties of *Colocasia antiquorum*. This convention was retained by Engler who added four varieties to *C. antiquorum*. In 1939, Hill felt that if one polymorphic species is to be recognized as outlined in the International Rules of Botanical Nomenclature, then the name *Colocasia esculenta* should take precedence over *C. antiquorum* because *Arum colocasia* and *A. esculentum*, the first names used by Linnaeus to describe this genus, include the specific epithets *colocasia* and *esculentum* (Gomez-Beloz and Rivero, 2006).

2.10 Cultivars/varieties of *Colocasia esculenta* in Nigeria

All the five genera of *Colocasia* are vegetatively propagated (Plucknett, 1977). *Colocasia esculenta* is one of at least 10 species of *Colocasia*. Many varieties of *C. esculenta* are also known (Plucknett, 1983; Gomez-Beloz and Rivero, 2006). Osuji (2013) stated that only one species each of *Colocasia* and *Xanthosoma* exist in Nigeria. Ekanem and Osuji (2006) reported that National Root Crops Research Institute Umudike documented four cultivars of *C. esculenta* in Nigeria, namely 'Coco India', 'Ukpong', 'Ede Ghana' and 'Ede ofe'. Five cultivars of *C. esculenta*, three from Nsukka in Enugu State, namely: 'Nachi', 'Nworoko' and 'Odogolo' and two from Umudike in Abia State, namely: 'Nkpong' and 'Ugwuta' ('Coco India') have been reported (Ogbonna and Nweze, 2012; Orji *et al.*, 2014; Ogbonna *et al.*, 2015; Orji and Ogbonna, 2015). In addition, Bede *et al.* (2013) reported that two cultivars of *C. esculenta* tubers locally known as 'Coco India' and 'Ede Ofe' were obtained from the main market in Owerri, Southeastern Nigeria. They however, did not indicate whether the cultivars were grown in Owerri, Imo State. Ugwuaja and Chiejina (2011) reported that *Colocasia esculenta* var. *antiquorum* and *C. esculenta* var. *esculenta* are traditionally called 'Ugwuta' and 'Nkashi' ('Ede') respectively, in Nsukka area of Enugu State. Ugbajah (2013) reported that three cultivars of *C. esculenta* are grown in Dunukofia Local Government Area of Anambra State. They include: 'Akanoke', 'Coco India' and 'Edeofe'. In addition, 'edeofe' is the most popularly grown cultivar followed by 'Coco India' and 'Akonoke' ('nwine'). Bassey *et al.* (2016) identified four distinct cultivars of *C. esculenta* in Akwa Ibom State, Nigeria. They are 'Congoma', 'Nkenge', 'Panya' and 'Uyo local'. The cultivars are named based on their localities indicating that there are no unified names for cultivars of *C. esculenta* in Nigeria. This makes their identification and collection for study difficult.

Due to variance within *Colocasia*, a debate continues today whether there are two distinct species of the aroid, namely *Colocasia esculenta* and *C. antiquorum*, or one polymorphic species, either *C. esculenta* or *C. antiquorum*, with several varieties (Plucknett, 1983). This variability is common among crops that have been cultivated for a long time. Owing to their polyploidy nature, there is a wide variety of *Colocasia* making Linnean taxonomic application difficult at best (Léon, 1977; Doku, 1981). For now, *C. esculenta* can be considered the major species with two varieties, *Colocasia esculenta* var. *esculenta* and *C. esculenta* var. *antiquorum*. Onyilagha *et al*. (1987) conducted a preliminary investigation, monitored and recorded 17 characters to help in the classification of some of the cultivars. They reported that *C. esculenta* can be grouped into two separate species.

2.11 Common Names of Plants

Plants are normally provided with local names by the different people of the various regions in their lanquages. These common or local names of the plants have their own weakness. There are three main defects in common names (Pandey, 1981). They include: they may be quite indefinite; they are restricted to the people of one language or even one section of a country; and they are not regulated by any constituted authority. These local names cause a taxonomic disorder and make it difficult for a plant to be identified and collected for study.

2.12 Economic importance of *C. esculenta*

The economic importance of *C. esculenta* cannot be over emphasized. They are used as ornamental; human food (starch and vegetables); potential fodder; medicines: folklore; and vertebrate poisons: mammals (USDA, 2013). The corms and cormels are edible. The varieties undergo prolong boiling

before eaten except ‘kochuo’, which can be boiled or roasted to destroy the acrid calcium oxalate crystals. The leaves are used as vegetable. Tubers are used as industrial alcohol. Taro-lactin and taro-malt, prepared from the flour, are good foods for infants and invalids (Pandey, 1981). It is a prestige crop for gifting and feasting; parts of the plant are used medicinally and it features in the folklore of Oceania and South East Asia (Onwueme, 1999). Like other members of the family, the plant contains an irritant which causes intense discomfort to the lips, mouth and throat (Brown, 2000). This acridity is caused in part by microscopic needle-like raphides of calcium oxalate monohydrate and in part by another chemical, probably a protease (Bradbury and Nixon, 1998). Members of the Araceae family include food crops, ethnomedicinally invaluable genera and species, ornamental and other unexploited plants (Etukudo, 2003; Osuji, 2013). In addition, *C. esculenta* has a number of medicinal uses. Its corm is used as an abortifacient, to treat tuberculous ulcers, pulmonary congestion, crippled extremities, fungal abscesses in animals, and as an antihelmintic. Its foliage is used as a styptic and poultice. The stem sap is used by the Warao as a treatment for wasp stings (Wilbert, 1986). Juice of the petioles is used as astringent and styptic (Pandey, 1981).

In Igbo culture, this crop is mainly cultivated by women. Cocoyam is planted from March to June and harvested in November/December. Indian cocoyam (or ‘cocoindia’) a *Colocasia* spp. is planted in March and can be harvested as early as July/August/September. Cocoyam grows well in wet, damp or shady places (Okeke *et al*., 2009). Moreover, they are used as special delicacy in Southeastern Nigeria. They are cooked and pounded into ‘fufu’ and consumed with traditional soups/sauces. Some, for instance, ‘ede-ofe’ are used as thickening agents in popular traditional soups, ‘ofe onugbu’ (bitter-leaf soup) and ‘ofe ora’. ‘Ofe onugbu’ is a popular and important soup/sauce among the Igbos and is served at very important functions. Ugbajah (2013) reported that ‘ede-ofe’ cultivar is commonly used

as paste for soup thickening, boiled and mixed with vegetables for occasions as special delicacy. In addition, the fresh tender leaves and petioles are used as vegetables during periods of scarcity. Cocoyams are also prepared in the form of dried chips, normally produced by prolonged cooking, slicing and sun drying. In Anambra State, the dried chips are known as 'asisa – ede' and are eaten during the lean planting season. It is also a very popular food in Enugu State and is known as 'Achicha'. Cocoyams are mainly stored as dried chips. These are placed in cool, dry places or over the fire place and taken when needed, especially during the lean planting season.

2.13 Speciation

Speciation is a central but elusive issue in evolutionary biology. Over the past sixty years, the subject has been studied within a framework conceived by Ernst Mayr and Theodosius Dobzhansky and subsequently developed further by numerous other workers. In the "isolation" theory, the evolution of reproductive isolation is a key element of speciation; natural selection is given only secondary importance while gene flow is considered prohibitive to the process (Schilthuizen, 2000).

Reproductive isolation is critical to the diversification of species. Post pollination barriers may be important in limiting gene flow between closely related species, but they are relatively cryptic and their evolution is poorly understood (Yost and Kay, 2009). Empirical estimates of the relative importance of different barriers to gene flow between recently diverged species are important for understanding processes of speciation (Kay, 2006). Kay and Schemske (2008) stated that the importance of reinforcement, that is, natural selection that strengthens reproductive isolation between incipient species, remains controversial. They further reported that reinforcement may be particularly

important in rapidly diverging lineages where ecological factors play a primary role in reproductive isolation, as may often be the case in tropical communities.

Evolutionists have long recognized the role of reproductive isolation in speciation, but the relative contributions of different reproductive barriers are poorly understood (Ramsey *et al.*, 2003). In addition, they reported that ecological factors resulting from adaptive divergence are the primary isolating barriers in this system. Nosil *et al.* (2005) described the reproductive barriers that derive from the reduced survival of immigrants upon reaching foreign habitats that are ecologically divergent from their native habitat. Theoretical studies of speciation have been dominated by numerical simulations aiming to demonstrate that speciation in a certain scenario may occur. What is needed now is a shift in focus to identifying more general rules and patterns in the dynamics of speciation. The crucial step in achieving this goal is the development of simple and general dynamical models that can be studied not only numerically but analytically as well (Gavrilets, 2003).

The dynamics of parapatric speciation are modelled as a biased random walk performed by the average genetic distance between the residents and immigrants. If a small number of genetic changes are sufficient for complete reproductive isolation, mutation and random genetic drift alone can cause speciation on the time-scale of ten to 1,000 times the inverse of the mutation rate over a set of loci underlying reproductive isolation. Even relatively weak selection for local adaptation can dramatically decrease the waiting time to speciation. The actual duration of the parapatric speciation process (that is, the duration of intermediate forms in the actual transition to a state of complete reproductive isolation) is shorter by orders of magnitude than the overall waiting time to speciation. For a wide

range of parameter values, the actual duration of parapatric speciation is of the order of one over the mutation rate. In general, parapatric speciation is expected to be triggered by changes in the environment (Gavrilets, 2000a).

Geographic variation may ultimately lead to the splitting of a subdivided population into reproductively isolated units in spite of migration (Gavrilets *et al.*, 2000b). Gavrilets (1998) stated that a classical view of speciation is that reproductive isolation arises as a by-product of genetic divergence. Distinctive features of the simulations are the consideration of the complete process of speciation (from initiation until completion), and of a large number of loci, which was only one order of magnitude smaller than that of bacteria. It is demonstrated that rapid speciation on the time-scale of hundreds of generations is plausible without the need for extreme founder events, complete geographic isolation, the existence of distinct adaptive peaks or selection for local adaptation. The plausibility of speciation is enhanced by population subdivision. Simultaneous emergence of more than two new species from a subdivided population is highly probable. Numerical examples relevant to the theory of centrifugal speciation and to the conjectures about the fate of 'ring species' and 'sexual continuums' are presented.

Martin and Willis (2010) investigated the evolution of intrinsic postzygotic isolation within and between populations of *Mimulus guttatus* and *M. nasutus* and suggested that intrinsic postzygotic isolation is common in hybrids between these *Mimulus* species, yet the particular hybrid incompatibilities responsible for effecting this isolation differ among the populations tested. Hence, they concluded that they evolve and spread only at the local scale. The spread and fixation of hybrid incompatibility alleles in geographically structured species depends on the selection experienced by

such factors. Therefore, studies of geographical variation in reproductive isolation provide insight into the evolutionary dynamics of hybrid incompatibilities.

Today, most evolutionary biologists consider that, for sexually reproducing organisms, speciation involves the evolution of reproductive isolating barriers that reduce the formation of fertile hybrids, and studies typically reveal multiple prezygotic and postzygotic barriers contributing to the total isolation between species (Lowry *et al*., 2008; Martin and Willis, 2010). Furthermore, there are many polymorphic epistatic hybrid incompatibility alleles in each species. Different populations must contain unique combinations of these alleles such that there is high fertility within populations but partial hybrid sterility in most interpopulation or interspecific crosses.

2.14.0. Theory and Plasticity of Phenotype

Phenotype is the product of the interaction of genotype and environment, and that any given genotype will produce different phenotypes in different environments (Briggs and Walters, 1997). Each genotype is likely to have a characteristic breadth of phenotype plasticity, itself under genetic control. In extreme sites plants with a narrow range of responses may be selected, whereas in less extreme or variable sites plants with a wider spectrum of responses might be at a selective advantage.

2.14.1. Hypothesis and Plasticity of Phenotype

1. Different characters of the phenotype show different degree of plasticity: Here plasticity can be observed in vegetative parts, including plant height, the number of shoots, leaves and flowers.

2. The extent of phenotype differs in different taxa: This is beautifully illustrated by the group of species of *Ranunculus* subgenus *Batrachium*, they exhibit heterophylly; species growing on land or in very shallow water produce only floating leaves, while taxa from deep or swift flowing water develop only finely divided submerged leaves. In contrast, species inhabiting shallow water produce both types of leaves.

3. Phenotypic plasticity is under genetic control (Briggs and Walters, 1997).

Experiments with *Polygonum amphibium* have also yielded valuable information on phenotypic plasticity. Plants growing on land and in water have very different phenotypes. If ramets of cloned material are separately grown in conditions simulating land, waterlogged and submerged conditions, then the degree to which a particular individual may produce both the 'land' and 'water' phenotype may be investigated. Some studies suggested that different individuals (presumably different genotypes) show different degrees of plasticity. Considering a general model of plasticity, it has been discovered that different genotypes, faced with the same range of different test conditions produce a difference repertoire of responses and some are able to produce a wider range of phenotypes than others. Clearly, many interacting factors must be considered including levels of heterozygosity, degree of relatedness of the taxa, as well as ecological factors. Plasticity remains a comparatively neglected area in the study of plants variation. Genecologists have not, in general, given enough attention to phenotypic plasticity (Briggs and Walters, 1997).

Phenotypic plasticity could be examined through the eyes of the plant physiologist. An intriguing example of physiological plasticity is that there is variability in the expression in cyanogenic plants of *Tifolium repens*. Moreover, some cyanogenic plants of *Lotus corniculations* are of stable phenotype,

whilst others are cyanogenic only at certain times of the year and under some conditions. It is cyanogenic free at just those times when grazing pressure is likely to be low and the risk of damage to plant by other factors is at its highest. This physiological flexibility should be seen as an adaptively significant (Briggs and Walters, 1997).

The ability to express a genotype as different phenotypes according to external conditions is referred to as phenotypic plasticity or one may refer to plastic responses (Stace, 1980). Turesson referred to different phenotypes which were the product of differing environments as ecophenes, and F.E. Clements called them ecads. Certain environments are well known to give rise to extreme adaptations to shady/sunny, alpine / low land and wet / dry condition. Certain genera or species are also notorious for producing a wide range of ecophenes, and the taxonomist has to be on the look-out for them. In *Epilobium* sun-plants have small, thick leaves much anthocyanins, many hairs, and a short stature, whereas shade-plants in the opposite characters. Since one of the most important diagnostic characters in *Epilobium* is the type of indumentums, it is vital to examine the quality of the hairs, not their quantity, when making determinations (Stace, 1980). In many annual grasses and other plants dry conditions promote a dwarf habit, a normally tall plant with a well branched inflorescence often appearing as a midget a few centimeters high with a single spikelet or flower. Fortunately the measurements of the individual parts of the latter are usually not modified, though they may be.

Plasticity is extremely common in bryophytes, for example, the genera *Hypnum*, *Sphagnum* and *Scapania*, where a great number of taxa have been described based on them (Stace, 1980). In the 1920s H. Buch pioneered the study of plasticity in bryophytes, conducting cultivation experiments on a range of liverwort genera. Many examples are known also in marine, fresh-water and terrestrial algae

and in lichens; in the later case the nature of the substrate (example, rock or tree-trunk) can be the important factor. Plastic responses are by no means confined to exomorphology, for chemical and anatomical characters are frequently affected.

Certain genera of flowering plants adopt different phenotypes according to the time of year at which they germinate and flower the-so-called seasoning variants (Stace, 1980). This phenomenon, often referred to as pseudoseasonal polymorphism, is particularly notable in montane taxa, which may be subjected to different lengths of season and temperature regimes according to the altitude at which they grow and the agricultural practices to which they are subjected; *Gentianella, Melampyrum* and *Rhinanthus* are good examples. Since these variants are often separated spatially they have mostly been given different names, frequently at the species level. In modern Floras they are often treated as varieties or subspecies, but in most cases they have not been investigated properly (the three above are difficult to cultivate) and are often probably just ecophenes.

It has been pointed out by several workers that plasticity and ecotypification are alternative adaptive strategies which are both important in evolution. Particularly perplexing are cases where certain ecophenes can mimic genuine ecotypes, for example, dwarf variants of *Prunella vulgaris* adapted for growth in close-cut lawns, or of *Cytisus scoparius* adapted for growth on exposed maritime shingle. In such examples the variant might be a genetically determined dwarf or a dwarf ecophene, and only experimental cultivation will distinguish the two.

The majority of taxonomists are of the opinion that ecophenes should not be given taxonomic status. There are a great many taxa (mostly infraspecific) based upon ecophenes, and when a taxonomist discovers their background they are usually no longer recognized as distinct, but are relegated as mere synonyms. Inevitably this process of discovery is slow, as most species have not yet been systematically cultivated. Probably the rate of description of new taxa which are in fact ecophenes still exceeds the rate of their relegation to synonymy. This is certainly so where little biosystematic research has been undertaken, particularly in the tropics and in lower plants (Stace, 1980).

Although in vascular plants there is almost universal acceptance that ecophenes should not be taxonomically named, there are some exceptions. Bryologists realize that the proportion of infraspecific taxa (and species) which are ecophenes is far higher than in vascular plants, but nowadays there is some agreement that, where detected, they should lose their status. In the algae the situation is more difficult still, because of lack of experimental evidence. The lichens are of interest since lichenologists have for many years deliberately given names in this group such a practice are being abandoned. Ecophenes are entities of a quite different nature from genetically determined variants, but they can be conspicuous in nature and they are of ecological importance (Stace, 1980).

Ecotype sometimes known as ecospecies describe a genetically distinct geographic variety, population or race within species (or among closely related), which is adapted to specific environmental conditions (Stace, 1980). Typically, they exhibit phenotypic difference (such as in morphology or physiology) stemming from environmental heterogenecity and are capable of interbreeding with other geographically adjacent ecotypes without loss of fertility or vigor. A subunit, race or variety or a plant

ecospecies, that is, restricted to one habitat; equivalent to a taxonomic subspecies. A group of similar populations within one and the same plant species that are adapted to certain climatic, edaphic, or cenotic conditions and that have developed, under these conditions hereditary morphological, physiological, biochemical, and other features. Thus, and ecotype is isolated with respect to distribution, genotypically it is an intraspecific subdivision, which distinguishes it from a biotype (Stace, 1980).

The term 'ecotype' was introduced in the 1920's by the Swedish scientist G. Turesson. Different plants have different number of ecotypes. The ecotypic composition of a species becomes more varied as its geographic range of ecological amplitude increase. For example, 36 ecotypes have been distinguished in Pine *Pinus silvestris* and 27 in the spruce *Picea abies*. Ecotypic polymorphism is most clearly manifested at the centre of speciation and morphogenesis. In the medic Medicaho falcate, for example, there are many ecotypes in the Caucasus and only a few in the northern USSR. Parallel ecotypic differentiation is observed in many species. Thus, the wormwood *Arthemisia campestris*, sheep sorrel (*Rumex acetosella*), and *Sitene uniflora*, that grow in bright dry habits where there are strong winds, develop ecotypes with procumbent stems (Stace, 1980).

Three main groups of ecotypes are distinguished: climatic, edaphic, and biotic climatic, or geographic, ecotypes occupy a separate part of the area of distribution of a species and originated under the influence of specific climatic conditions; for example, the awnless brome (*Bromus inermis*), the southern ecotype differs from the northern one by its nanism, narrow rough leaves, and wax coating. Edaphic ecotypes develop under the influence of soil and ground conditions, such as the pine ecotype on the chalky outcrops of the Don River; it has ever been described as the independent species *Pinus cretacea*. Biotypic, or cenotic, ecotypes appear and develop mainly under the influence of plants

together with it's given species from plant communities, for example, the field and forest ecotypes of the cocksfoot (*Dactylis glomerata*) and the forest and dune ecotypes of the narrow-leaved hawkweed (*Hieracium umbellatum*). The development of an ecotype is a lengthy process. If an ecotype has progressive characteristics, which permit it to extend the range of the species it may give rise to a near species, and consequently an ecotype is one of the stages and process of speciation (Stace, 1980).

2.15.0. Hybridization in Plants

Hybridization between species is common in plants. In biology and specifically, genetics, the term hybrid has several meanings, all referring to the offspring of sexual reproduction (Rieger *et al*., 1991). Many plant genera and species have their origins in polyploidy. Autopolyploidy results from the sudden multiplication in the number of chromosomes in typical normal populations caused by unsuccessful separation of the chromosomes during meiosis. Tetraploids (plants with four sets of chromosomes rather than two) are common in a number of different groups of plants and over time these plants can differentiate into distinct species from the normal diploid line. In *Oenothera lamarchiana* the diploid species has 14 chromosomes; this species has spontaneously given rise to plants with 28 chromosomes that have been given the name *Oenthera gigas*. When hybrids are formed between the tetraploids and the diploid population, the resulting offspring tend to be sterile triploids, thus effectively stopping the intermixing of genes between the two groups of plants (unless the diploids, in rare cases, produce unreduced gametes). Another form of polyploidy called allopolyploidy occurs when two different species mate and produce polyploid hybrids. Usually the typical chromosome number is doubled, and the four sets of chromosomes can pair up during meiosis, thus the polyploids can produce offspring. Usually, these offspring can mate and reproduce

with each other but cannot back-cross with the parent species. Allopolyploids may be able to adapt to new habitats that neither of their parent species inhabited.

Sterility in a non-polyploid hybrid is often a result of chromosome number; if parents are of differing chromosome pair number, the offspring will have an odd number of chromosomes, leaving them unable to produce chromosomally balanced gametes. While this is undesirable in a crop such as wheat, where growing a crop which produces no seeds would be pointless, it is an attractive attribute in some fruits. Triploid bananas and watermelons are intentionally bred because they produce no seeds (and are parthenocarpic).

2.15.1. Heterosis

Hybrids are sometimes stronger than either parent variety, a phenomenon most common with plant hybrids, which when present is known as hybrid-vigor (heterosis) or heterozygote advantage (Strokes *et al*., 2007). A transgressive phenotype is a phenotype displaying more extreme characteristics than either of the parent lines (Rieseberg, 1999). Plant breeders make use of a number of techniques to produce hybrids, including line breeding and the formation of complex hybrids. An economically important example is hybrid maize (corn), which provides a considerable seed yield advantage over open pollinated varieties. Hybrid seed dominates the commercial maize seed market in the United States, Canada and many other major maize producing countries (Wayne, 2004).

2.15.2. Limiting factors

A number of conditions limit the success of hybridization; the most obvious is great genetic diversity between most species. In plants, barriers to hybridization include blooming period differences,

different pollinator vectors, inhibition of pollen tube growth, somatoplastic sterility, cytoplasmic-genic male sterility and structural differences of the chromosomes (Hermsen and Ramanna, 1976). Darwin's origin of species is often criticized for having little to say about speciation. The complaint focuses in particular on Darwin's supposed failure to explain the evolution of the sterility and inviability of interspecific hybrids.

2.16 Morphological Characters

Morphological characters have been observed to be helpful in plant classification and identification. Morphology of roots has been reported useful in plant taxonomy. Singh (2004) indicated that rhizome characteristics are important for identification of various species of the genus *Iris*. Similarly, bulb structure (whether bulbs are clustered on rootstock or not) is an important taxonomic criterion in the genus *Allium*. Sharma (1993) specified that underground parts, such as roots and tubers are of some taxonomic value in plants. He added that tubers are helpful in the taxonomy of *Dioscorea* and Cyperaceae, whereas in *Ranunculus* and *Aristolochia*, the species are delimited on the basis of the shape of the root.

Morphological characters of some of the cultivars of *C. esculenta* were reported. Onyilagha *et al*. (1987) reported that ‘Nwine’ and ‘Ukpong’ cultivars of *C. esculenta* possessed pink tuber skin, long or club shaped cormels and non-irritation in the throat when boiled and eaten. Orji and Ogbonna (2015) determined the morphological characters of ‘Nachi’, ‘Nkpong’,’Nworoko’, ‘Odogolo’ and ‘Ugwuta’. They stated that the leaf lamina of ‘Nkpong’ was pale green in colour while others were dark green. The petiole colour of ‘Nkpong’ and ‘Odogolo’were pale green; ‘Nworoko’ and ‘Nachi’

were brown while that of 'Ugwuta' was brownish green. The petiole junction colour of all the cultivars was brown. Their corm was oval in shape. In addition, 'Nachi', 'Nkpong' and 'Odogolo' had rough corm skin. The flesh colour of 'Nworoko', 'Odogolo' and 'Ugwuta' was deep green, 'Nkpong' was milk in colour while 'Nachi' was light green. Ugwuaja and Chiejina (2011) reported that *Colocasia esculenta* var. *antiquorum* and *C. esculenta* var. *esculenta* are traditionally called 'Ugwuta' and 'Nkashi' ('Ede') respectively, in Nsukka area of Enugu State. Ugwuoke *et al.* (2008) reported the two generally known cocoyam varieties as *Colocasia esculenta* var. 'Ede ofe' and *C. esculenta* var. 'Ugwuta' which they collected from the experimental farm of the Department of Crop Science, University of Nigeria, Nsukka, Enugu State.

2.17 Anatomical characters

Comparative plant anatomy seeks to measure differences and similarities of structure between and among plants in an attempt, thereby, to reckon the degree of genetic relationship of those plants (Stern, 1978). Besides, the approach is evolutionary and rests on the premise that similar structures will have evolved in organisms if they are genetically related to one another. The more numerous the similar structures, the closer the relationship of the organisms while the fewer the similarities, the more distant the relationship.

In addition, leaf anatomy provides various characters of taxonomic significance. Leaf characters, such as arrangement, type, form, duration and venation are widely used in both the classification and identification (Sharma, 1993). Moreover, characters such as epidermal structures, nature and type of stomata, venation pattern, trichome type and petiole anatomy have been largely employed. In *Ulmus* and *Betula*, the species are delimited only on the basis of leaf characters. In *Trifolium*, the species were

separated on the basis of stipule morphology. *Dalbergia* species were distinguished on the basis of their leaflet size, shape and arrangement on the rachis. Singh (2004) reported that leaves are important for identification in palms, *Salix* and *Populus*. The genus *Azadirachta* has been separated from *Melia* among other features by the presence of unipinnate leaves, as against bipinnate in the latter. Similarly, the genus *Sorbus* has been separated from *Pyrus*, and genus *Sorbiaria* separated from *Spiraea* on the basis of pinnate leaves. Stipules were important sources for identification in *Viola* and *Salix*. Leaf venation was important for the identification of the species in *Ulmus* and *Tilia*. Interpetiolar stipules were useful for identification within the family Rubiaceae. Foliar anatomy had been used widely in several taxonomically different groups such as Euphorbiaceae, Cyperaceae and Poaceae of Angiosperms and Coniferae of Gymnosperms. The rejection of *Sanmiguelia* and *Furcula* as angiosperm fossils from Triassic era has largely been on the basic detailed study of the venation pattern of leaf (Hickey and Doyle, 1977; Singh, 2004).

Moreover, Sharma (1993) noted that in determining relationship between different Genera, Orders and other taxonomic categories, the anatomical characters are most useful. In addition, anatomical features have played an increasingly important role in elucidation of phylogenetic relationships. Some of the basic evidently anatomical characters of well-established taxonomic value are the type, size, shape, wall sculpture and pattern of wood cells, stellar patterns, types of vascular bundles, rays, ground tissue and parenchyma, epidermal and mesophyll tissue, stomata, trichomes, sclereids, nodes, and phloem cells (Pandey, 1981; Sharma, 1993). Okeke (2004) used anatomical parameter among others in separating the three taxa: *Dioscorea cayenensis*, *D. pruinosa and D. rotundata*. However, Green and Okoli (2007) suggested varietal status for *D. cayenensis*, on the basis of epidermal and starch grain evidence.

Patterns of the distribution of Sclerenchyma in *Carex* and *Festuca* have been used in distinguishing species. Sclerenchyma was also used in differentiating two genera of Velloziaceae Viz: *Vellozia* and *Barbacensis* (Sharma, 1993). *Dioscorea* species were also distinguished on the basis of stem anatomy. In addition, transformation of cortex into tissue of *Casuarina*, structure of stem endodermis in families such as Asteraceae and Piperaceae, presence of bicollateral vascular bundles in two alternate rings in Cucurbitaceae, and occurrence of cortical and medullary bundles in some families such as Amaranthaceae, Chenopodiaceae and Nyctaginaceae, are some of the features of taxonomic importance. Nonetheless, various workers observed that anatomical features have been noted to be quite conservative in showing the extent of relationships between and within taxa (Stace, 1980; Nyananyo and Osuji, 2007).

2.18 Calcium Oxalate

Calcium oxalate crystals are widespread in flowering plants, including both dicotyledons and monocotyledons (Prychid and Rudall, 1999). They were first discovered by Leeuwenhoek in the 17th century. The calcium oxalate accumulation is linked to the detoxification of calcium (Ca^{2+}) in the plant (Martin *et al*., 2012). Many plants accumulate crystalline calcium oxalate into surplus calcium (Franceschi, 1989; Fink, 1991). In higher plants, calcium oxalate typically develops within intravacuolar membrane chambers of specialized cells (Webb, 1999). In addition, calcium oxalate crystals are by far the most prevalent and widely distributed mineral deposits in higher plants. Calcium oxalate is the most abundant insoluble mineral found in plants and it is common among many plant families (Doege, 2003). The placement, size and shape of calcium oxalate crystals within some plant tissues suggest that they may play an important role in defense (Franceschi, 2001; Doege, 2003). Some or all of the crystals which may be situated at oblique angles within the cell or the ends may be

interdigitate, and are referred to as the non defensive raphide idioblasts whereas those with needle like calcium oxalate crystals, aligning parallel with the long axis of the idioblast and fill up nearly the entire cell, are said to be defensive raphide idioblasts (Saadi and Mondal, 2012a).

Many plants accumulate calcium oxalate as it has been reported in more than 1000 different genera of plants (Franceschi and Nakata, 2005); and in more than 215 higher plant families (Franceschi and Horner, 1980; Lersten and Horner, 2006) including gymnosperms and angiosperms. In angiosperms, crystal formation is generally intracellular and crystals form inside the vacuoles of specialized cells called idioblast. However, in gymnosperms, most of the crystals form in the cell wall (Kinzel, 1989). Almost all members of the aroid family contain minute crystals of calcium oxalate, distributed throughout their tissues, which may be implicated in the irritating quality found in many Araceae species (Okeke *et al.*, 2009).

Plant crystals display an astonishing variety of morphologies, most of which comform to one of the following categories defined by botanists (Franceschi and Horner, 1980). They include: (1) prisms, consisting of simple regular prismatic shapes; (2) druses, which are spherical aggregates of crystals; (3) styloids, acicular crystals that form singly; (4) raphides, acicular crystals that form in bundles and (5) crystal sand, small tetrahedral crystals that form in clusters. These variety of shapes are consistent and repeatable from one generation to the next, demonstrating that the physiological and genetic parameters controlling them are consistent (Prychid and Rudall, 1999).

Although the morphology of raphides and styloids look alike, yet they can be differentiated. Raphides are bundles of narrow, elongated needle-shaped crystals, usually of similar orientation, with pointed ends at maturity, whereas styloids also known as ‘pseudoraphides', are thicker than raphides and usually solitary within a cell (Prychid and Rudall, 1999). In addition, styloids may have pointed or squared ends, and may be elongated or cuboidal. They vary considerably in size and shape; in cross section they may be square or rectangular, occasionally with the longer walls convex; in longitudinal section typically longer and slender (100–300 μm or longer), with pointed, forked or sometimes square ends. Wu and Cutler (1985) found that variation in styloid size and shape has some taxonomic application among species of *Iris*. Franceschi and Nakata (2005) reported that Araceae has numerous druses, multi-crystal druses and needle shaped raphide crystals of calcium oxalate present in the tissue. Druses are multiple crystals that are thought to have precipitated around a nucleated site to form a crystal conglomerate (Horner *et al*., 1981). They may have a similar defensive function to that of raphides, as they also have sharp points resulting in considerable irritation if eaten (Prychid and Rudall, 1999). In addition, they are common in dicotyledons but relatively rare in monocotyledons, where they are almost entirely restricted to the first-branching taxa, *Acorus*, some Araceae and *Tofieldia*.

Key attributes for differentiating raphides include size, cross-section and termination morphology, all of which appear to vary to differing degrees depending on taxa of origin (Saadi and Mondal, 2011). In addition, they enumerated four types of raphides. They include: Type I Raphides: It is the most common raphide form and consists of four-sided single crystals that have two symmetrical pointed ends; Type II Raphides: Which are also four-sided, have one pointed and one bidentate or forked end (Prychid and Rudall, 1999). This type of raphide has so far only been recorded in a few families such

as the Vitaceae (Cody and Horner, 1983; Webb, 1999). The bidentate end is formed by crystal twinning (Arnott and Webb, 2000); Type III Raphides: The third form is crystals with six to eight sides and symmetrical pointed ends. This raphide type is known to occur in the Agavaceae (Wattendorff, 1976), Typhaceae (Horner *et al*., 1981) and Dioscoreaceae; Type IV Raphides: The fourth raphide form comprises twinned crystals with H-shaped cross-sections and asymmetrical ends (one wedge-shaped and the other sharply pointed) (Bradbury and Nixon, 1998; Kostman and Franceschi, 2000). Grooved raphides occur in Araceae (Prychid and Rudall, 1999). In addition, raphides in Araceae may also be barbed, such as those of *Alocasia*, *Colocasia*, *Dieffenbachia* and *Xanthosoma*, in which the tips of the barbs are slightly hooked and oriented away from the tapering end and towards the abruptly pointed end of the raphide.

Moreover, the sizes and appearances of calcium oxalate crystals (COC) can differ within families, genus and species, and these characteristics might be genetically controlled. Genetic regulation of CaOx formation is indicated by constancy of crystal morphology within species, cell specialization, and the remarkable coordination of crystal growth and cell expansion (Meric, 2009). Using a variety of approaches, researchers have begun to unravel the exquisite control mechanisms exerted by cells specialized for CaOx formation that include the machinery for uptake and accumulation of Ca, oxalic acid biosynthetic pathways, and regulation of crystal growth. The presence or absence of micro-characters in plant system like calcium oxalate crystals has been used for understanding the evolutionary relationships of plant species (Saadi and Mondal, 2011). In monocotyledonous leaves, styloids are usually found either in parenchymatous bundle sheath cells around vascular strands or in crystal idioblasts in adjacent mesophyll tissues, although, in *Xanthorrhoea,* the styloids in the leaf are frequently epidermal (Rudall and Chase, 1996). Calcium oxalate crystals may have taxonomic

potential for both botanist and taxonomist (Horner and Wagner 1995; Saadi and Mondal, 2011). The frequency of occurrence, quantity and distribution of oxalates of calcium are important taxonomic characters, which have been clearly used to delimit cultivars as well as characterize plant germplasm (Osuji *et al*., 1997; Osuji, 2006). In addition, Ilarslan *et al*. (2001) suggested that crystal formation within the cell is under genetic control; hence, the type, presence or absence of crystals may be represented as a taxonomic character (Prychid and Rudall, 1999; Lersten and Horner, 2000).

2.19 Chemical Characters

Chemotaxonomy of plants is an expanding field of study and seeks to utilize chemical information to improve upon the classification of plants (Singh, 2004). Chemical evidence has, in fact been used ever since man first began to classify plants as edible and inedible, obviously based on their chemical differences. Pandey (1981) reported that the science of chemical taxonomy was based on the classification of plants on the basis of their chemical constituents which were deeply concerned with the molecular characteristics. The method of chemical taxonomy is simple in principle and is based on the investigation of the distribution of chemical compounds or groups of biosystematically related plants. Moreover, the chemotaxonomic studies include the investigations of the pattern of the compounds existing in plants, and in all the individual parts of the plants, such as bark, wood, leaves, and roots. The chemical constituents usually differ much in different organs. Thus, such investigations are needed for obtaining real evidence relationships or otherwise of plants (Pandey, 1981).

Furthermore, Sharma (1993) enumerated some of the major classes of the chemical constituents as flavonoids, alkaloids, amino acids, fatty acids, aromatic compounds, terpenoids, polysaccharides and

carotenoids. In addition, Singh (2004) listed phenolics, glucosinolates and terpenes, among others as chemical characters. Pandey (1981) reported that various researchers have given a review of phytotaxonomical chemistry and emphasized on the role of various chemicals in plant taxonomy. Sharma (1993) revealed that the most widely and effectively used compounds in chemotaxonomy are the flavonoids; and some of the specific examples of the use of flavonoids in chemotaxonomy were mentioned. Isoflavone iridin was found only in the section *Pogonivis* of genus *Iris*; South American species of *Eucryphia* of the family Eucryphiaceae may easily be distinguished on the basis of their flavonoid glycosides; presence of five leaf flavonoids (tricin, luteolin, glycosyl flavones, bisulphate complex and 5-glucoside) in both Poaceae and Araceae, link them together; species of *Spirodella*, unidentifiable on morphological grounds, may be distinguished on the basis of their flavonoid chemistry. Besides, examples to indicate the use of chemistry in solving taxonomic problems according to Sharma (1993) include: Caryophyllales produce betalains and not anthocyanins; Polygonales produce anthocyanins and not betalains; Juglandales are aromatic plants while Fagales are non–aromatic; highly aromatic compounds are found in Lamiaceae, and Sapindaceae have plenty of tannins. Pandey (1981) noted that isoquinonline (alkaloids) is found in the families of Ranales; retanone in the families of Leguminales; biflavonyls in *Casuarina equisetifolia* (Casuarinaceae). Meanwhile, Gershenzon and Mabry (1983) have provided a comprehensive review of the significance of secondary metabolites in higher classification of angiosperms. They reported that tropane alkaloids of Solanaceae and Convolvulaceae were similar, suggesting a close relationship. Thorne (2003) claimed that chemical and other evidence supported the placement of Cruciferae and Capparaceae in the Order Capperales (on the basis of the presence of glucosinolates), and Pavaveraceae and Fumariaceae in the Order Pavaverales or Suborder Pavaverineae of Ranunculales. Sharma (1993) recorded that because of the presence of an iridoid compound, aucubin, Genus *Buddleia* was transferred from Loganiaceae to Buddleiaceae, near Scrophulariaceae. In the taxonomy of Asteraceae

and tribe Genisteae of Fabaceae, the petal carotenoids, which form a prominent group of terpenoids, proved very useful.

2.20 Cytological Characters

When a species with many varieties are considered, the morphological data cannot be sufficient to obtain a clear representation of the phylogenetic relationships between species. In these cases cytotaxonomy can be an effective tool and it can allow a more accurate knowledge of the relationships (Dewey, 1984; Gianfranco *et al*., 2008). Cytotaxonomy is a branch of cytogenetics, devoted to the comparative study of karyological features for systematic and evolutionary purposes (Siljak-Yakovlev and Peruzzi, 2012). Today, a number of information can be obtained by chromosome studies; among which include, chromosome number, size and structure and chromosome behaviour at meiosis. These are good taxonomic characters because hereditary materials are housed in the chromosome. Karyotypes can provide information about taxonomic relationships, genetic aberrations, and the evolutionary origins of species (Young *et al.*, 2012).

The chromosomes have been considered as the physical bases of heredity because they have a specific organization, individuality, functions and capable of self reproduction. Their main chemical constituent is DNA, a universally accepted genetic or heredity material, found to carry genetic information from one generation to next generation. They occur in all living beings in a specific number and organization and usually fall into different categories: 1. Prokaryotic chromosome: Most contain single main DNA molecule. 2. Eukaryotic chromosome: In contrast to the single main DNA. Molecule of most prokaryotic, the chromosome genome is partitioned into several molecules of DNA

or chromosomes. The chromosomes occur in the nucleus of a eukaryotic cell. In a chromosome, a single, linear and double – stranded DNA molecule is associated with almost equal amount of histone proteins. There is also certain non-histone or acidic proteins in the chromosomes which act as structural, enzymatic and regulatory proteins (Verma and Agarwal, 2009).

Chromosome identification is critical for cytological analyses, as well as subsequent studies in genomics, taxonomy, and the evolution of polyploidy, enabling an understanding of the relationship between visible landmarks and genetic or physical map features (Harper and Cande, 2000). Chromosome size and morphology may help indicate evolutionary relationships among plant species. In plant taxonomy, breeding and genetic studies, information about chromosome karyotypes can be useful in species identification and analysis of hybrid populations (Qu *et al*., 2004). Somatic cells of higher plants usually have chromosome in pairs; that is, two of each kind of chromosome present in each cell (Verma and Agarwal, 2009). Species in which there are two sets of chromosomes are referred to as diploid, given the symbol '2n'. About half of plant species are diploid, with two sets of chromosome per nucleus or cell while 'n' normally signifies the gametic or haploid number.

There is variation in the chromosome number of *C. esculenta*. Chromosome numbers reported include 2n = 21, 22, 26, 28, 38, and 42 (Coates *et al*., 1988; Parvin *et al.*, 2008). Moreover, the basic chromosome numbers of x=12 and x=14 for *C. esculenta* were reported by earlier workers. The somatic chromosome numbers 28 and 42 are considered to be diploid and triploid respectively (Kuruvilla and Singh, 1981; Coates *et al*., 1988; Lebot and Aradhya, 1991). Lee (1999) reported that the greatest variation in chromosome number occurs is India. The "Polynesian taros" primarily all have twenty-eight chromosomes, while generally there is a greater concentration of 42-chromosome types in East Asia. It has been speculated that the 28-chromosome cultivars preceded the 42-chromosome types into the

Pacific islands. The disparity in numbers may be due to the fact that taro chromosomes are liable to unpredictable behaviour during cell divisions. The most commonly reported results are 2n = 28 or 42; however, Ekanem and Osuji (2006) reported chromosome count of 2n = 24 for four cultivars of *C. esculenta* cultivated in Southeastern Nigeria namely: 'Coco India', 'Ede Ukpong', 'Ede Ghana' and 'Ede Ofe'. Chromosome number of 2n=28 was reported for three species of *Colocasia* (Yang *et al*., 2003). They include *Colocasia gaoligongensi*, *C. gigantea* and *C. gongii*.

Having gone through the literature, it became clear that local names of *C. esculenta* varieties have their weaknesses because they originated from different localities based on their dialects and languages. Consequently, the names are restricted only to the people of the dialects and languages. In addition, they are not regulated by any constituted authority. Obviously therefore, there is confusion in the taxonomy of *C. esculenta*. Moreover, there is scanty of information on the taxonomic investigation of the varieties cultivated in Nigeria. It therefore, became crucially important to study varieties of *C. esculenta* cultivated in Anambra State with a view to taxonomically elucidating them.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

Anambra State is one of the five states that make up the Southeastern states of Nigeria. Geographically, Southeastern states of Nigeria extend from latitudes 4° 40’ to 7° 20’ north latitude, and 6° 00’ to 8° 20’ east longitude (Okeke *et al.,* 2009). The states cover an area of about 50 000 km^2 of Nigeria’s total area of 923 768 km^2. Anambra State extends from latitude 6º 20’ 00’’ N, longitude 7º 00’ 00’’ E (NGA, 2012). Two seasons occur in a year, rainy season with annual rainfall, ranging from 1,400 mm in the north to 2,500 mm in the south; and dry season lasting for about four months, November to February. As a result, the natural vegetation of Anambra State is mainly rainforest.

Anambra State is divided into three senatorial zones namely; Anambra North, Anambra Central and Anambra South. Three towns, each from these three senatorial zones were selected as three geographical locations for this study. They include: Umuikwu – Anam is situated in Anambra West Local Government Area. It is bounded by famous River Niger on the south. Agulu is in Aniocha Local Government Area. Its geographical coordinates are 6º 07’ 0’’ North, 7º 4’ 0’’ East. The famous Agulu Lake is located along Awka road in Agulu. Uga is situated in Aguata Local Government Area. Its geographical coordinates are 5º 56’ 0’’ North, 7º 5’ 0’’ East. The famous ‘Obizi’ spring is located in this town.

3.2.0 Sources of Materials

Cormels of *Colocasia esculenta* varieties were obtained from the local farmers in three geographical locations of Anambra State, namely: Umuikwu-Anam in Anambra West LGA (Anambra North); Agulu in Anaocha LGA (Anambra Central) and Uga in Aguata LGA (Anambra South).

3.2.1 Umuikwu-Anam in Anambra West LGA (Anambra North)

Collection Numbers	Varieties
ACE-41	*Colocasia esculenta* var. *antiquorum* (Eddoe, 'Ugwuta', 'Ede ofe', 'Ede ofe green')
ACE-42	*C. esculenta* var. *esculenta* (Dasheen, 'Nkashi', 'Nachi','Ede ofe purple')
ACE-43	'Kochuo' ('Coco India')
ACE-44	'Nwine' ('Akonoke', 'Nkpong')

3.2.2 Agulu in Anaocha LGA (Anambra Central)

Collection Numbers	Varieties
ACE- 45	*Colocasia esculenta* var. *antiquorum* (Eddoe, 'Ugwuta', 'Ede ofe', 'Ede ofe green')
ACE-46	*C. esculenta* var. *esculenta* (Dasheen, 'Nkashi', 'Nachi', 'Ede ofe purple')
ACE-47	'Kochuo' ('Coco India')

ACE-48 'Nwine' ('Akonoke', 'Nkpong')

ACE-49 'Akpa – ahuri' ('Ogeriobosi', 'Nworoko')

3.2.3 Uga in Aguata LGA (Anambra South)

Collection Numbers Varieties

ACE-50 *Colocasia esculenta* var. *antiquorum* (Eddoe, 'Ugwuta', 'Ede ofe', 'Ede ofe green')

ACE-51 *Colocasia esculenta* var. *esculenta* (Dasheen, 'Nkashi', 'Nachi', 'Ede ofe purple')

ACE-52 'Kochuo' ('Coco India')

ACE-53 'Nwine' ('Akonoke', 'Nkpong')

ACE-54 'Ogeriobosi' ('Nworoko')

ACE-55 'Opa'

Five varieties, namely, *Colocasia esculenta* var. *antiquorum* (eddoe) traditionally known as 'akikara' in Aguata area of Anambra State, 'Ugwuta' in Nsukka area of Enugu State and 'Ede ofe green' in National Root Crops Research Institute, Umudike; *Colocasia esculenta* var. *esculenta* (dasheen) traditionally known as 'mgbagwurike' in Amesi, Anambra State, 'Nkashi' and 'Nachi' in Nsukka area of Enugu State and 'Ede ofe purple' in National Root Crops Research Institute (NRCRI), Umudike; 'Kochuo' ('Coco India'), 'Nwine' ('Akonoke', 'Nkpong') and 'Ogeriobosi' ('Nworoko') were identified and the morphological features documented and compared with other cultivars from Nsukka

in Enugu State and National Root Crops Research Institute, Umudike in Abia State. They were then grown in a common environment in Uga, Anambra State. The specimens' identification and the voucher specimens' authentication were performed by C.A. Ezeabara in liaison with Prof. C.U. Okeke, Plant Taxonomists, and deposited in the herbarium of Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

3.3 Morphological Study

The corms (main stems), cormels, petioles and leaves were measured. The plants were four months old when the measurements of leaves and petioles were done whilst matured corms and cormels were measured when the plants were harvested. The coleoptile was removed before corm was measured; third cormels, fully opened active leaves and petioles from the base were measured. The petiole measurement started from the ligule to the base of the leaf. The fresh corms and third cormels from the base of the main stem were peeled with a knife. Observations and measurements of the habit and plants' parts were done using eye lens, thread and ruler. Photographs of the habit and plant parts were then taken with a digital camera (Sony DSC-W230, China). The characters were then interpreted.

3.4 Anatomical Studies

Method described by Ndukwu and Okoli (1992) was employed. Fresh leaves, petioles and roots of the five varieties of *C. esculenta* were collected, and then free-hand-sectioned transversely, with a new sharp razor blade. A drop of water was placed on a clean slide; the specimen section was mounted on it, then stained with a drop of 0.1% safranin solution and counter stained with a drop of 1% alcian blue

solution. The specimen was then carefully covered with a coverslip and observed under an OLYMPUS (XSZ-107BN, China) light microscope at X4, X10 and X40 magnifications. For measuring the length and width of the pores, the slides were observed under an OLYMPUS (XSZ-107BN, China) light microscope fitted with eyepiece graticule (Erma-Tokyo, Japan) at X40 magnification. They were studied and photomicrographs taken with digital camera (Sony DSC-W230, China). The characters were then interpreted.

3.5 Histochemical Localization of Calcium Oxalate Crystals

Method outlined by Ndukwu (1992) was used. Fresh petioles of five varieties of *C. esculenta* were collected and fixed in freshly prepared FAA (1 part formalin, 1 part glacial acetic acid and 18 parts 70% ethanol v/v) for 24 hrs. The materials were rinsed twice in deionized water before sectioning. Transversely free-hand sections were made. The sections were lightly stained with 0.1% safranin solution, counter stained with a mixture of 6% w/v hydrogen peroxide (H_2O_2) and silver nitrate ($AgNO_3$) in bright light supplied by 100 W electric bulb fastened in a clamp stand. The slides were left in the bright light source for 30 minutes. Immersion oil was added and covered with coverslips. They were observed under an OLYMPUS (XSZ-107BN, China) light microscope at X100 magnification. Photographs of the informative sections were taken with digital camera (Sony DSC-W230, China). The features were then interpreted.

3.6 Phytochemical Study

3.6.1 Preparation of Plant Materials for Phytochemical and Proximate Analyses

Leaves, petioles, corms, cormels and roots of all the varieties of *C. esculenta* were used for the studies. The petioles were sliced with a knife in order to increase the surface area and sun-dried for a week, whereas the tubers were sun-dried for six days. The leaves and roots were air-dried at room temperature for five days. All the dried samples were ground into fine powder using a manual grinder (Corona, USA.). The powdered plant parts samples were used for the analyses.

3.6.2 Extraction

Each of the dried powdered plant materials (100 g) was packed into a soxhlet apparatus (2 L) and extracted exhaustively with 500 ml of diethyl ether (75^{o}C) for 6 hrs. The ether was evaporated using a Cole–Parmer Desiccator (EW-06525-24, USA) and then left overnight at laboratory temperature for evaporation of the remaining ether. The test solution of each extract was prepared by dissolving 10 g of crude plant extract separately in 100 ml sterile distilled water in a 250 ml Erlenmeyer flask in a water bath (1083, Germany) at 80^{o}C for 2 hrs. Extracts were subsequently filtered through four folds of cheese cloth.

3.6.3 Qualitative Phytochemical Analyses

The homogenous sample of each of the samples of the leaves, petioles, tubers and roots of the five varieties of the species of *C. esculenta* was subjected to phytochemical analyses for qualitative determinations, according to the methods described by Harborne (1973), Trease and Evans (1989), and Sofowora (1993). The performed qualitative tests were briefly described as:

1. Test for Alkaloids: To 0.5 g of each extract, 3 mls of 1% aqueous hydrochloric acid solution was added and stirred. To 1 ml of the filtrate collected in different test tubes, few drops of Meyer's reagent, Hager's reagent and Picric acid solution were added. The presence of precipitate in most of the reagent mixtures indicated the presence of alkaloids.

2. Test for Flavonoids: Two grams (2 g) of the extract was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath (1083, Germany). The mixture was filtered while hot and then cooled; 5 mls of 20% sodium hydroxide (NaOH) was added to an equal volume of the detanned extract. A yellow solution indicated the presence of flavonoids.

3. Test for Saponins: In a test tube, 0.5 g of the extract was shaken with water. Frothing, which persists on warning was taken as evidence for the presence of saponins.

4. Test for Tannins: To 0.5 g of prepared extract, 1ml of distilled water and 2 drops of ferric chloride were added. A blue–black, green or blue–green precipitate indicated the presence of tannins.

5. Test for Cyanogenic glycosides: Qualitative estimation of cyanogenic glycosides in the sample was investigated using the method outlined by AOAC (1995) and Katoch (2011). Filter paper strips were dipped in 1% picric acid solution and allowed to dry. After drying, they were further dipped into 10% Na_2CO_3 solution and dried again. The strips were stored in stoppered bottle. The sample was moistened with H_2O and allowed to hydrolyze in stoppered test tube containing sodium picrate paper. A piece of moistened sodium picrate paper was inserted in test tube and a great care was taken not to allow it to come in contact with the sample. Two (2) drops of chloroform was added into the stopper tube, hermitically.

The sodium picrate paper gradually turned orange and then brick red. This indicated that the plant material contained cyanogenic glycosides. The rapidity of change in colour depends upon the amount of free HCN present.

3.6.4 Quantitative Phytochemical Analysis

1. Alkaloid Determination

Method outlined by Obadoni and Ochuko (2001) was used.

Five (5) grams each of the samples were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 hrs. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

2. Determination of Flavonoids

The flavonoid content of the sample of the plant was determined by method as was described by Kumaran and Karunakaran (2006).

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415 nm. To 100 µl of the plant extracts in methanol (10 mg/ml), in methanol and a drop of acetic acid, was mixed, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in three triplicates.

3. Determination of Total Saponins

The saponin content of the sample was determined by method outlined by Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded.

The purification process was repeated, and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.

4. Determination of Total Tannins

Five hundred milligrams (500 mg) of the sample was weighed into a 50 ml plastic bottle. Fifty milliliters (50 ml) of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

5. Determination of Hydrogen Cyanide (HCN)

This was determined by Alkaline Pikrate Colorimeter method of Trease and Evans (1989). Using 250 ml conical flask, 1.02 g of the sample was dispersed in 50 ml of disstilled water. An alkaline Pikrate paper

was hung over the sample mixture and the blank in their respective flasks. The set up was incubated overnight and each Pikrate paper was eluted (or dipped) into a 60 ml of distilled water. A standard cyanide solution was prepared and diluted to a concentration of 0.05 mg/ml. The absorbance of the eluted sample solution and of the standard were measured in a Jenway Digital Spectophotometer (6051, UK) at 540 ɲm wavelength with the reagent blank at zero. Hydrogen Cyanide (HCN) mg/kg were computed thus:

$$\text{HCN mg/kg} = \frac{1000}{W} \times \frac{au}{as} \times c \times D$$

Where:

W = weight of sample analyzed

au = absorbance of test sample

as = absorbance of standard HCN solution

c = concerntration of standard in mg/ml

D= dilution factor, where applicable.

6. Determination of Oxalate

This was done using the method of Oke *et al.* (1996) and Onwuka (2005). Two grams (2 g) of each sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask. Ten (10) ml of 6 M HCL was added and the suspension was digested at 100^{o}C for 1 hour. It was cooled and made up to 250 ml mark of the volumetric flask before filtration. Duplicate portions of 125 ml filtrate were measured

into beakers and four drops of methyl red indicator was added. This was followed by the addition of concentrated NH_40H solution (drop wise) unit the test solution changed from salmon pink colour to a faint yellow colour (P^H 4.0-4.5). Each portion was heated to 90^{o}C cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^{o}C and 10 ml of 50% $CaCl_2$ solution was added while it was stirred continuously.

After heating, it was cooled and left overnight at 5^{o}C. The solution was centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate was completely dissolved in 10 ml of 20% (v/v) H_2So_4 solution. The filtrate resulting from the digestion process was titrated against 0.05 M standardized $KMnO_4$ solutions to a faint pink colour which persisted for 30 seconds.

The percentage calcium oxalate content was calculated with the formula:

$$\text{Percentage (\%) oxalate} = \frac{\text{titre} \times 0.00225 \times 100}{W}$$

Where: W= weight of sample

7. Determination of Phytate Content

The colorimetric method was used to determine the phytate content of each sample (Onwuka, 2005). Two grams (2 g) of each sample was mixed with 0.2 N HCl to form 1.25 w/v mixture. The mixture was shaken and allowed to stand for 30 minutes at room temperature. It was filtered with whatman no 42 filter paper to obtain the extract used in the analysis.

An aliquot (0.5 ml) of the extract was mixed with lml of Fenn solution in a test tube. It was heated in water GFL water bath (1083, Germany) for 30 minutes and then cooled in ice for 15 minutes. It was then allowed to attain room temperature (32^{o}C). It was later treated with 2ml of 2,2-Bipyridine solution, mixed well and its absorbance was read in a Jenway Digital Spectrophotometer (6051, UK) at 510 μm, meanwhile a standard phytate solution (sodium phytate) was prepared and diluted to 0.5 mg/ml. One (1) ml of the standard solution was treated as described above and its absorbance was also read. The percentage phytate content was calculated with the formula given below:

$$\text{Percentage (\%) phytate} = \frac{100}{W} \times \frac{au}{as} \times \frac{C}{100} \times \frac{Vt}{Va}$$

Where: W = weight of sample

au = absorbance of sample

as = absorbance of standard solution

Vt = total extract volume

Va = volume of extract analyzed

3.7.0 Proximate Analysis

Analyses of the proximate contents of the leaves, petioles, cormels and roots of five varieties of the species of *C. esculenta* were carried out according to the methods described by Onwuka (2005) with the exception of fat determination.The methods are described below:

3.7.1 Moisture Content Determination

The dishes were washed thoroughly and dried in the oven. They were latter put inside the dessicator to cool. After which they were weighed. Sample was put into the weighed dish and weight taken. The sample was dried in the oven at 70^{o}C for 2 hrs and at 105^{o}C for the next 4 hrs. The sample was cooled in the dessicator and the dry weight of sample plus dish taken. The moisture content was calculated as follows:

$$\text{Percentage (\%) moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:-

W_1 = Initial weight of empty crucible

W_2 = Weight of crucible + sample before drying

W_3 = Final weight of crucible + sample after drying

3.7.2 Ash Content Determination

Five grams (5 g) of finely ground dry sample was weighed into a tarred silica crucible. The sample was charred on a heater inside a fume cupboard, to dry off most of the smoke. The sample was transferred into a pre-heated muffle furnace at 550^{o}C. It was left at this temperature for 2 hours. After which it was cooled in a dessicator and re-weighed.

$$\text{Percentage (\%) Ash} = \frac{\text{Weight of ash}}{\text{Weight of original sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where:-

W_1 =Weight of empty crucible

W_2 =Weight of crucible +sample before drying

W_3 = Weight of crucible + ash

3.7.3 Crude Fibre Content Determination

Water reflux was boiled for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution. The solution was filtered through two folds of cheese cloth on a fluted funnel. The residue was washed with boiling water until there was no longer acid. The residue was transferred to a beaker and boiled for 30 minute with 200 ml of a solution containing 1.25 g of carbonate–free NaOH per 100 ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven and weighed. It was finally incinerated, cooled and weighed.

The loss in weight after incineration x 100 was the percentage of crude fibre.

3.7.4 Crude Protein Content Determination

A measured weight (2 g) of sample was weighed into a 250 ml beaker. After which 75 ml of hot water was added and brought to boil. It was stirred vigorously and added 25 ml of 6% copper sulphate solution. It was again brought to boil, stirred vigorously and added 25 ml of the 1.25% sodium

hydroxide solution. The mixture was stirred vigorously, removed from the flame and allowed to settle. It was filtered through a 15 cm, No. 4 Whatman paper. The precipitate was cleaned from the sides of the beaker. The paper was washed free from sulphate with very hot water 6 times. It was allowed to drain well, and then transferred to a kjeldahl flask containing about 10 g anhydrous sodium hydroxide and a trace of selenium. Thirty milliliters (30 ml) of conc. H_2SO_4 was added, nitrogen content determined and hence the protein content of the sample.

Percentage (%) protein = % N x F

Where:-

F = Conversion factor (6.25)

$$\text{Percentage (\%) Nitrogen} = \frac{V_S - V_B \times N_{acid} \times 0.028 \times 100}{W}$$

Where:-

V_S = Volume of acid required to titrate sample in milliliters

V_B = Volume of acid required to titrate blank in milliliters

Nacid = normality of acid (0.1N)

W = weight of sample in grams

Therefore, Percentage (%) protein = % Nitrogen x 6.25.

3.7.5 Fat Content Determination

The method of Pearson (1973) was employed. The method is based on the principle that non-polar components of the samples are easily extracted into organic solvents. Three (3) grams, (moisture – free) of each sample, was placed into labeled fat-free thimbles. These were then weighed, plugged with glass wool and introduced into the soxlet extractors containing 160 ml petroleum ether (b.p 75^{o}C). Clean dry receiver flasks were also weighed and fitted to the extractors. The extraction units were then assembled, and cold water was allowed to circulate, while the temperature of the water bath was maintained at 60^{o}C. Extraction was carried out for eight hours. At the end of this time, the thimbles containing the samples were removed and placed in an oven at 70^{o}C for three hours and dried to constant weight.

$$\text{Percentage (\%) Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.7.6 Carbohydrate Content Determination

Carbohydrate content was determined by difference method:

Percentage (%) Carbohydrate = 100 – (% Moisture + % Ash + % Crude fibre + % Crude protein + % Fat).

3.7.7 Determination of Minerals

With the exception of calcium, magnesium and iron, all other mineral contents of these samples were done following the dry ash extraction method outlined by James (1995) and Kirk and Sawyer (1998). A measured weight of these samples were burnt to ashes (as in ash determination) thereby remaining all

the organic materials leaving the organic ash. The resulting ashes were each dissolved in 5 mls of dilute (0.1 M) hydrochloric acid solution and then diluted to 100 mls in a volumetric flask. This extract was used in specific analysis for the different mineral elements.

1. Determination of Calcium and Magnesium by Complexiometric Titration

The Versanate EDTA titrimetric method of Udoh and Oguwale (1986) was employed. A measured volume of 20 mls of each extract was dispersed into conical flask and treated with pinches of masking agents (hydroxylamine hydrochloride, sodium cyanide and sodium potassium ferrocyanide). The flask was shaken and the mixture dissolved, and 20 mls of ammonia was added to it to raise the P^H to 10.00 (a point at which both calcium and magnesium form complexes with EDTA).

The mixture was titrated against 0.02 N EDTA solution using Eriochrome Black T as indicator. A reagent blank was also titrated and titration in each case was done deep red to a permanent blue point. The titration value represents both calcium and magnesium ions in test sample. A repeat titration was done to determine Ca^{2+} alone in the test samples.

However, titration of mg^{2+} alone was done in similarity with the above titration procedures but in this case, 10% NaOH was used in place of the ammonia buffer and Solechrome dark blue indicator was used in place of Eriochrome Black T indicator. From the values obtained Ca^{2+} and Mg^{2+} in the samples were calculated using the formula:

$$\text{Mg/Ca (Mg/100)} = \frac{100}{W} \times \text{T-B (N} \times \text{Mg/Ca)} \times \frac{Vt}{Va}$$

Where:

W = Weight of sample

T = Titre value of sample

B = Titre value of blank

Mg = Magnesium equivalence

Ca = Calcium equivalence

N = Normality of titrant (0.02 N EDTA).

Vt = Total extract volume

Va = Volume of extract analysed

2. Determination of Sodium and Potassium by Flame photometry

The instrument, Jenway digital flame photometer, was set up according to the manufactures instruction. It was switch on and allowed 15 minutes to equilibrate. Meanwhile standard sodium and potassium solutions were prepared separately and diluted in series to contain 10,8,6,4, and 2 pp of sodium and potassium, respectively. After caliberating the instrument, 1 ml of each standard was aspirated into it and sprayed over the non-luminious flame. The optical density of the resulting emission from each standard solution was recorded. Before flaming, the appropiate element filter (Na or K) was put in place with the standards measured, the test sample extracts were measured in time and they were plated into standard curves which was used to extrapolate the content of each test element.

3. Determination of Phosphorus

Phosphorus in the test sample was determined by the molybdovanadate colourimetric method (James, 1995). Two milligrams (2 mg) of the dry ash digest of the sample was dispersed into a 50 ml volume flask. At the same time the same volume of water and standard phosphorus solution were measured into different flasks to serve as reagent blank and standard, respectively. Two millilitres (2 ml) of the phosphorus colour reagent (molybdovanadate solution) was added to each of the flask and allowed to stand at room temperature (32^{o}C) for 15 minutes. The content of each flask was diluted to the 50 ml mark with distilled water and its absorbance was measured in a Jenway Digital Spectrophotometer (6051, UK) at a wavelength of 540 nm with the phosphorus content was calculated using the formula as shown.

$$P\ (mg/100g) = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where:

W = Weight of sample ashed

Au = absorbance of test sample

as = absorbance of standard phosphorus solution

e = concentration of standard phosphorus solution

Vt = total extract volume

Va = volume of extract analyzed

4. Determination of Iron (Fe)

Five grams (5 g) of the sample was taken in a 100 ml micro-Kjeldahl flask. A glass bead and 10 ml of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled. Then 1 ml volume of concentrated sulfuric acid was added carefully, followed by the addition of 2 ml of concentrated HF, and heating was continued for at least 30 min and then cooling. The content of the flask was reduced to iron (II) using sodium azide solution and excess azide was removed by boiling and then the content was filtered. The solution of the flask was then neutralized with dilute NH_4OH in the presence of 2 ml of a 0.01% (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 50 ml calibrated flask and made up to the mark with deionized water.

A suitable aliquot (2 ml) of the final solution was pipetted into a 10 ml calibrated flask and the iron content was determined as described above using tartrate or EDTA as masking agent (Stahr, 1991; Ahmed and Roy, 2009).

3.7.8 Determination of Vitamin Contents

The vitamins A, C and E assays were performed with the methods of Pearson (1973) and Ogugua *et al*. (2013). While vitamins B_1, B_2 and B_3 contents were determined using the methods described by Okwu and Josiah (2006).

1. Determination of Vitamin A

A quantity of 1.0 g of ground sample was macerated with 20 ml of petroleum ether. This was decanted into a test tube and then evaporated to dryness. A measured amount of 0.2 ml of chloroform-acetic anhydride (1:1, v/v) was added to the residue. An amount of 2 ml of TCA-chloroform in like (1:1 v/v)

was added to the resulting solution and absorbance was measured at 620 nm. Vitamin A standard was prepare in like manner and the absorbance taken at 620 nm. The concentration of vitamin A in the sample was extrapolated from the standard curve.

2. Determination of Vitamin B_1 (Thiamine) Content

The colorimeter method described by Okwu and Josiah (2006) was used. Five grams of each sample was extracted by homogenizing it is 50 ml of normal ethanolic sodium hydroxide (NaOH) solution. The homogenate was filtered through whatman no 42 filter paper and the residue was washed with the extractant solution and was obtained. A portion of the extract (10 ml treated with equal volume (10 ml) of dilute potassium ferrocyanide in a 50 ml volumetric flask, meanwhile a standard thiamin solution and the extracting solution were used to set up the standard and reagent blank, respectively. Both were treated as described above and the absorbance was read in a Jenway Digital Spectrophotometer (6051, UK) at a wavelength of 360 nm. The reagent blank was used to the zero the reading of the instrument before readings were taken. The formula given as follow was used to calculate the thiamin content.

$$\text{Thiamin Mg/100g} = \frac{100}{W} \times \frac{au}{as} \times c \times \frac{vt}{va} \times D$$

Where:

W = weight of sample used

au = absorbance of sample

as = absorbance of standard

c = concentration of standard (mg/ml)

vt = total volume of extract

va = volume of extract analyze

D = dilution factor, where applicable

3. Determination of Vitamin B_2 (Riboflavin) Content

This was done using the colorimetric method as described by Okwu and Josiah (2006). Five grams (5 g) of each sample was mixed with 50 ml of 50% ethanol solution and shaken very well. It was allowed to stand at room temperature (30^{o}C) for an hour, and filtered through Whatman No 42 filter paper to obtain the extract (filtrate). An aliquot was treated with equal volumes (10M) of (5%) potassium permanganate ($KMnO_4$) solution, then 10 ml of 30% hydrogen peroxide (H_2O_2) was added to it and it was allowed to stand in a GFL electric water bath (1083, Germany) for 30 minutes. After that 2 ml of 40% sodium sulphate (Na_2SO_4) solution was added to it, mixed very well and made up to 50 ml, by diluting water in a volumetric flask.

Meanwhile, 1 ml of a standard in riboflanin and 10 ml of the extracting solution (50% ethanol) were put in separate beakers and treated as described above, to form the standard and the reagent blank respectively, the reagent blank used to zero the instrument while the absorbance of the standard solution and the samples were read in turns at a wavelength of 510 nm.

The formula given as shown below was used to calculate the riboflavin content.

$$\text{Riboflavin (mg/100g)} = \frac{100}{w} \times \frac{au}{as} \times C \times \frac{vt}{va} \times D$$

Where:

W = weight of sample analyzed

au = absorbance of sample

as = absorbance of standard

c = concentration of standard (mg/ml)

vt = total volume of extract

va = volume of extract analyzed

D = dilution factor, where applicable

4. Determination of Vitamin B_3 Content (Niacin)

Five grams of each sample was extracted with 50ml of normal sulpuric acid solution (NH_2S0_4) by allowing standing for 30 minutes with intermittent shaking at 10 minutes interval, it was filtered and the residue was washed with the extractant acid solution until 50 ml extract was obtained.

A 10 ml portion of the extract was dispersed into a 50 ml volumetric flask. Similarly, standard niacin solution and 10 ml of the extracting solution were put in separate volumetric flasks to serve as the standard and reagent blank, respectively.

Three drops of ammonia solution was added to each flask and mixed well. It was acidified with 10 ml of 0.02 NH_2SO_4 solution. It was then treated with 10 ml of normal potassium ferrocyanide solution (1N K_2Fe CN). The mixture was made up to 50 ml in each flask with distilled water before its respective absorbance was read at 470 nm in a Jenway Digital Spectrophotometer (6051, UK). The reagent blank was used to set the instrument at zero. The formula below was used to calculate the niacin content in the sample.

$$\text{Niacin (mg/100g)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{vt}{va} \times D$$

Where:

W = weight of sample (g)

au = absorbance of sample

as = absorbance of standard

c = concentration of standard (mg/ml)

vt = total volume of extract

va = volume of extract analyzed

D = dilution factor, where applicable

5. Determination of Vitamin C

A measured weight of 1 g of sample was macerated with 20 ml of 0.4% oxalic acid. This was filtered and to 1 ml of filtrate was added 9 ml of Indolephenol reagent. The standard solution of vitamin C was prepared similarly and the absorbances of the standard solution and the sample were read at 520 nm. The concentration of vitamin C was extrapolated from the standard curve of vitamin C.

6. Determination of Vitamin E

A measured 1 g of the sample was macerated with 20 ml of ethanol and then filtered. A quantity of 0.2% ferric chloride in ethanol and 1 ml of 0.5% α-α-dipyridine added to 1 ml of the filtrate. This was diluted

to 5 ml with distilled water. Absorbance was taken at 520 nm. The standard solutions were prepared similarly and the concentration of vitamin E extrapolated from the standard curve.

3.8 Cytological Study

Mitotic chromosome spreads were generated following a protocol by Ndukwu and Okoli (1992) and Osuji (2003). The cormels from each variety were planted in plastic pots (90 x 145 mm), filled with sandy loamy soil to a depth of 45 mm, and allowed to sprout. Following sprouting (emergence of plumule), the plants were uprooted by pouring water to the slant plastic pots so that soil runs out with the water. Fresh root tips were then excised (15 mm) from 8 am to 3.00 pm at hourly interval; and pretreated in 8-hydroxyquinoline for 4 hrs. They were later fixed in Carnoy's fluid (3:1 ethanol: acetic acid) for 24 hrs. Storage of root tips pending squashing was stored in 70% ethanol solution. The roots were hydrolyzed in 0.5% aqueous HCl (for 5 min) in a watch glass. About 1 mm tips of hydrolyzed roots were excised and placed on a clean glass slide (22x22 mm) and a drop of FLP-orcein was added to it. A coverslip was placed over the stained tissue and even pressure was applied to generate mitotic chromosome spreads. A drop of Immersion oil was added on top of the coverslips. Chromosome slides were then observed microscopically in light microscope, an OLYMPUS (XSZ-107BN, China) at X100 magnification. Photographs of informative slides were taken with digital camera (Sony DSC-W230, China). The characters were then interpreted.

3.9 Statistical Analysis

SPSS software version 20 was used for the statistical analysis. One-Way-Anova was used to analyze the data at 0.05 and 0.01 level of probability. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means, and data were expressed as mean $\pm$ standard deviation of triplicate determinations.

CHAPTER 4

RESULTS

Morphological Characters

Morphological observation had that the leaves of all the varieties were thick, succulent and downward-pointing, with reticulate venation. Three strong midribs arose from the point of attachment to the petiole, the main midrib pointed towards the basal lobe, while other two smaller midribs extended to the two posterior lobes, with one midrib on each lobe. The primary lateral veins originated from the three midribs, while the veinlets emanated from these primary lateral veins. Leaves margin were entire; the leaf surfaces were sagittate, not shiny; the abaxial and adaxial surfaces of the leaf blades were glabrous with the exception of abaxial surface of 'ogeriobosi'; the anterior lobes were twice as large as the posterior lobes which were round in shape; the petiole attachments were peltate (Plates 1-10).

The base of petiole of *Colocasia esculenta* var. *antiquorum* was deep green, the colour of the petiole and leaf was pale-green with net venation (Plates 1 and 2). The abaxial surface of the leaf blade was glabrous (Plate 2).

Plate 1: Habit of *Colocasia esculenta* var. *antiquorum*

Plate 2: Abaxial leaf surface of *Colocasia esculenta* var. *antiquorum*

The petiole of *Colocasia esculenta* var. *esculenta* was pale green and the leaf colour was deep green with net venation (Plates 3 and 4). The abaxial surface of the leaf blade was glabrous (Plate 4).

Plate 3: Habit of *Colocasia esculenta* var. *esculenta*

Plate 4: Abaxial leaf surface of *Colocasia esculenta* var. *esculenta*

The petiole colour of ‘kochuo’ was green and the leaf colour was deep green (Plates 5 and 6). The leaf had a purplish dot on the centre of the adaxial surface (Plate 5). Abaxial leaf surface was glabrous and had a conspicuous purplish colour at the point of attachment to the petiole (Plate 6).

Plate 5: Habit of ‘kochuo’

Plate 6: Abaxial leaf surface of ‘kochuo’

The petiole of ‘nwine’ was yellowish-green in colour and the leaf colour was pale green with net venation (Plates 7 and 8). The abaxial leaf surface was glabrous (Plate 8).

Plate 7: Habit of ‘nwine’

Plate 8: Abaxial leaf surface of ‘nwine’

The petiole of ‘ogeriobosi’ was purple in colour and the colour of the leaf was deep green (Plates 9 and 10). There was presence of foliaceous (leaf–like) appendages at the veins of the abaxial surface of the leaf (Plate 10).

Plate 9: Habit of ‘ogeriobosi’

Plate 10: Abaxial leaf surface of 'ogeriobosi'

Colocasia esculenta var. *antiquorum* had large corm which was more or less orbicular (approximately circular) in shape with numerous small round cormels (Plate 11).

Plate 11: Corm and cormels of *Colocasia esculenta* var. *antiquorum*

The corm of *Colocasia esculenta* var. *esculenta* was large and oval with few more or less cylindrical shaped cormels (Plate 12).

Plate 12: Corm and cormels of *Colocasia esculenta* var. *esculenta*

'Kochuo' had a large oval shaped corm with cormels that had numerous vertical brownish stripes on the surface (Plate 13).

Plate 13: Corm and cormels of 'kochuo'

‘Nwine’ possessed large corm with slender cormels (Plate 14).

Plate 14: Corm and cormels of ‘nwine’

‘Ogeriobosi’ had the largest corm, oval in shape with largest cylindrical cormels (Plate 15).

Plate 15: Corm and cormels of ‘ogeriobosi’

Corm shapes of *Colocasia esculenta* var. *esculenta,* 'nwine' and 'ogeriobosi' were more or less oval, whereas those of *Colocasia esculenta* var. *antiquorum* and 'kochuo' were orbicular. *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *antiquorum* had the smallest cormel in comparison with cormels of others. The cormel of 'ogeriobosi' was biggest when compared with others and the cormel of 'nwine' was elongated/slender (Plate 16).

Plate 16: Shape, size and texture of corms and cormels of varieties of *Colocasia esculenta*

A. ***Colocasia esculenta* var. *antiquorum***

B. ***Colocasia esculenta* var. *esculenta***

C. **'kochuo'**

D. **'nwine'**

E. **'ogeriobosi'**

Petiole length of ‘ogeriobosi’ at 63.3±3.83 cm was the highest, whereas the least was *Colocasia esculenta* var. *antiquorum* at 26.67±2.20 cm (Table 1). The petiole colour of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* were the same whereas others differed. There was significant difference among the leaf length of all the varieties at $p<0.05$. The leaf length of ‘ogeriobosi’ at 49.9±3.55 cm was the highest, whereas the least was ‘kochuo’ at 35.6±4.80 cm. There was no significant difference between the leaf width of *Colocasia esculenta* var. *antiquorum* at 36.90±4.25 cm and *Colocasia esculenta* var. *esculenta* at 36.00±2.65 cm, whereas the leaf width of ‘nwine’ at 45.27±3.66 cm was the highest. The leaves of *Colocasia esculenta* var. *esculenta*, ‘kochuo’ and ‘ogeriobosi’ were deep green in colour, whereas those of *Colocasia esculenta* var. *antiquorum* and ‘nwine’ were pale green. There was significant difference among the corm length of all the varieties; and it ranged from 4.10±0.10 cm in *Colocasia esculenta* var. *antiquorum* to 8.60±0.35 cm in ‘ogeriobosi’. There was also significant difference among the corm width of all the varieties. The corm width of ‘kochuo’ at 6.33±1.27 cm was the highest, while the least value was found in *Colocasia esculenta* var. *esculenta* at 4.47±0.72 cm. In addition, there was significant difference among the cormel lengths of all the varieties, which ranged from 3.70±0.96 cm in *Colocasia esculenta* var. *antiquorum* to 7.03±0.36 cm in ‘ogeriobosi’. The colour of the peeled fresh tubers of all the varieties varied with *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* having the same colour (Table 1).

Table 1: Morphological characters (cm) of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

Varieties	Petiole length	Petiole colour	Leaf Length	Leaf Width	Leaf Colour	Corm length	Corm width	Cormel length	Peeled tuber (Fresh)
Antiq	26.67±2.20[a]	Pale green	42.80±5.86[a]	36.90±4.25[a]	Deep green	4.10±0.10[a]	5.23±1.28[a]	3.70±0.96[a]	Green
Esc	38.07±2.22[b]	Pale green	44.80±4.33[b]	36.00±2.65[a]	Pale green	6.17±0.50[b]	4.47±0.72[b]	5.00±1.35[b]	Green
'Kochuo'	40.33±2.35[c]	Green	35.60±7.70[c]	34.13±4.80[b]	Deep green	4.86±1.95[c]	6.33±1.27[c]	5.45±3.02[c]	Purple
'Nwine'	41.40±5.65[d]	Yellowish green	41.40±4.23[d]	45.27±3.66[c]	Pale green	4.55±0.87[d]	5.67±0.59[d]	6.63±1.42[d]	Pink
'Ogeriobosi'	63.30±3.83[e]	Purple	49.90±3.55[e]	35.80±3.64[d]	Deep green	8.60±0.35[e]	5.53±0.76[e]	7.03±0.36[e]	Milk

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Data are means ± standard deviation of triplicates determinations. Columns with the same letter are not significantly different ($p>0.05$).

Anatomical Characters

The arrangements of the mesophyll were the same in the tranverse sections of leaf of all the varieties. The vascular bundles were small in size, collateral and closed (Plates 17-21).

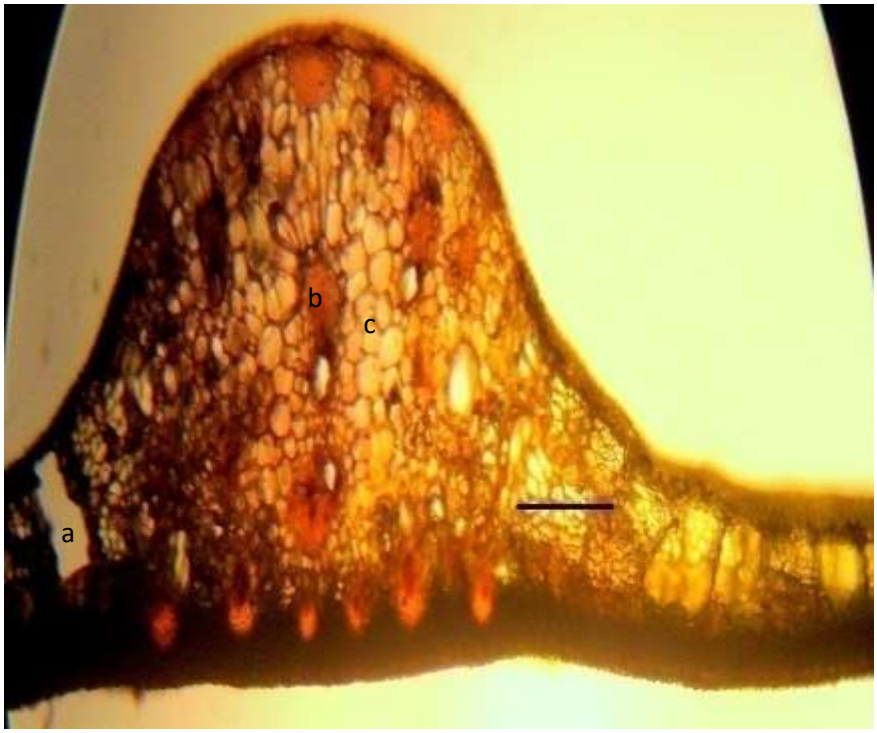

Plate 17: Transverse section of leaf of *Colocasia esculenta* var *antiquorum* X40.

Scale bar = 32 µm

a=air space

b=vascular bundle

c=mesophyll

Plate 18: Transverse section of leaf of *Colocasia esculenta* var *esculenta* X40.

Scale bar = 32 µm

a=air space

b= vascular bundle

c= mesophyll

Plate 19: Transverse section of leaf of 'kochuo' X40. Scale bar = 32 µm

a=air space

b= vascular bundle

c= mesophyll

Plate 20: Transverse section of leaf of 'nwine' X40. Scale bar = 32 µm

a=air space

b= vascular bundle

c= mesophyll

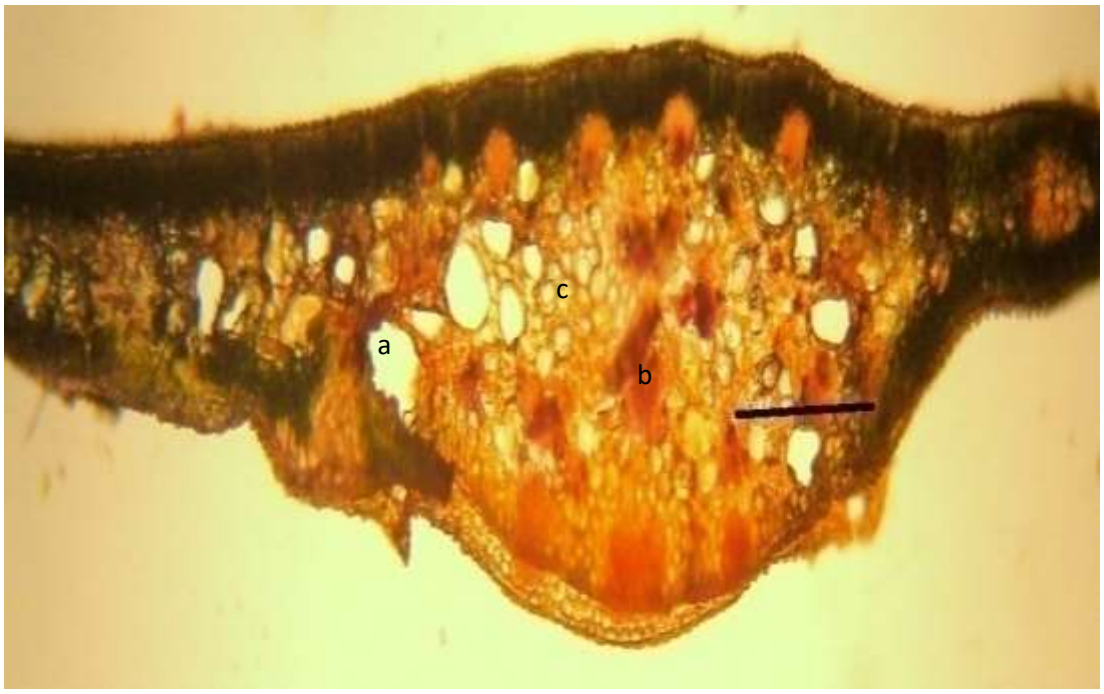

Plate 21: Transverse section of leaf of 'ogeriobosi' X40. Scale bar = 32 µm

a=air space

b= vascular bundle

c= mesophyll

Epidermal Studies

The epidermal observations (Plates 22 and 31) revealed that there were stomata on both upper (adaxial) and lower (abaxial) leaf surfaces. Both the adaxial and abaxial leaf surfaces of all the varieties possessed anomocytic stomatal type. The outline of the stoma was oval while the stomatal pore was narrowly elliptic.

The stomata were present in both adaxial and abaxial surfaces of *Colocasia esculenta* var. *antiquorum*. The outline of the stomata was oval while the stomatal pore was narrowly elliptic. Both adaxial and abaxial leaf surfaces possessed anomocytic stomatal type (Plates 22 and 23).

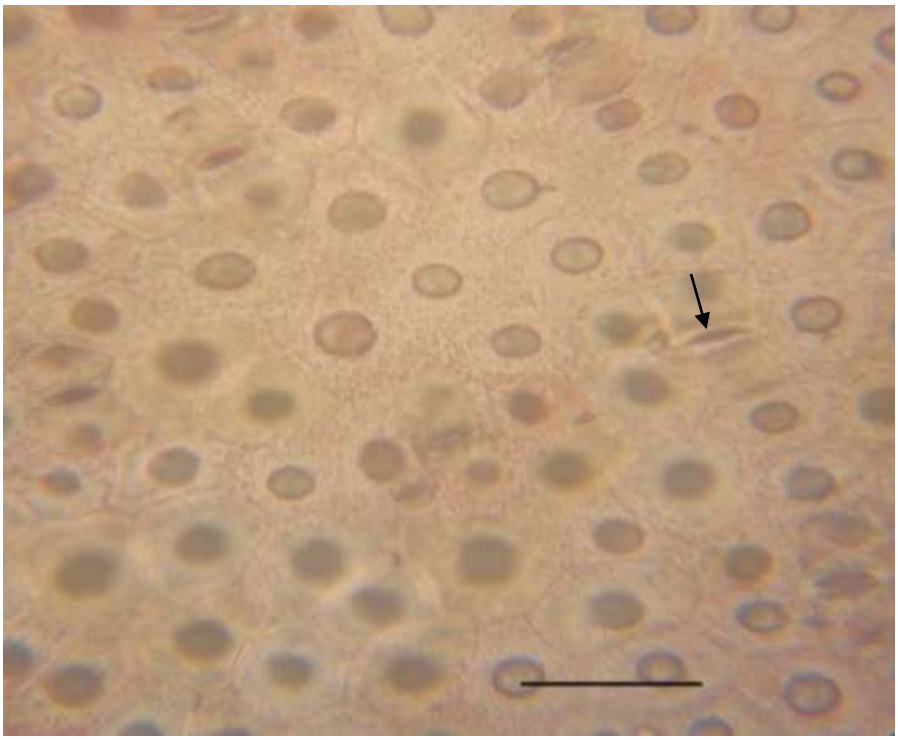

Plate 22: Epidermal features of adaxial leaf surface of *Colocasia esculenta* var. *antiquorum* X100.

Scale bar = 24 µm

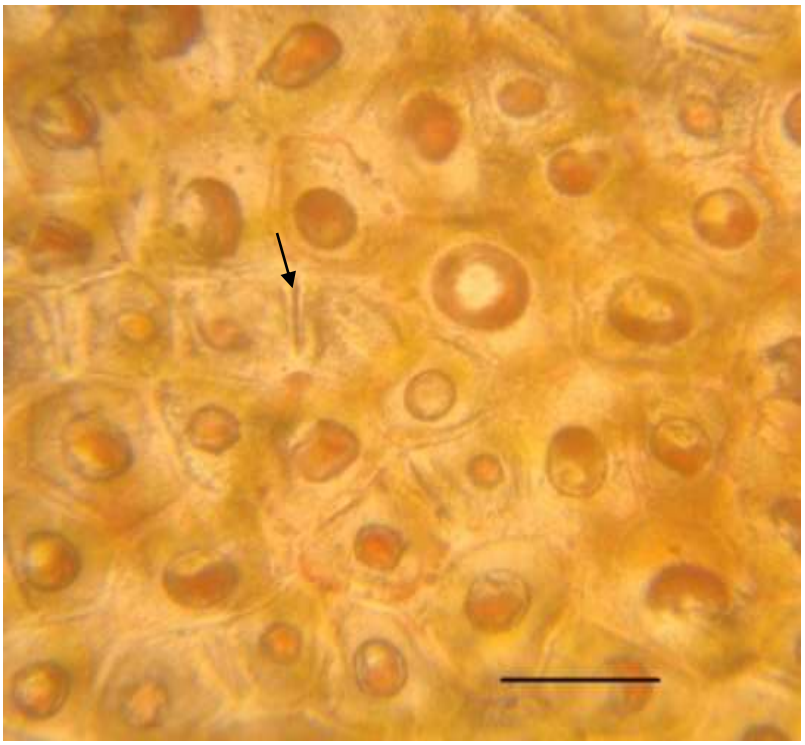

Plate 23: Epidermal features of abaxial leaf surface of *Colocasia esculenta* var. *antiquorum* X100.

Scale bar = 24 µm

The stomata were present in both adaxial and abaxial surfaces of *Colocasia esculenta* var. *esculenta*. The outline of the stomata was oval while the stomatal pore was narrowly elliptic. Both adaxial and abaxial leaf surfaces possessed anomocytic stomatal type (Plates 24 and 25).

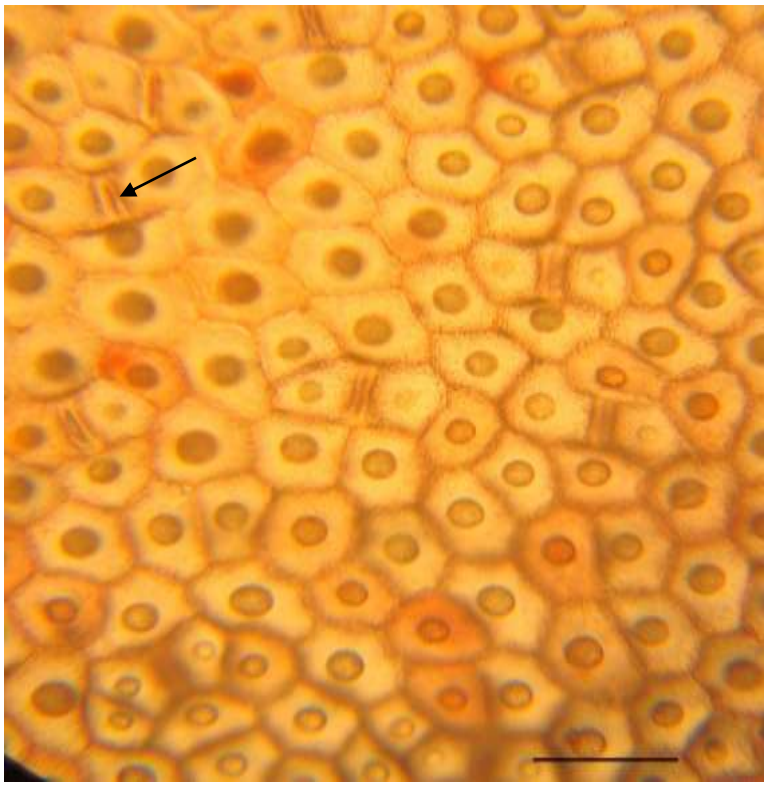

Plate 24: Epidermal features of adaxial leaf surface of *Colocasia esculenta* var. *esculenta* X100.

Scale bar = 24 µm

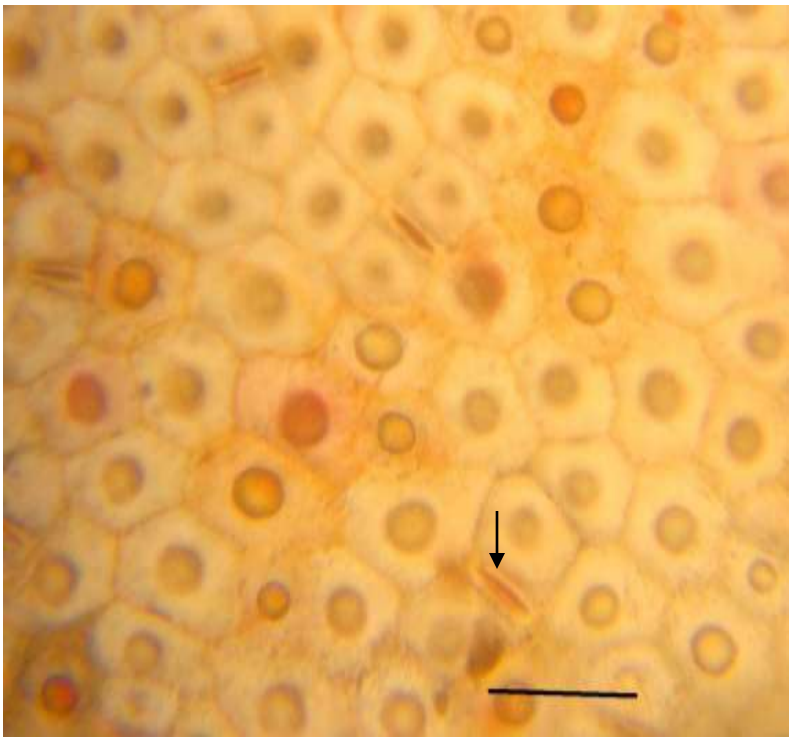

Plate 25: Epidermal features of abaxial leaf surface of *Colocasia esculenta* var. *esculenta* X100.

Scale bar = 24 µm

The stomata were present in both adaxial and abaxial surfaces of ‘kochuo’. The outline of the stomata was oval while the stomatal pore was narrowly elliptic. Both adaxial and abaxial leaf surfaces possessed anomocytic stomatal type (Plates 26 and 27).

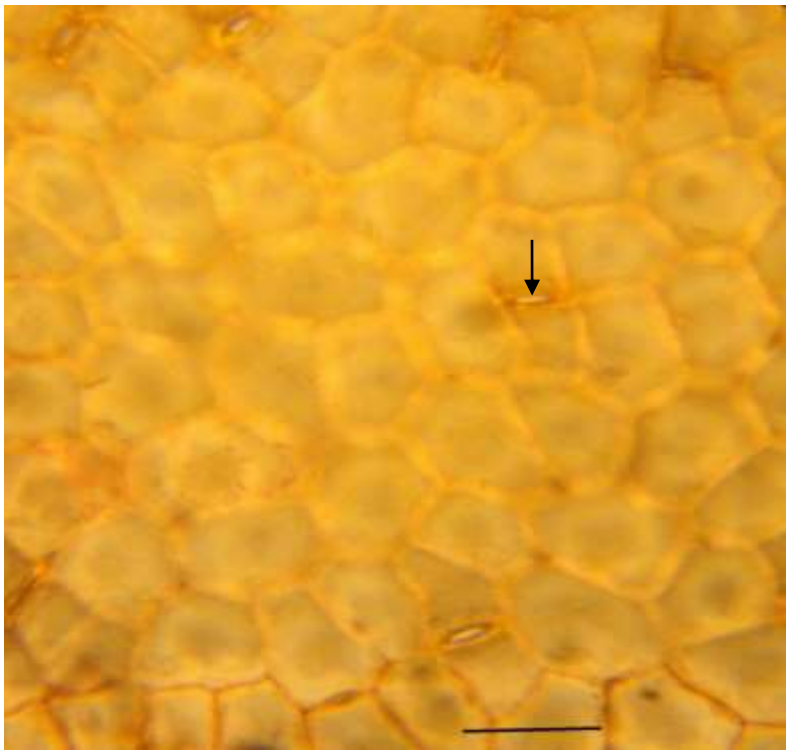

Plate 26: Epidermal features of adaxial leaf surface of ‘kochuo’ X100.

Scale bar = 24 µm

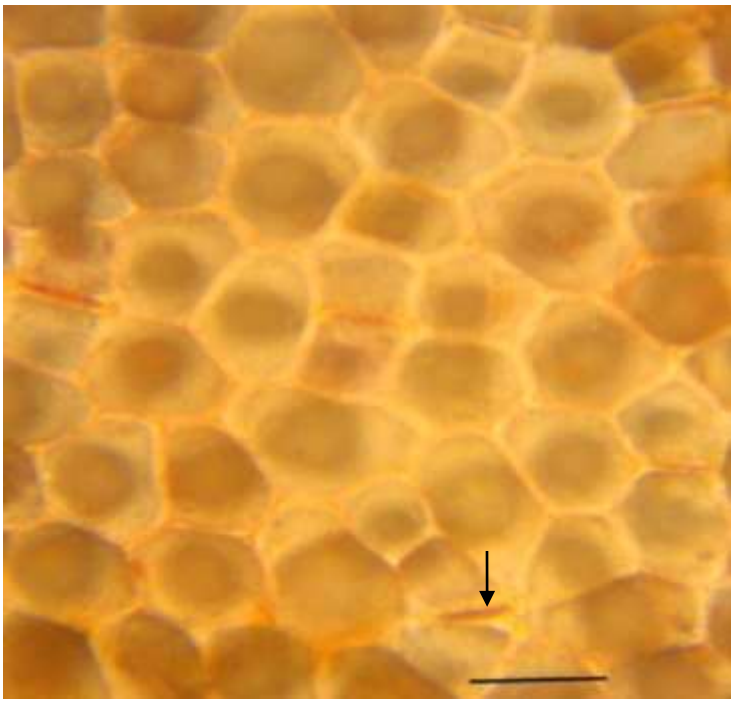

Plate 27: Epidermal features of abaxial leaf surface of 'kochuo' X100.

Scale bar = 24 µm

The stomata were present in both adaxial and abaxial surfaces of'nwine'. The outline of the stomata was oval while the stomatal pore was narrowly elliptic. Both adaxial and abaxial leaf surfaces possessed anomocytic stomatal type (Plates 28 and 29).

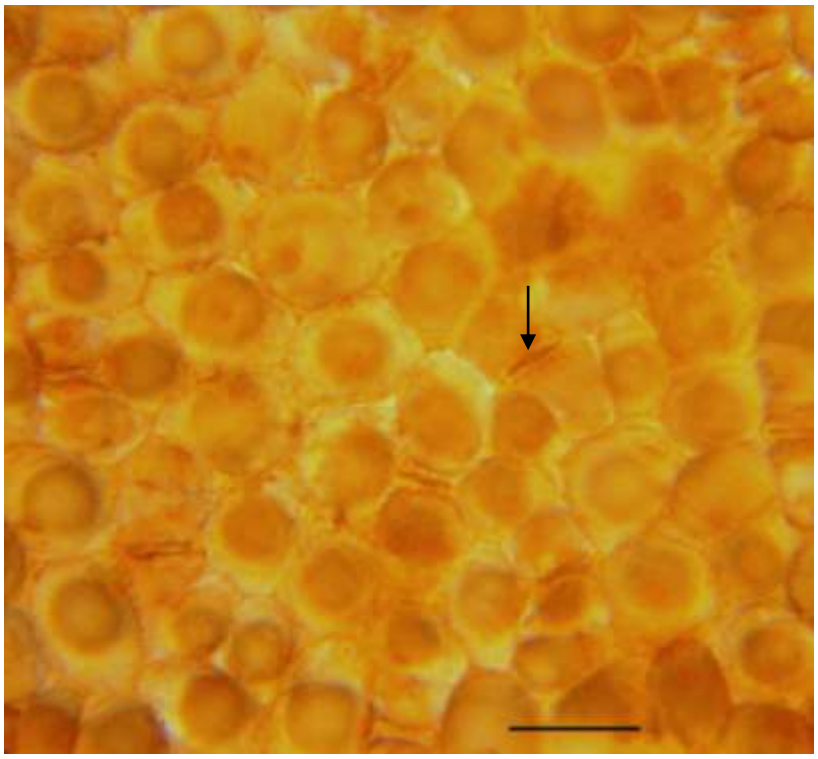

Plate 28: Epidermal features of adaxial leaf surface of 'nwine' X100.

Scale bar = 24 µm

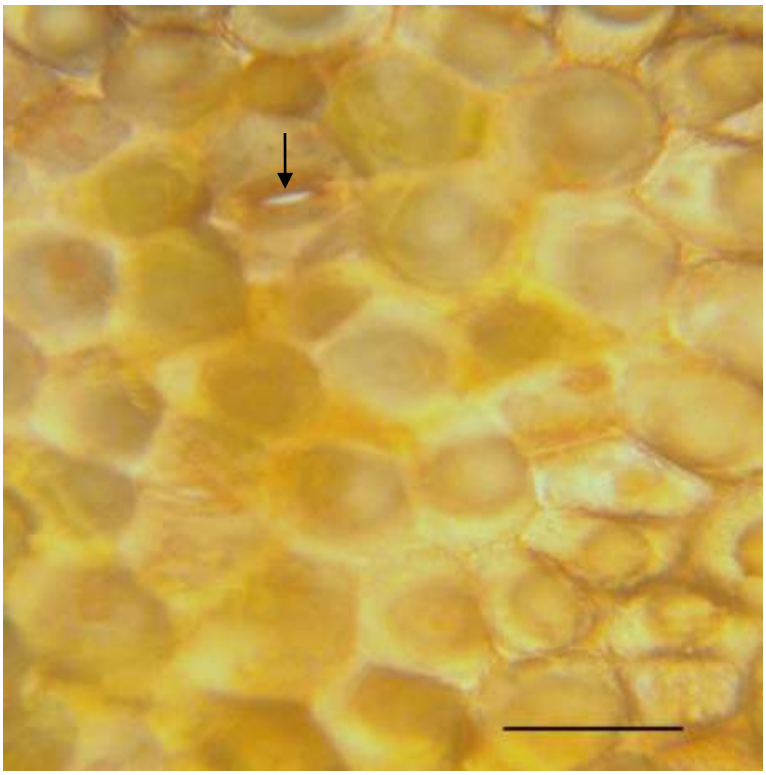

Plate 29: Epidermal features of abaxial leaf surface of 'nwine' X100.

Scale bar = 24 µm

The stomata were present in both adaxial and abaxial surfaces of ‘ogeriobosi’. The outline of the stomata was oval while the stomatal pore was narrowly elliptic. Both adaxial and abaxial leaf surfaces possessed anomocytic stomatal type (Plates 30 and 31).

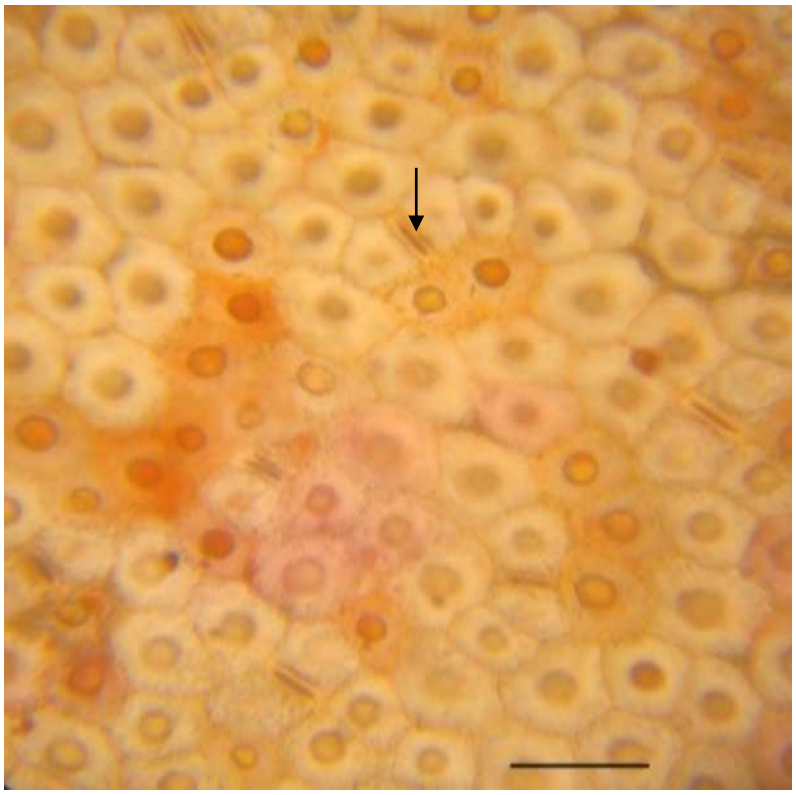

Plate 30: Epidermal features of adaxial leaf surface of ‘ogeriobosi’ X100.

Scale bar = 24 µm

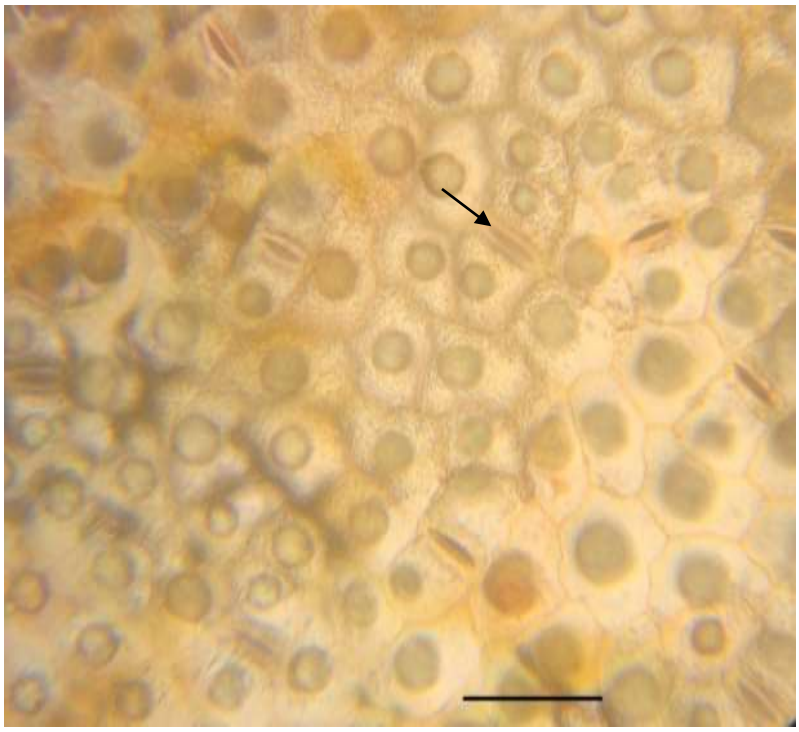

Plate 31: Epidermal features of abaxial leaf surface of 'ogeriobosi' X100.

Scale bar = 24 µm

Transverse Sections of the Petiole

In the transverse sections of the petiole, the pores/vessels were of various sizes and not occluded with tyloses (Plates 32-36). The arrangements of the pores in all the varieties were exclusively solitary (Plates 32-35) with the exception of pores in ‘ogeriobosi’ which were in clusters (Plate 36). The pores/vessels of *C. esculenta* var. *antiquorum*, ‘nwine’ and ‘ogeriobosi’ were round (Plates 32, 35 and 36), while those of *C. esculenta* var. *esculenta* and ‘kochuo’ were oval (Plates 33 and 34) in shape. The pores varied in size (Plates 32-36), largest vessels were discovered in ‘kochuo’ (Plate 34), whereas the smallest were found in ‘ogeriobosi’ (Plate 36).

The arrangement of the pores/vessels of *Colocasia esculenta* var. *antiquorum* was exclusively solitary (Plate 32). The length of vessels varied from 480 µm – 132 µm while the width was from 360 µm – 120 µm (Plate 32 and Table 2).

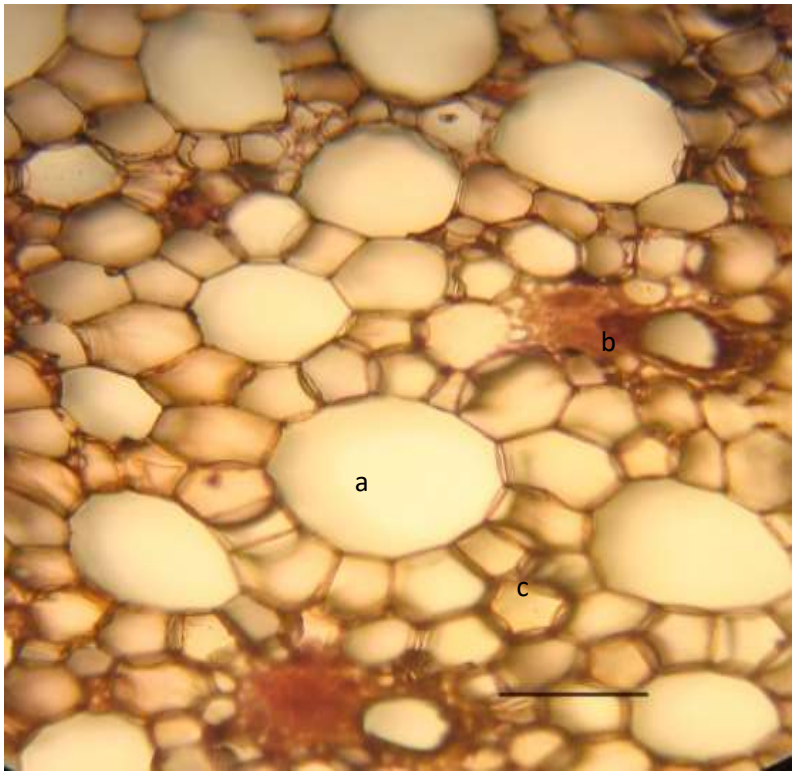

Plate 32: Transverse section of petiole of *Colocasia esculenta* var. *antiquorum* X400.

Scale bar = 16 µm

a=pore/vessel

b=vascular bundle

c=parenchyma

The arrangement of the pores/vessels of *Colocasia esculenta* var. *esculenta* was exclusively solitary (Plate 33). The range of the length of vessels was from 552 µm – 168 µm while the width was from 288 – 144 µm (Plate 33 and Table 2).

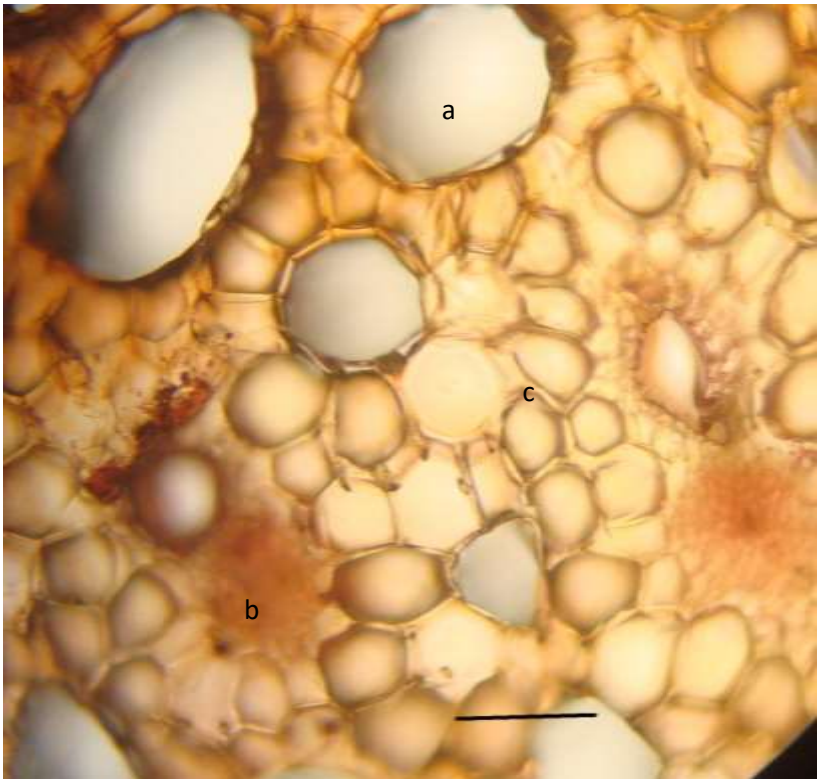

Plate 33: Transverse section of petiole of *Colocasia esculenta* var. *esculenta* X400.

Scale bar = 16 µm

a=pore/vessel

b=vascular bundles

c=parenchyma

The arrangement of the pores/vessels of ‘kochuo’ was exclusively solitary. The range of the length of vessels was from 504 – 144 µm while the width was from 456 – 120 µm (Plate 34 and Table 2). The largest vessels were discovered in ‘kochuo’ (Plate 34).

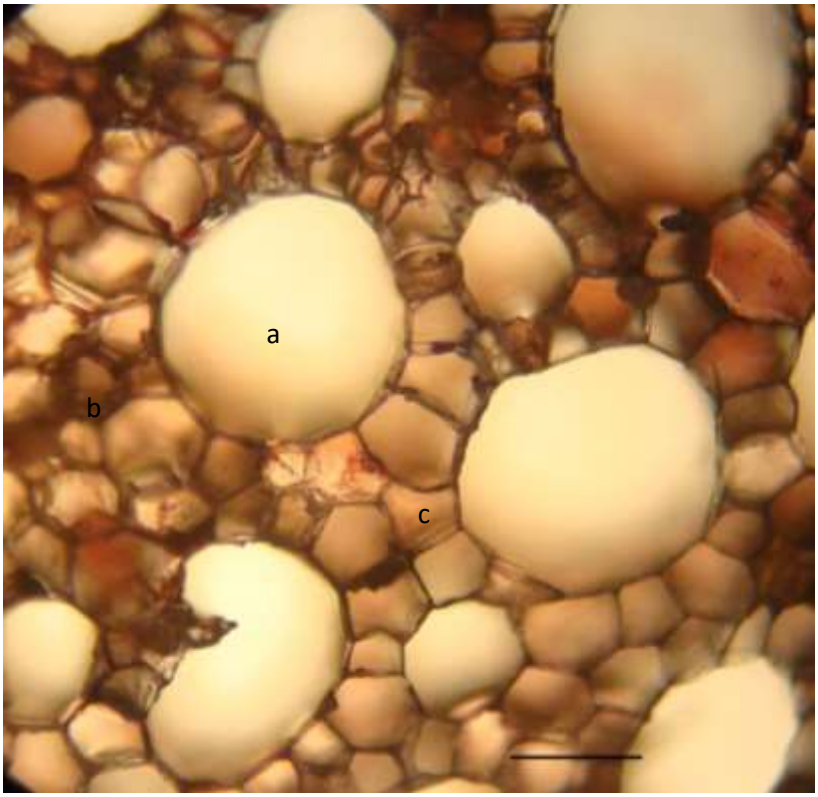

Plate 34: Transverse section of petiole of ‘kochuo’ X400. Scale bar = 16 µm

a=pore/vessel

b=parenchyma

The arrangement of the pores/vessels of ‘nwine’ was exclusively solitary (Plate 35). The length of vessels varied from 492 – 156 µm while the width was from 396 – 132 µm (Plate 35 and Table 2).

Plate 35: Transverse section of petiole of ‘nwine’ X400. Scale bar = 16 µm

a=pore/vessel

b= vascular bundle

c= parenchyma

The arrangement of the pores/vessels of ‘ogeriobosi’was in clusters. The pores found in ‘ogeriobosi’ were small in size (Plate 36). The length of vessels varied from 216 – 120 µm while the width was from 168 – 96 µm (Plate 36 and Table 2).

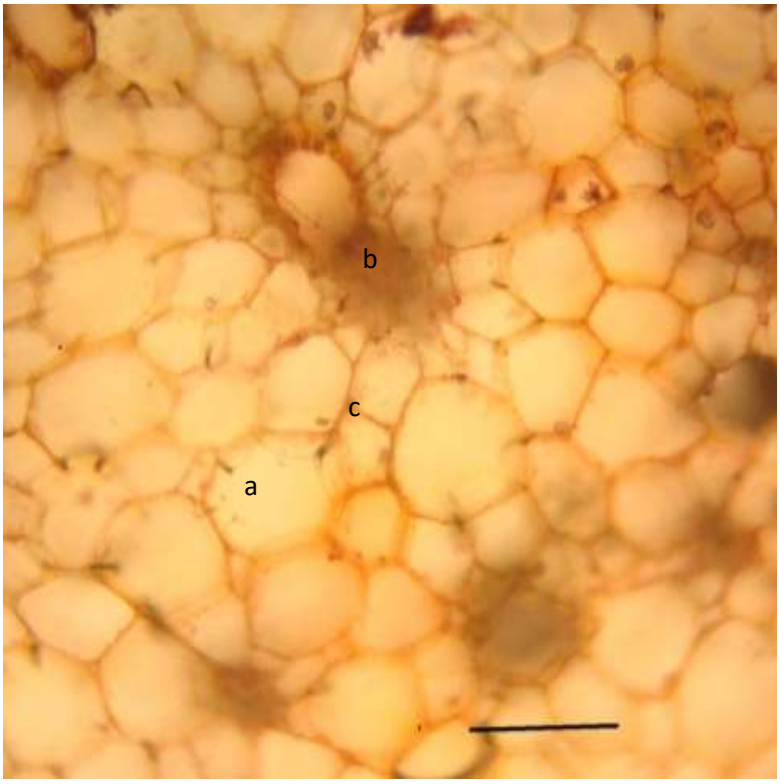

Plate 36: Transverse section of petiole of ‘ogeriobosi’ X400. Scale bar = 16 µm

a= pore/vessel

b=vascular bundle

c=parenchyma

The length of vessels of *Colocasia esculenta* var. *antiquorum* varied from 480 µm – 132 µm while the width was from 360 µm – 120 µm (Table 2). The range of the length of vessels of *Colocasia esculenta* var. *esculenta* at 552 µm – 168 µm was highest whereas that of 'ogeriobosi' at 216 µm – 120 µm was the lowest. The width of vessels of 'ogeriobosi' at 168 µm – 96 µm was also the smallest range (Table 2).

Table 2: Size (µm) of vessels of petioles (T/S) of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and Ogeriobosi'

S/N	*Colocasia esculenta* varieties	Length	Width
1	*Colocasia esculenta* var. *antiquorum*	132 – 480	120 – 360
2	*Colocasia esculenta* var. *esculenta*	168 – 552	144 – 288
3	'Kochuo'	144 – 504	120 – 456
4	'Nwine'	156 – 492	132 – 396
5	'Ogeriobosi'	120 – 216	96 – 168

Transverse Sections of Root

In transverse sections of the root, the centre of roots of all the varieties was occupied by large and well developed pith (Plates 37- 41). The roots of *Colocasia esculenta* var. *antiquorum*, 'kochuo' and 'ogeriobosi' had air-chambered cortex (Plates 37, 39 and 41).

The centre of root of *Colocasia esculenta* var. *antiquorum* was occupied by large and well developed pith. It had air chambered cortex (Plate 37).

Plate 37: Transverse section of root of *Colocasia esculenta* var. *antiquorum* X100.

Scale bar = 24 µm

a=pith

b=metaxylem

c=protoxylem

d=pericycle

e= air chamber

f=cortex

The centre of root of *Colocasia esculenta* var. *esculenta* was occupied by large and well developed pith (Plate 38).

Plate 38: Transverse section of root of *Colocasia esculenta* var. *esculenta* X100.

Scale bar = 24 µm

a=pith

b=metaxylem

c =protoxylem

d=pericycle

e=cortex

The centre of root of ‘kochuo’ was occupied by large and well developed pith. It had air chambered cortex (Plate 39).

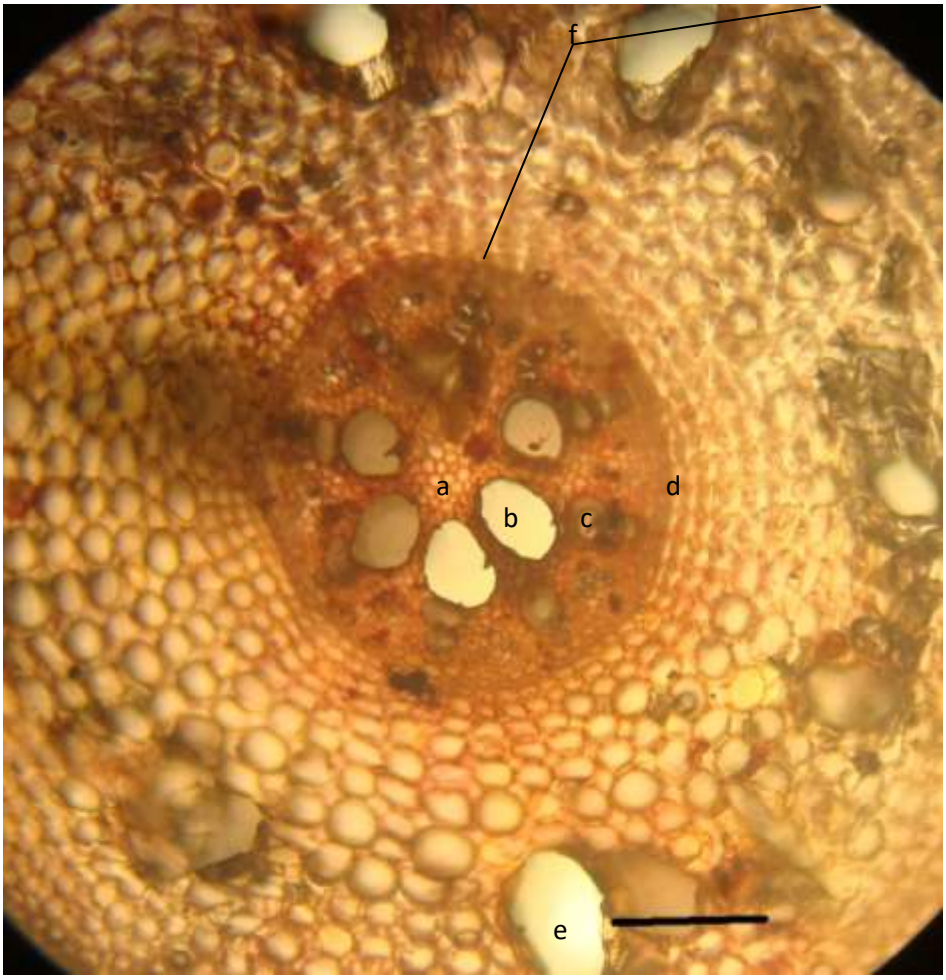

Plate 39: Transverse section of root of ‘kochuo’ X100. Scale bar = 24 µm

a=pith

b= metaxylem

c=protoxylem

d=pericycle

e=air chamber

f= cortex

The centre of root of ‘nwine’ was occupied by large and well developed pith (Plate 40).

Plate 40: Transverse section of root of ‘nwine’ X100. Scale bar = 24 µm

a=pith

b=metaxylem

c=protoxylem

d=pericycle

e=cortex

The centre of root of ‘ogeriobosi’ was occupied by large and well developed pith. It had air chambered cortex (Plate 41).

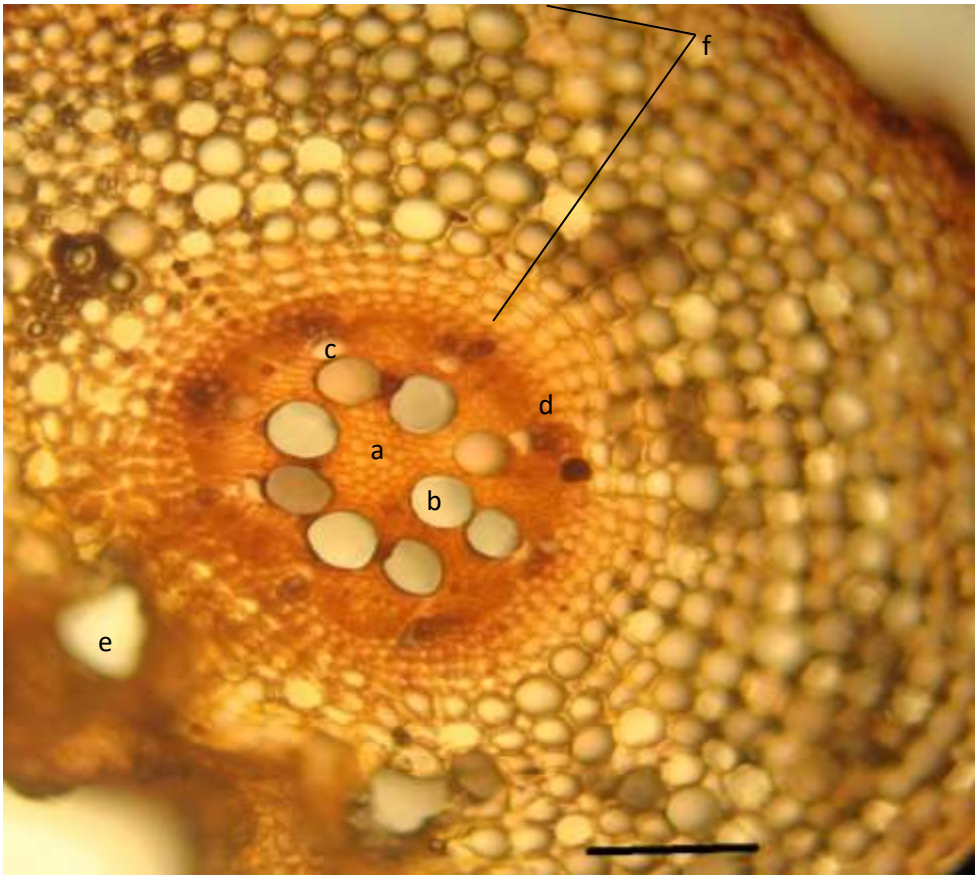

Plate 41: Transverse section of root of ‘ogeriobosi’ X100. Scale bar = 24 µm

a=pith

b=metaxylem

c=protoxylem

d=pericycle

e=air chamber

f=cortex

Histochemical Characters

Crystal sands were only seen in petioles of *Colocasia esculenta* var *esculenta* and ‘nwine’ (Plates 45 and 51) but were not seen in *Colocasia esculenta* var. *antiquorum*, ‘kochuo’ and ‘ogeriobosi’. Druses and rosettes were only present in petioles of ‘kochuo’ (Plates 48 and 49). A bar of styloid was only found in petiole of *C. esculenta* var. *antiquorum* (Plate 42b). Raphides were present in the petioles of all the varieties with the exception of ‘nwine’ (Plates 42a, 44, 47 and 53). Prismatic oxalate crystals (rhombohedral) were found in all the varieties (Plates 42c, 43, 46, 50, 52 and 54).

Raphides were present in the petiole of *Colocasia esculenta* var. *antiquorum* (Plates 42a). A bar of styloid was only found in petiole of *C. esculenta* var. *antiquorum* (Plate 42b). Prismatic oxalate crystal (rhombohedral) was also found (Plate 42c).

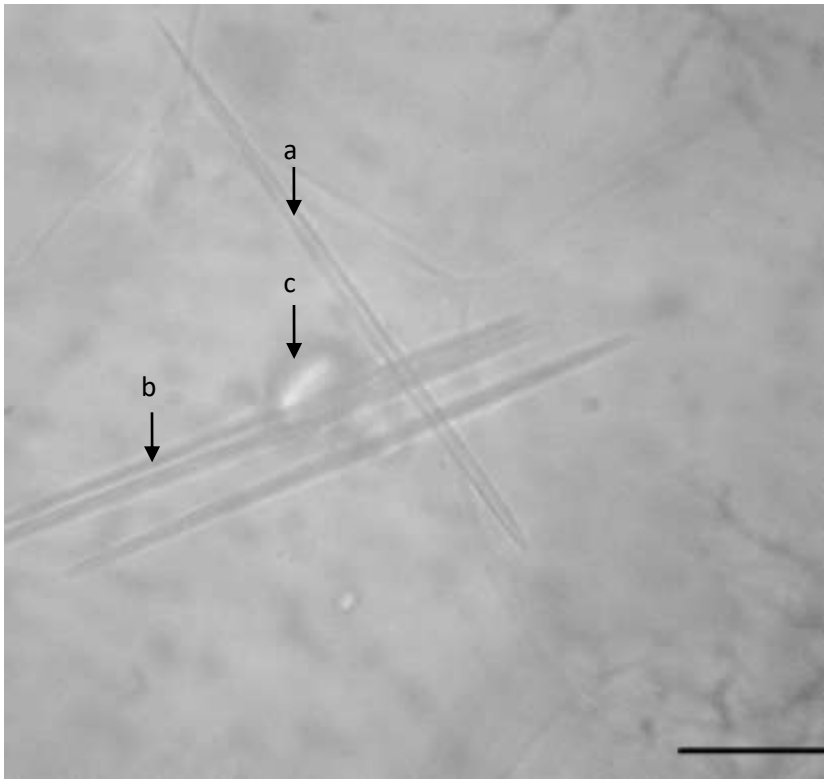

Plate 42: Forms of calcium oxalate crystals in petiole of *Colocasia esculenta* var. *antiquorum* X1000.

(a). Raphides

(b). Styloid

(c). Rhombohedral

Scale bar = 8 µm

Prismatic oxalate crystals (rhombohedral) present in petiole of *Colocasia esculenta* var. *antiquorum* (Plate 43).

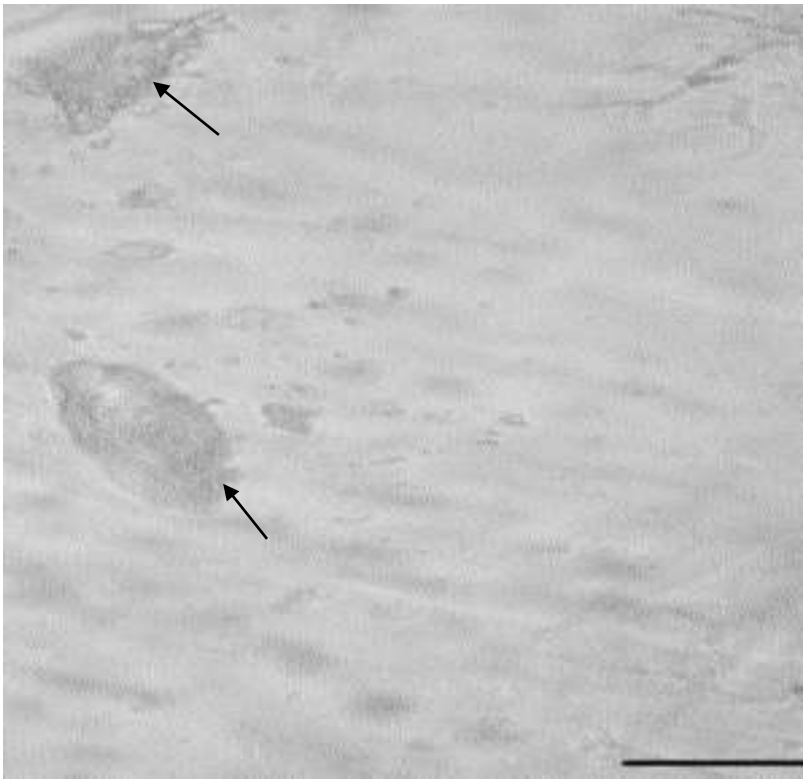

Plate 43: Prisms in the petiole of *Colocasia esculenta* var. *antiquorum* X1000.

Scale bar = 8 µm

Raphides were present in the petiole of *Colocasia esculenta* var. *esculenta* (Plate 44)

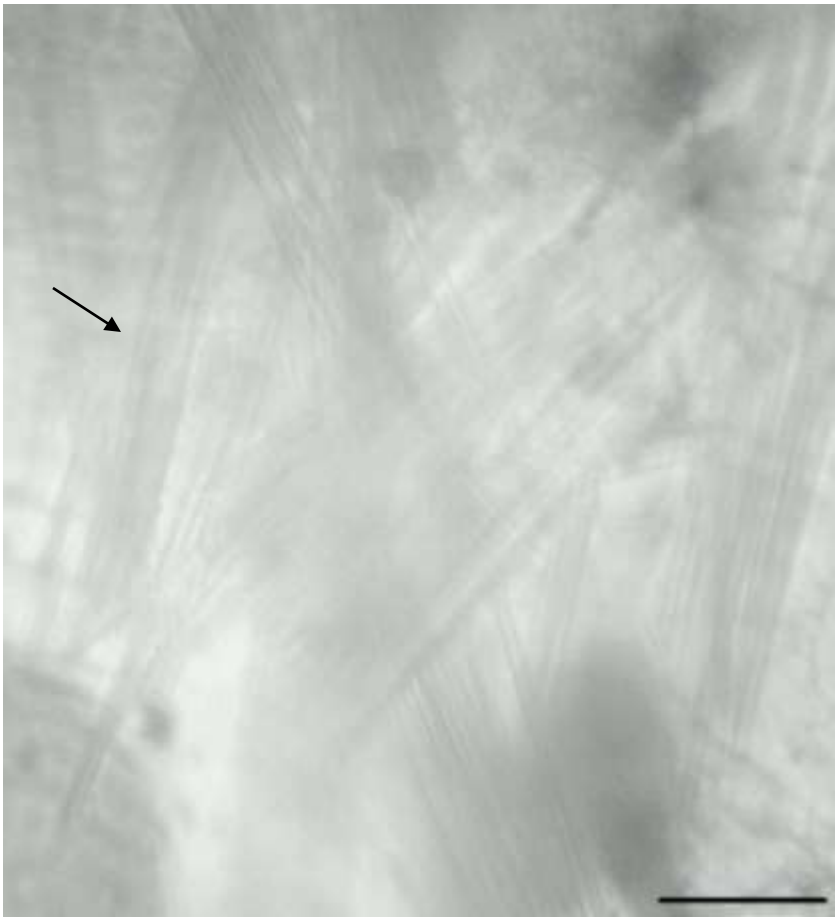

Plate 44: Raphides scattered in the petiole of *Colocasia esculenta* var. *esculenta*

X1000.

Scale bar = 8 µm

Crystal sands were seen in petiole of *Colocasia esculenta* var. *esculenta* (Plate 45).

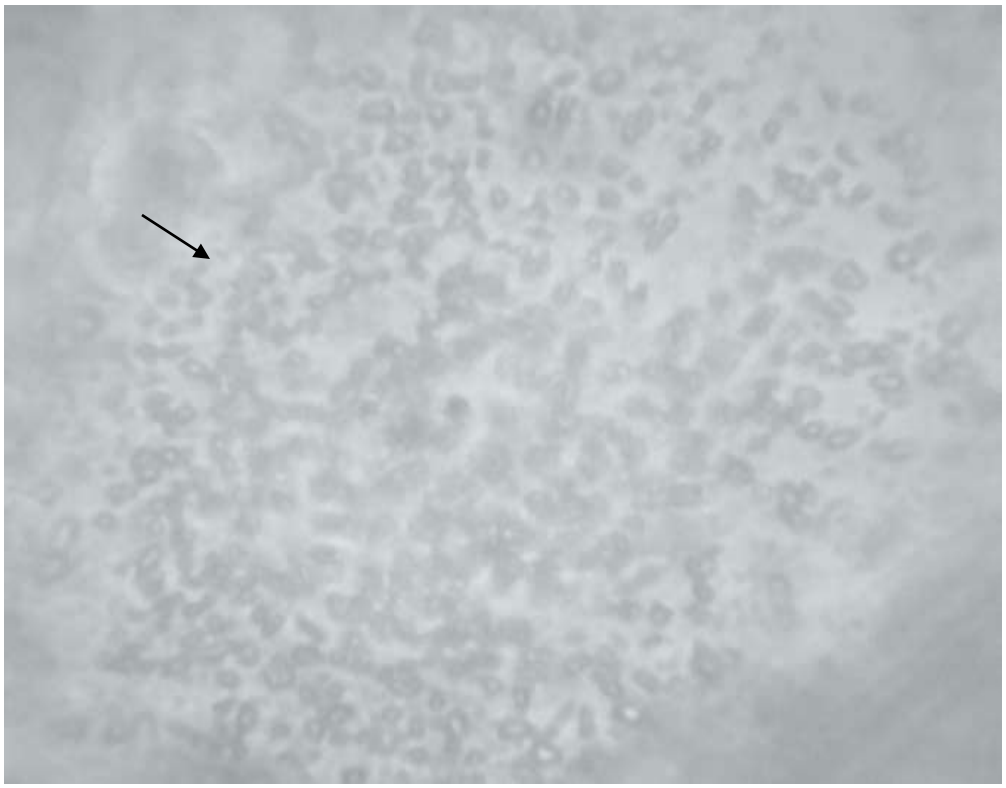

Plate 45: Crystal sands in petiole of *Colocasia esculenta* var. *esculenta* X1000.

Scale bar = 8 µm

Prismatic oxalate crystal (rhombohedral) found in petiole of *Colocasia esculenta* var. *esculenta* (Plate 46).

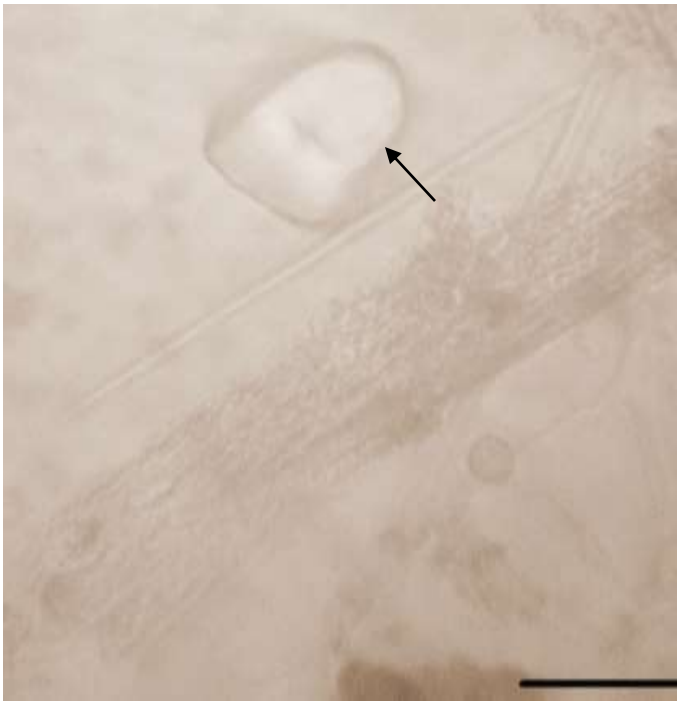

Plate 46: Prism in petiole of *Colocasia esculenta* var. *esculenta* X1000.

Scale bar = 8 µm

Raphide bundle present in petiole of ‘kochuo’ (Plate 47).

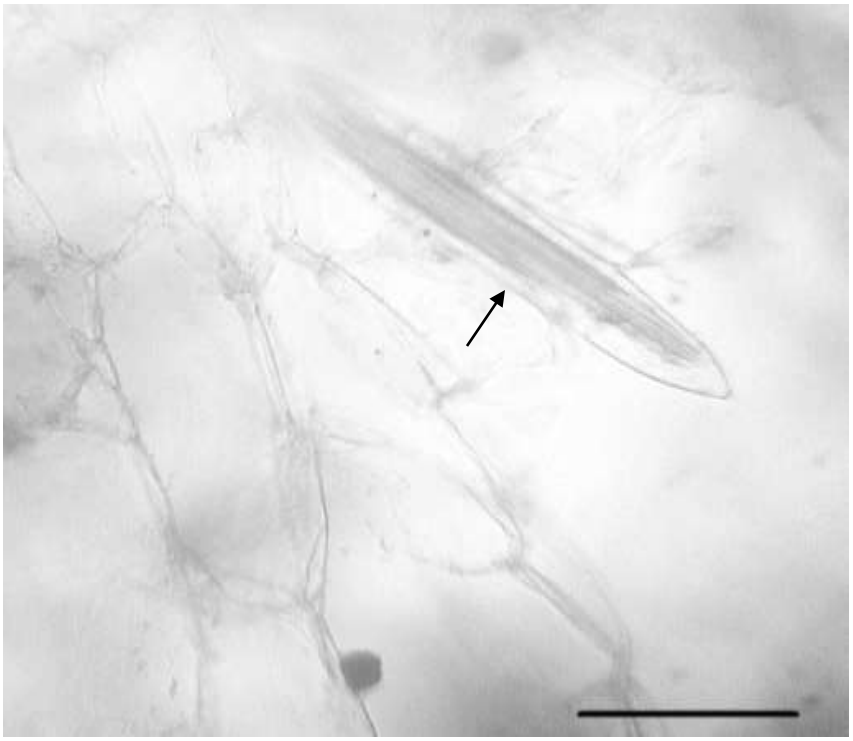

Plate 47: Raphide bundle in the petiole of ‘kochuo’ X1000.

Scale bar = 8 µm

Druse present in petiole of ‘kochuo’ (Plate 48).

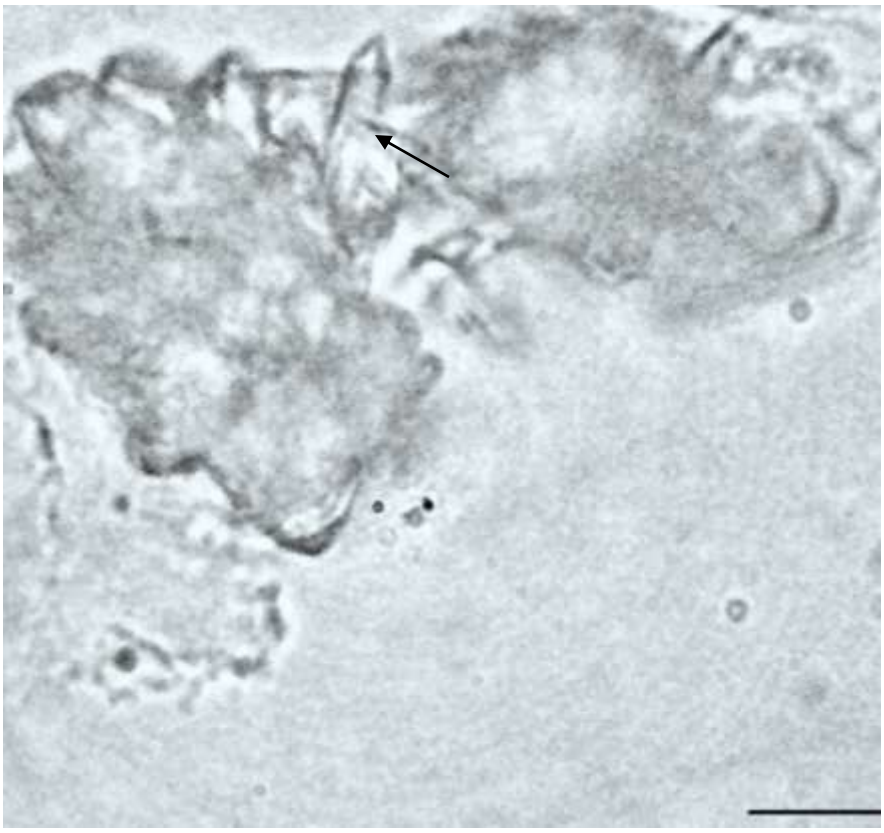

Plate 48: Druse in the petiole of ‘kochuo’ X1000. Scale bar = 8 µm

Rosette present in petiole of ‘kochuo’ (Plate 49).

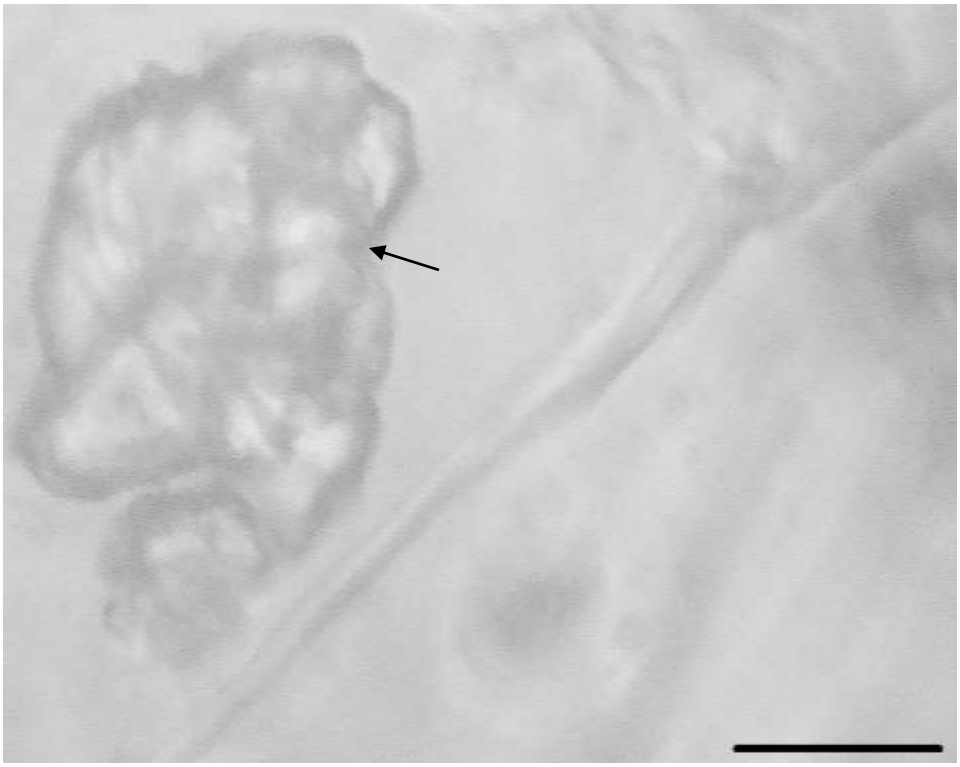

Plate 49: Rosette in the petiole of ‘kochuo’ X1000. Scale bar = 8 µm

Prismatic oxalate crystals (rhombohedral) present in petiole of ‘kochuo’ (Plate 50).

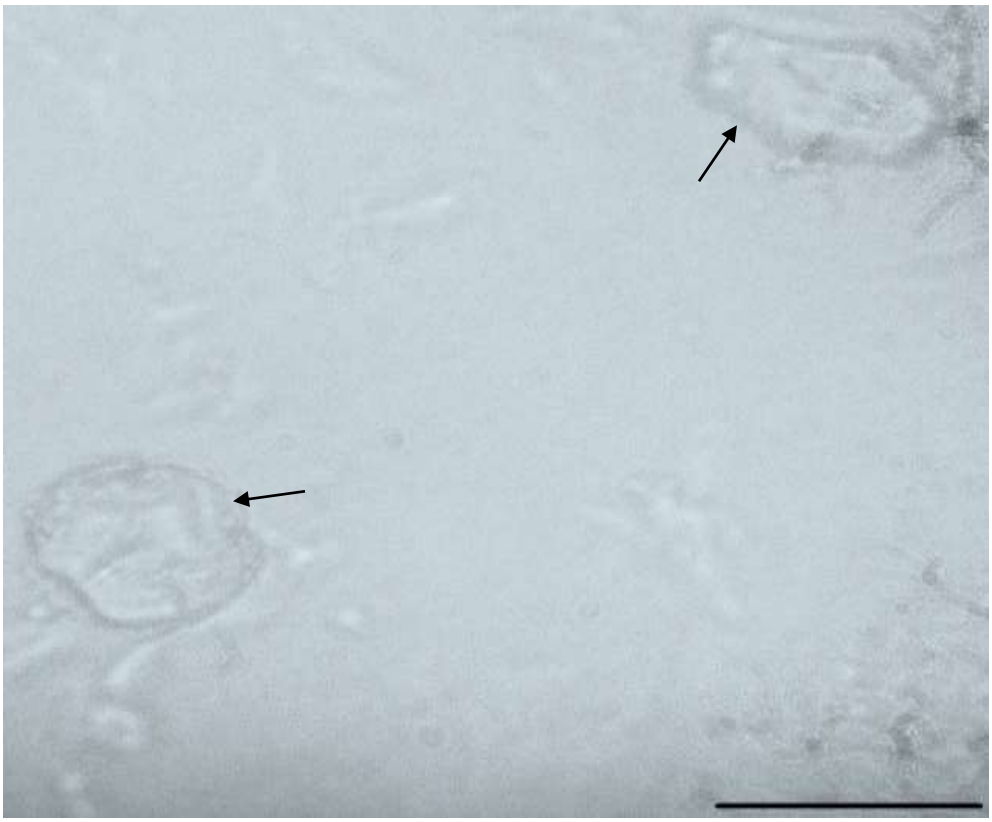

Plate 50: Prisms in the petiole of ‘kochuo’ X1000. Scale bar = 8 µm

Crystal sands were seen in petiole of ‘nwine’ (Plate 51).

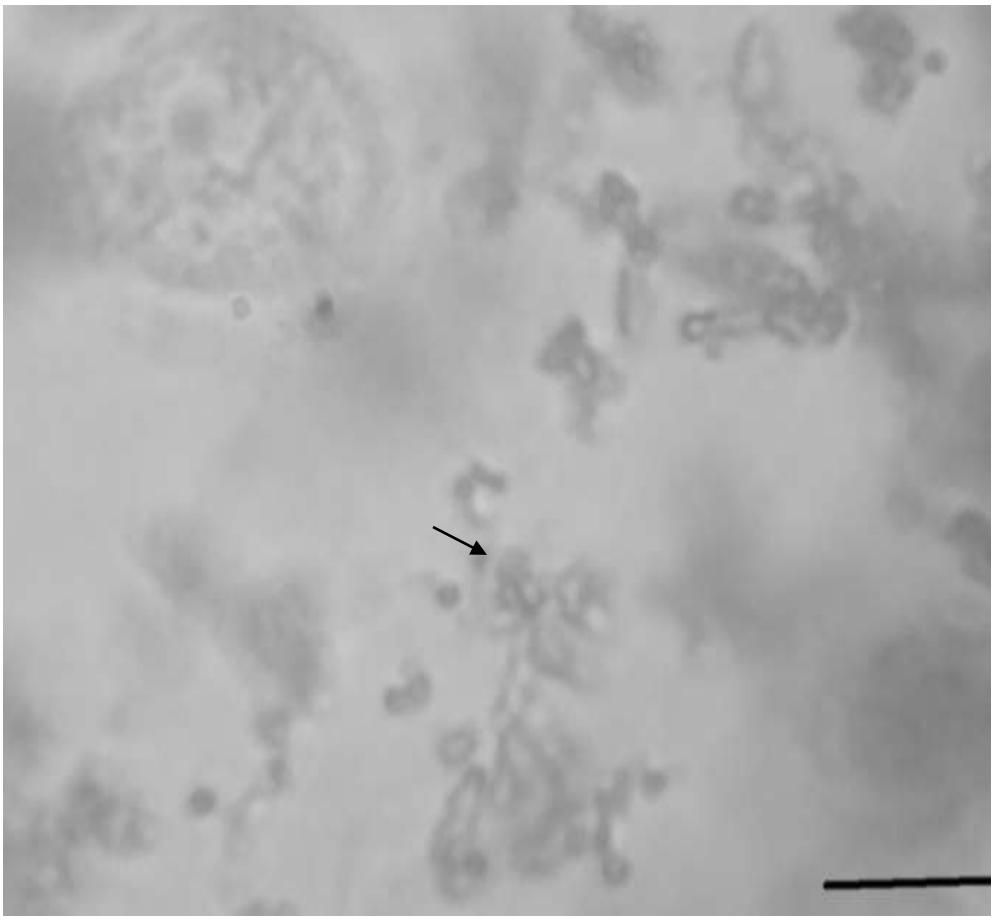

Plate: 51: Crystal sands in petiole of ‘nwine’ X1000.

Scale bar = 8 µm

Prismatic oxalate crystal (rhombohedral) found in petiole of ‘nwine’ (Plate 52).

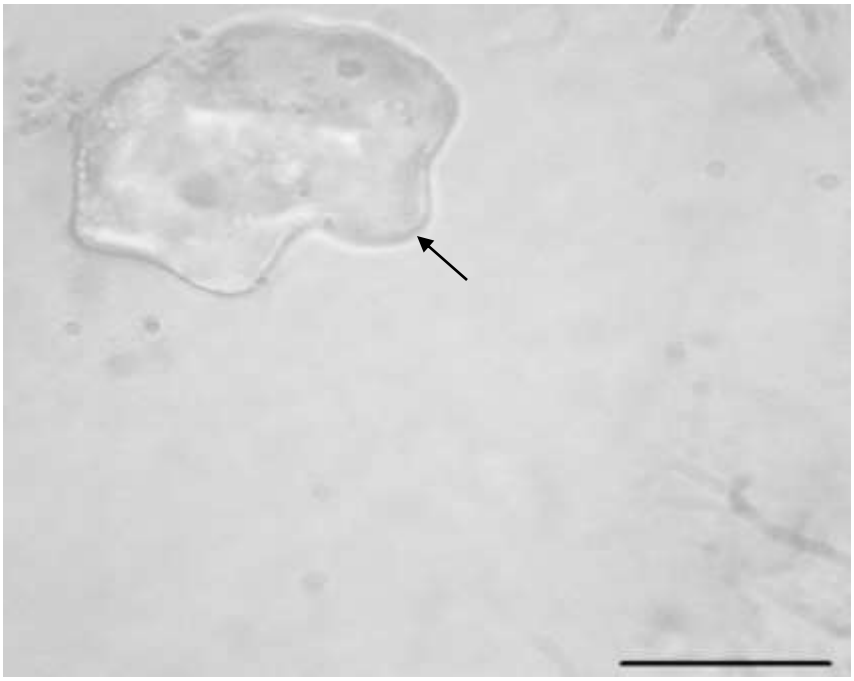

Plate 52: Prism in petiole of ‘nwine’ X1000. Scale bar = 8 µm

Raphides were present in the petiole of ‘ogeriobosi’ (Plate 53).

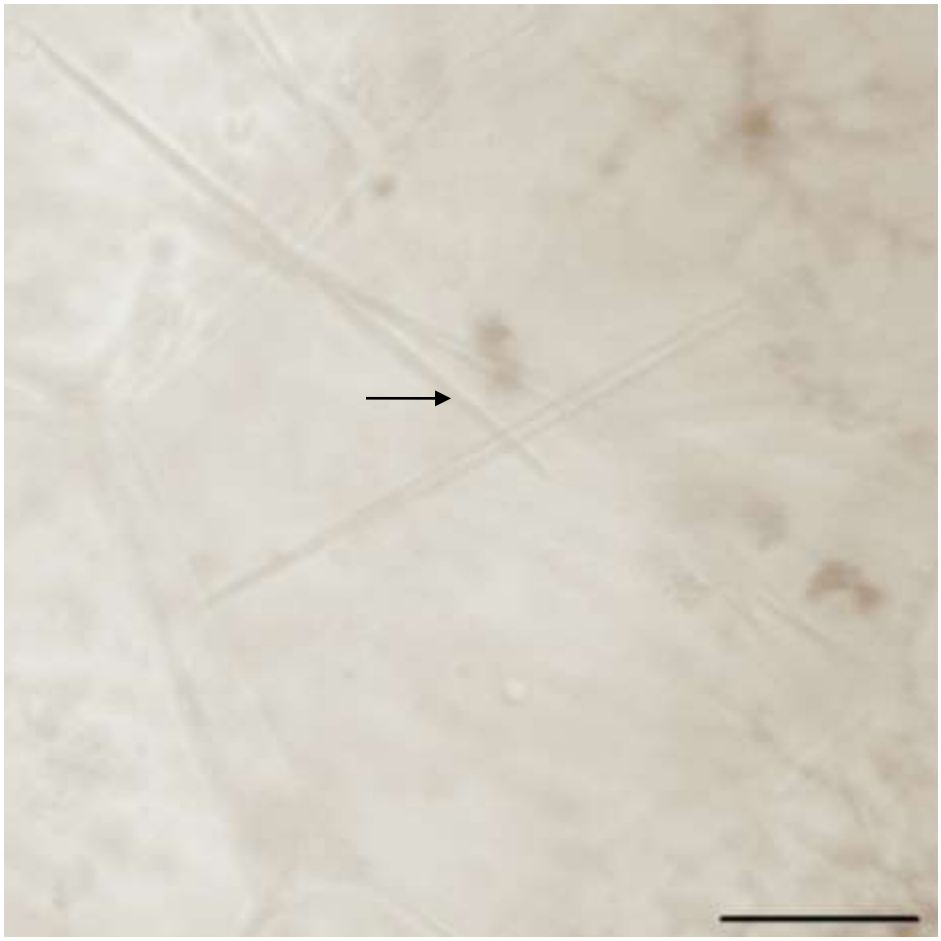

Plate 53: Raphides in petiole of ‘ogeriobosi’ X1000. Scale bar = 8 µm

Prismatic oxalate crystal (rhombohedral) found in petiole of ‘ogeriobosi’ (Plate 54).

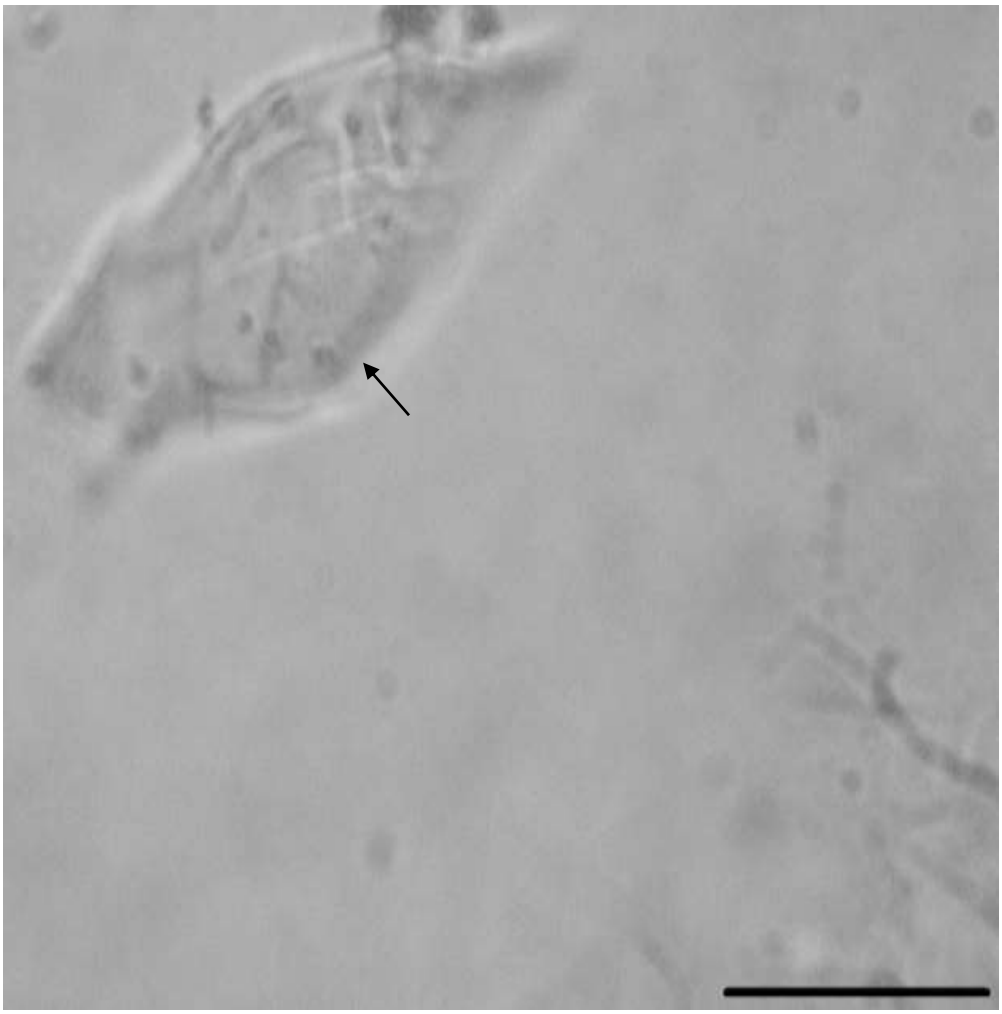

Plate 54: Prism in the petiole of ‘ogeriobosi’ X1000. Scale bar = 8 µm

Cytological Characters

The chromosomes were visible in *Colocasia esculenta* var. *antiquorum* at 1.00 pm. Chromosome count of 2n=24 was observed in *Colocasia esculenta* var. *antiquorum* (Plate 55).

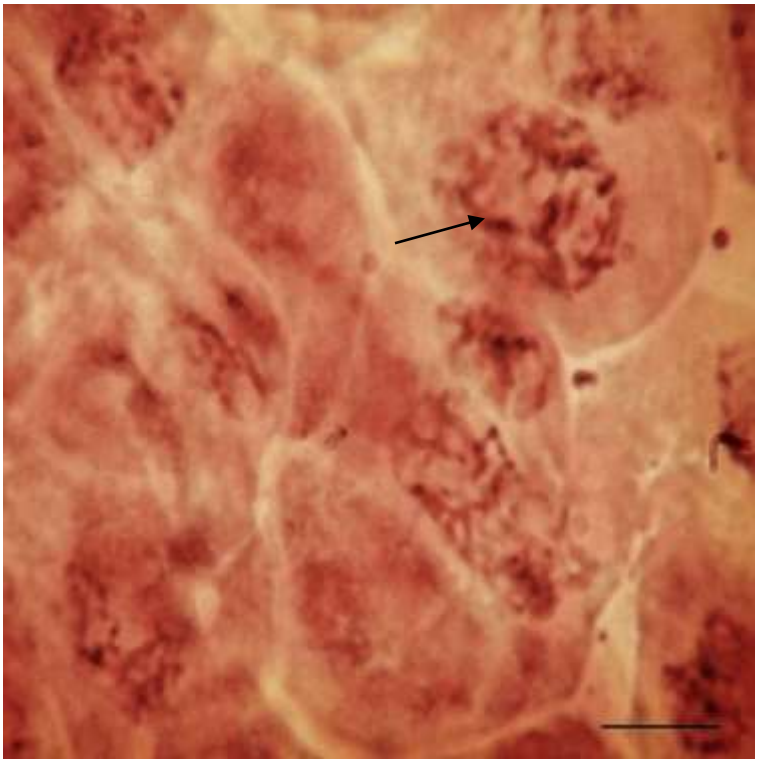

Plate 55: Chromosomes of *Colocasia esculenta* var. *antiquorum* X1000.

Scale bar = 8 µm

The chromosomes were visible in *Colocasia esculenta* var. *esculenta* at 3.00 pm. Chromosome count of 2n=24 was observed in *Colocasia esculenta* var. *esculenta* (Plate 56).

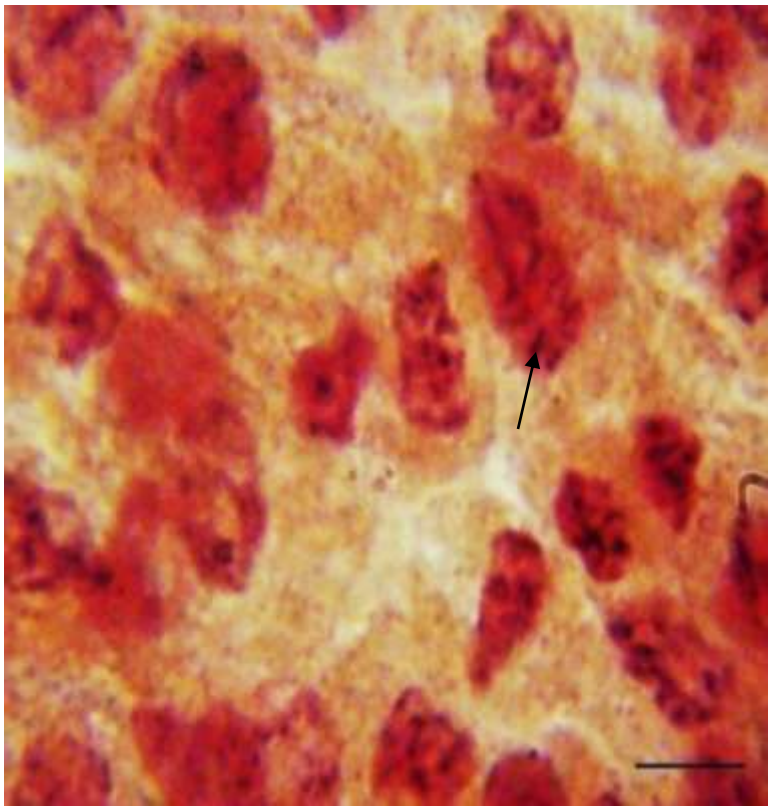

Plate 56: Chromosomes of *Colocasia esculenta* var. *esculenta* X1000.

Scale bar = 8 µm

The chromosomes were visible in ‘nwine’ at 12.00 noon. Chromosome count of 2n=24 was observed in ‘nwine’ (Plate 57).

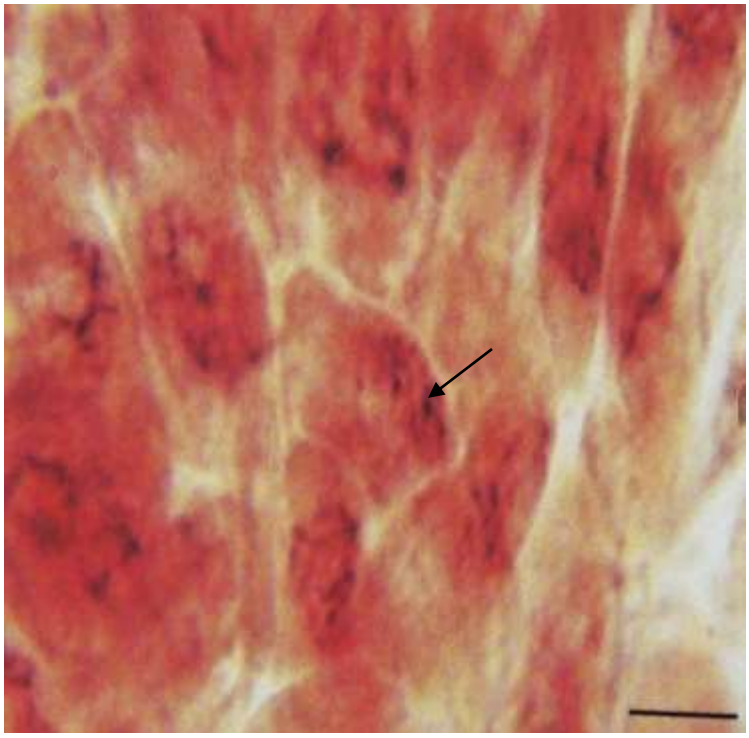

Plate 57: Chromosomes of ‘kochuo’ X1000. Scale bar = 8 µm

The chromosomes were visible in ‘kochuo’ at 2.00 pm. Chromosome count of 2n=24 were observed in ‘nwine’ (Plate 58).

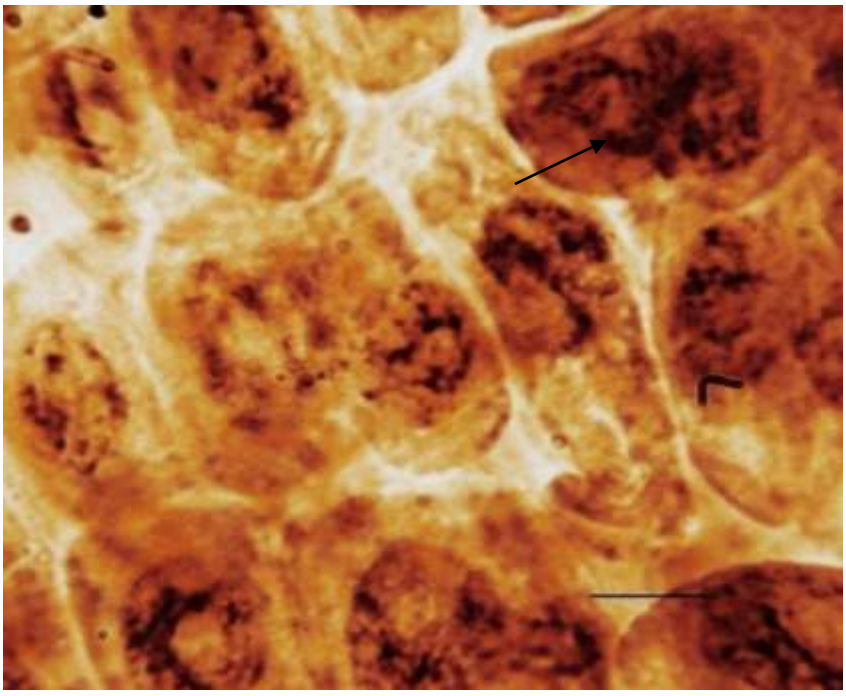

Plate 58: Chromosomes of ‘nwine’ X1000. Scale bar = 8 µm

The chromosomes were visible in ‘ogeriobosi’ at 3.00 pm. Chromosome count of 2n=28 was found in ‘ogeriobosi’ (Plate 59).

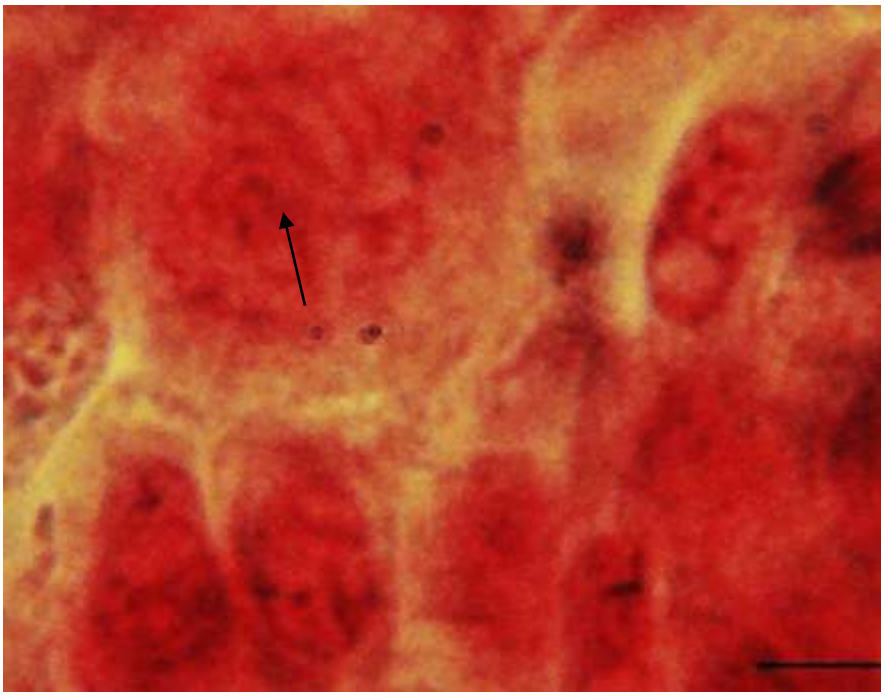

Plate 59: Chromosomes of ‘ogeriobosi’ X1000. Scale bar = 8 µm

Phytochemical Compositions

Significant difference existed among the tannin contents of all the varieties (Table 3). The highest level was found in 'ogeriobosi' at 0.65±0.03% while the least was present in *Colocasia esculenta* var. *antiquorum* at 0.41±0.01%. There was no significant difference among the flavonoid levels of *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'nwine'. The greatest flavonoid content at 0.78±0.01% was found in 'ogeriobosi' and least in *Colocasia esculenta* var. *antiquorum* at 0.55±0.02%. There was no significant difference between the alkaloid content of *Colocasia esculenta* var. *esculenta* and 'kochuo'. The highest concentration of alkaloid at 0.94±0.02% was present in 'ogeriobosi', whereas the least was present in *Colocasia esculenta* var. *antiquorum* at 0.70±0.05%. There was no significant difference between the saponin level of *Colocasia esculenta* var. *esculenta* and 'kochuo'. The greatest value of saponin was present in 'ogeriobosi' at 1.41±0.01%, while the least was present in *Colocasia esculenta* var. *antiquorum* at 1.25±0.02%. Highest concentration of hydrogen cyanide was found in *Colocasia esculenta* var. *esculenta* at 5.81±0.01 mg/kg, whereas the least was found in *Colocasia esculenta* var. *antiquorum* at 4.51±0.01 mg/kg. There was no significant difference between the hydrogen cyanide content of 'nwine' and 'ogeriobosi'. There was no significant difference between the oxalate concentrations of *Colocasia esculenta* var. *esculenta* at 0.54±0.02% and 'nwine' at 0.57±0.04%. Highest concentration of oxalate was present in 'ogeriobosi' at 0.77±0.03% whereas the least concentration was present in *Colocasia esculenta* var. *antiquorum* at 0.47±0.01%. There was no significant difference among the phytate content of 'kochuo', 'nwine' and 'ogeriobosi'. The greatest level of phytate was found in *Colocasia esculenta* var. *esculenta* at 0.59±0.04% while the least was found in *Colocasia esculenta* var. *antiquorum* at 0.38±0.02% (Table 3).

Table 3: Mean phytochemical composition of leaf of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (%)						
Varieties	**Tannin**	**Flavonoid**	**Alkaloid**	**Saponin**	**HCN (mg/kg)**	**Oxalate**	**Phytate**
Antiq	0.41±0.01[a]	0.55±0.02[a]	0.70±0.05[a]	1.25±0.02[a]	4.51±0.01[a]	0.47±0.01[a]	0.38±0.02[a]
Esc	0.52±0.01[b]	0.71±0.02[b]	0.91±0.02[b]	1.35±0.02[b]	5.81±0.01[b]	0.54±0.02[b]	0.59±0.04[b]
'Kochuo'	0.61±0.03[c]	0.71±0.04[b]	0.90±0.03[b]	1.35±0.03[b]	5.51±0.09[c]	0.66±0.02[c]	0.48±0.01[c]
'Nwine'	0.57±0.01[d]	0.73±0.01[b]	0.86±0.01[c]	1.31±0.01[c]	5.33±0.06[d]	0.57±0.04[b]	0.51±0.01[c]
'Ogeriobosi'	0.65±0.03[e]	0.78±0.01[c]	0.94±0.02[b]	1.41±0.01[d]	5.28±0.03[d]	0.77±0.03[d]	0.50±0.02[c]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

There was no significant difference between the tannin content of root of *Colocasia esculenta* var. *esculenta* and 'nwine' at $p>0.05$ (Table 4). High significant difference did not exist between them and tannin level of 'ogeriobosi' at $p>0.01$. The least tannin content was present in the root of *Colocasia esculenta* var. *antiquorum.* There was no significant difference among the flavonoid values of *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *esculenta*, 'kochuo' and 'ogeriobosi' at $p>0.05$. In addition, the difference among the alkaloid and flavonoid contents of all the varieties was not highly significant at $p>0.01$. There was significant difference among the alkaloid level of all the varieties at $p>0.05$; with the highest level in root of 'ogeriobosi' and least in root of *Colocasia esculenta* var. *antiquorum*. There was no significant difference among the saponin value of *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'ogeriobosi' with the least value in *Colocasia esculenta* var. *antiquorum*. There was no significant difference between the hydrogen cyanide levels of *Colocasia esculenta* var. *esculenta,* 'kochuo' and 'ogeriobosi' at $p>0.05$. The least hydrogen cyanide was found in the root of *Colocasia esculenta* var. *antiquorum* at 3.46±0.02 mg/kg while the greatest level was detected in 'nwine'. Significant difference existed among the oxalate concentration of all the varieties at both $p<0.05$ and $p<0.01$, with the greatest value in root of *Colocasia esculenta* var. *esculenta* at 1.79±0.01% and least in *Colocasia esculenta* var. *antiquorum* at 1.53±0.02%. There was no significant difference between the phytate concentrations of root of *Colocasia esculenta* var. *antiquorum* at 0.37±0.02% and 'kochuo' at 0.38±0.02% at $p>0.05$, whereas there was significance difference between them and that of 'nwine' at $p<0.05$ but not highly significant at $p>0.01$. There was significant difference between the *Colocasia esculenta* var. *antiquorum* and 'ogeriobosi' but highly significant at $p<0.01$ (Table 4).

Table 4: Mean phytochemical composition of root of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (%)						
Varieties	**Tannin**	**Flavonoid**	**Alkaloid**	**Saponin**	**HCN (mg/kg)**	**Oxalate**	**Phytate**
Antiq	0.34±0.01^{a}	0.58±0.02^{a}	0.80±0.16^{a}	1.19±0.01^{a}	3.46±0.02^{a}	1.53±0.02^{a}	0.37±0.02^{a}
Esc	0.44±0.01^{b}	0.61±0.01^{a}	0.84±0.02^{a}	1.31±0.02^{b}	4.78±0.02^{b}	1.79±0.01^{b}	0.49±0.00^{b}
'Kochuo'	0.44±0.02^{b}	0.59±0.02^{a}	0.92±0.02^{a}	1.31±0.01^{b}	4.79±0.05^{b}	1.59±0.01^{c}	0.38±0.02^{a}
'Nwine'	0.38±0.02^{c}	0.56±0.04^{b}	0.85±0.02^{a}	1.24±0.02^{c}	4.93±0.04^{c}	1.66±0.02^{d}	0.47±0.04^{b}
'Ogeriobosi'	0.46±0.02^{b}	0.61±0.01^{a}	0.93±0.02^{a}	1.33±0.02^{b}	4.80±0.02^{b}	1.62±0.03^{e}	0.40±0.01^{a}
p>0.05	*	*	*	*	*	*	*
P>0.01	**	ns	ns	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$);**highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

There was significant difference among the tannin level of *Colocasia esculenta* var. *esculenta* and 'kochuo' at $p<0.05$ and there was no high significant difference at $p>0.01$ between 'kochuo' and 'ogeriobosi' which was the greatest value of tannin present in the petiole (Table 5). The least level of tannin was found in the petiole of *Colocasia esculenta* var. *antiquorum* at 0.29±0.01%. There was no significant difference between the flavonoid content of *Colocasia esculenta* var. *esculenta* and 'nwine' at $p>0.05$ while there was no highly significant difference between flavonoid content of 'kochuo' and 'ogeriobosi' which was the highest concentration. The least concentration of flavonoid was present in the petiole of *Colocasia esculenta* var. *antiquorum* at 0.47±0.02%. There was significant difference among the alkaloid level of all the varieties but among *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'nwine' at $p<0.01$, there was no high significant difference. The highest level of alkaloid was present in the 'ogeriobosi' at 0.84±0.02%, whereas the least was in *Colocasia esculenta* var. *antiquorum* at 0.66±0.05%. There was significant difference among the saponin content of all the varieties at $p<0.05$ but high significant difference did not exist among them at $p>0.01$. There was no significant difference among the hydrogen cyanide concentration of *Colocasia esculenta* var. *antiquorum,* 'kochuo' 'nwine' and 'ogeriobosi'. The greatest level of hydrogen cyanide was present in *Colocasia esculenta* var. *esculenta* at 2.34±0.01 mg/kg. There was also no significant difference among the oxalate concentration of *Colocasia esculenta* var. *antiquorum,* 'kochuo' 'nwine' and 'ogeriobosi' which happened to be the highest level of oxalate. The least value of oxalate was found in *Colocasia esculenta* var. *esculenta* at 0.53±0.01%. There was also no significant difference between the phytate of *Colocasia esculenta* var. *antiquorum* and 'kochuo' as well as between that of 'nwine' and 'ogeriobosi' at $p>0.05$. The highest value of phytate was present in *Colocasia esculenta* var. *esculenta* at 0.48±0.02% (Table 5).

Table 5: Mean phytochemical composition of petiole of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (%)						
Varieties	**Tannin**	**Flavonoid**	**Alkaloid**	**Saponin**	**HCN (mg/kg)**	**Oxalate**	**Phytate**
Antiq	0.29±0.01[a]	0.47±0.02[a]	0.66±0.05[a]	1.06±0.01[a]	1.74±0.01[a]	0.55±0.05[a]	0.32±0.05[a]
Esc	0.36±0.01[b]	0.53±0.01[b]	0.76±0.02[b]	1.17±0.01[a]	2.34±0.01[b]	0.53±0.01[b]	0.48±0.02[b]
'Kochuo'	0.38±0.03[b]	0.60±0.06[c]	0.77±0.02[b]	1.18±0.02[a]	1.93±0.02[a]	0.65±0.02[c]	0.35±0.01[a]
'Nwine'	0.33±0.02[c]	0.53±0.04[b]	0.73±0.01[b]	1.10±0.02[a]	1.92±0.03[a]	0.62±0.01[c]	0.40±0.03[c]
'Ogeriobosi'	0.38±0.01[b]	0.57±0.02[c]	0.84±0.02[c]	0.86±0.57[a]	1.71±0.28[a]	0.62±0.02[c]	0.40±0.02[c]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	ns	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

There was no significant difference between the tannin content of ‘kochuo’ and ‘ogeriobosi’ which was the highest tannin content present in the cormels of all the varieties (Table 6). The least level of tannin was present in *Colocasia esculenta* var. *antiquorum.* The highest flavonoid content was found in the cormels of ‘ogeriobosi’ and there was no significant difference between *Colocasia esculenta* var. *antiquorum* at 0.18±0.01% and ‘nwine’ at 0.19±0.01% as well as between *Colocasia esculenta* var. *esculenta* and ‘kochuo’. There was no significant difference between the alkaloid concentration of *Colocasia esculenta* var. *esculenta* and ‘kochuo’ at $p>0.05$; and ‘ogeriobosi’ at $p<0.01$.The highest level of alkaloid was found in the cormel of ‘ogeriobosi’ at 0.19±0.01%, whereas the least was found in the cormel of *Colocasia esculenta* var. *antiquorum* at 0.09±0.01%. Highest value of saponin was present in the cormel of ‘ogeriobosi’ at 0.63±0.02%, while the least was present in the cormel of *Colocasia esculenta* var. *antiquorum* at 0.49±0.01%.There was no significant difference between the saponin content of *Colocasia esculenta* var. *esculenta* and ‘kochuo’ at $p>0.05$ and high significant difference did not exist between the two and that of ‘ogerobosi’ at $p>0.01$. There was significant difference among the hydrogen cyanide of all the varieties with greatest level in the cormel of *Colocasia esculenta* var. *esculenta* at 1.75±0.01 mg/kg and the least in ‘nwine’ at 1.28±0.03 mg/kg. There was no significant difference between the oxalate content of *Colocasia esculenta* var. *esculenta* and ‘ogeriobosi’. The greatest value of oxalate was present in the cormel of ‘kochuo’ at 2.32±0.03% and least in *Colocasia esculenta* var. *antiquorum* at 1.69±0.04%. There was no significant difference between the phytate concentration of ‘nwine’ and ‘kochuo’. The highest phytate value was found in the cormel of *Colocasia esculenta* var. *esculenta* at 0.82±0.02% and least in *Colocasia esculenta* var. *antiquorum* at 0.63±0.02% (Table 6).

Table 6: Mean phytochemical composition of cormels of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

Varieties	Composition (%)						
	Tannin	Flavonoid	Alkaloid	Saponin	HCN (mg/kg)	Oxalate	Phytate
Antiq	0.12±0.01[a]	0.18±0.01[a]	0.09±0.01[a]	0.49±0.01[a]	1.64±0.01[a]	1.69±0.04[a]	0.63±0.02[a]
Esc	0.19±0.01[b]	0.24±0.01[b]	0.18±0.02[b]	0.62±0.01[b]	1.75±0.01[b]	2.16±0.01[b]	0.82±0.02[b]
'Kochuo'	0.20±0.01[c]	0.26±0.03[b]	0.18±0.02[b]	0.62±0.05[b]	1.35±0.02[c]	2.32±0.03[c]	0.70±0.02[c]
'Nwine'	0.16±0.02[d]	0.19±0.01[a]	0.12±0.01[c]	0.56±0.04[c]	1.28±0.03[d]	2.27±0.03[d]	0.75±0.02[d]
'Ogeriobosi'	0.21±0.01[c]	0.31±0.02[c]	0.19±0.01[b]	0.63±0.02[b]	1.35±0.02[c]	2.19±0.03[b]	0.77±0.04[d]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

Proximate Constituents

There was no significant difference at both ($p>0.05$ and $p>0.01$) between the phosphorus content of the cormels of ‘nwine’ and ‘ogeriobosi’; which were the highest values (Table 7). There was significant difference among the magnesium content of all the varieties of *Colocasia esculenta* investigated. The highest value was found in the cormels of ‘kochuo’ at 68.61±0.14 mg/100g and least in that of *Colocasia esculenta* var. *esculenta* at 63.48±0.03 mg/100g. There was also significant difference among the calcium content of the cormels of all the varieties with highest concentration in ‘kochuo’ at 419.44±0.21 mg/100g and least in *Colocasia esculenta* var. *esculenta* at 200.76±0.02 mg/100g. There was no significant difference among the iron content of the cormels of *Colocasia esculenta* var. *esculenta,* ‘nwine’ and ‘ogeriobosi’ which had the least level whereas the highest level was present in the cormels of ‘kochuo’. The greatest value of sodium was found in the cormels of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* and there was no significant difference between the two. The least value was present in ‘nwine’. The highest level of potassium was found in the cormels of ‘nwine’ at 165.63±0.30 mg/100g and least in that of ‘kochuo’ at 158.71±0.01 mg/100g (Table 7).

Table 7: Mean mineral composition of cormels of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**P**	**Mg**	**Ca**	**Fe**	**Na**	**K**
Antiq	224.79±0.08[a]	63.78±0.07[a]	224.73±0.06[a]	0.80±0.03[a]	98.32±0.07[a]	162.61±0.27[a]
Esc	223.51±0.03[b]	63.48±0.03[b]	200.76±0.02[b]	0.69±0.02[b]	98.42±0.03[a]	162.80±0.08[a]
'Kochuo'	225.61±0.24[c]	68.61±0.14[c]	419.44±0.21[c]	0.85±0.04[c]	96.53±0.24[b]	158.71±0.01[b]
'Nwine'	226.75±0.08[d]	61.60±0.15[d]	228.81±0.12[d]	0.71±0.01[b]	92.75±0.05[c]	165.63±0.30[c]
'Ogeriobosi'	226.79±0.05[d]	59.52±0.09[e]	416.75±0.05[e]	0.70±0.03[b]	95.33±0.06[d]	161.76±0.03[d]
p>0.05	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

The highest level of phosphorus was present in the root of ‘kochuo’ at 163.51±1.15 mg/100g and the least was in the root of ‘ogeriobosi’ at 159.30±0.02 mg/100g (Table 8). In addition, there was no significant difference between the phosphorus contents of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta.* Root of *Colocasia esculenta* var. *antiquorum* had the greatest magnesium level the least was found in root of ‘kochuo’. There was significant difference among the calcium contents of the root all the varieties with highest value in the root of ‘ogeriobosi’ at 132.65±0.05 mg/100g and least in that of *Colocasia esculenta* var. *esculenta* at 128.20±0.04 mg/100g. There was no significant difference between the iron level present in the root of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta,* were the greatest value. The least value was found in the root of ‘nwine’ at 0.69±0.01 mg/100g. There was significant difference among the potassium contents of the root all the varieties, with highest concentration in the root of *Colocasia esculenta* var. *antiquorum* at 208.62±0.15 mg/100g whereas the least concentration was in the root of ‘kochuo’ at 148.94±0.31 mg/100g (Table 8).

Table 8: Mean mineral composition of root of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**P**	**Mg**	**Ca**	**Fe**	**Na**	**K**
Antiq	161.75±0.08[a]	103.69±0.21[a]	128.69±0.08[a]	0.84±0.02[a]	194.61±0.27[a]	208.62±0.15[a]
Esc	162.32±0.02[a]	78.46±0.04[b]	128.20±0.04[b]	0.84±0.05[a]	135.82±0.02[b]	149.57±0.06[b]
'Kochuo'	163.51±1.15[b]	75.72±0.17[c]	129.33±0.06[c]	0.79±0.05[b]	145.90±0.05[c]	148.94±0.31[c]
'Nwine'	160.62±0.16[c]	81.66±0.04[d]	131.75±0.04[d]	0.69±0.01[c]	129.66±0.04[d]	153.69±0.21[d]
'Ogeriobosi'	159.30±0.02[d]	82.77±0.03[e]	132.65±0.05[e]	0.77±0.02[b]	147.62±0.02[e]	152.83±0.02[e]
p>0.05	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

The greatest level of phosphorus was found in the leaf of *Colocasia esculenta* var. *antiquorum* at 180.41±0.07 mg/100g and the least in that of *Colocasia esculenta* var. *esculenta* at 167.43±0.03 mg/100g (Table 9). There was no significant difference between the phosphorus contents of 'kochuo' and 'ogeriobosi'. Highest value of magnesium was present in the leaf of *Colocasia esculenta* var. *antiquorum* at 103.49±0.09 mg/100g and least was found in leaf of 'kochuo' at 89.39±0.30 mg/100g. Level of calcium was greatest in the leaf of *Colocasia esculenta* var. *antiquorum* at 465.82±0.02 mg/100g whereas the least was present in the leaf of 'nwine' at 445.56±0.24 mg/100g. There was no significant difference at $p>0.05$ among the iron contents of the leaves of *Colocasia esculenta* var. *esculenta,* 'kochuo' and 'ogeriobosi', which happened to be the greatest value. There was also no significant difference at $p>0.05$ between the iron contents of the leaves of *Colocasia esculenta* var. *antiquorum* and 'ogeriobosi, which was the least level. In addition, all the varieties were not highly significant at $p>0.01$. There was significant difference among the sodium contents of leaf of all the varieties with highest concentration in 'nwine' at 192.68±0.20 mg/100g and least in *Colocasia esculenta* var. *esculenta* at 185.78±0.04 mg/100g. Potassium content of the leaf was greatest in 'nwine' at 207.62±0.02 mg/100g and least in 'ogeriobosi' at 194.74±0.11 mg/100g (Table 9).

Table 9: Mean mineral composition of leaf of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**P**	**Mg**	**Ca**	**Fe**	**Na**	**K**
Antiq	180.41±0.07[a]	103.49±0.09[a]	465.82±0.02[a]	0.95±0.01[a]	189.56±0.07[a]	196.78±0.07[a]
Esc	167.43±0.03[b]	96.34±0.10[b]	452.79±0.03[b]	1.07±0.02[b]	185.78±0.04[b]	206.80±0.01[b]
'Kochuo'	174.55±0.65[c]	89.39±0.30[c]	458.10±1.13[c]	1.24±0.33[b]	189.34±0.09[c]	198.42±0.12[c]
'Nwine'	172.58±0.19[d]	92.62±0.19[d]	445.56±0.24[d]	0.93±0.02[a]	192.68±0.20[d]	207.62±0.02[d]
'Ogeriobosi'	174.62±0.02[c]	91.45±0.05[e]	459.31±0.04[e]	1.09±0.03[b]	190.53±0.09[e]	194.74±0.11[e]
p>0.05	*	*	*	*	*	*
p>0.01	**	**	**	ns	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

Highest concentration of phosphorus was found in the petioles of ‘kochuo’ and ‘nwine’ which there was no significant different between them (Table 10). The least concentration was present in the petiole of ‘ogeriobosi’. There was significant difference among the magnesium and calcium levels of all the varieties at $p<0.05$ and highly significant existed at $p<0.01$. Greatest value of magnesium was found in petiole of *Colocasia esculenta* var. *esculenta*, whereas least value was found in *Colocasia esculenta* var. *antiquorum*. Highest level of calcium was present in the petiole of *Colocasia esculenta* var. *antiquorum* at 376.68±0.24 mg/100g, while the least level was present in ‘ogeriobosi’ at 328.49±0.08 mg/100g. There was significant difference among the iron content of *Colocasia esculenta* var. *antiquorum*, ‘kochuo’, ‘nwine’ and ‘ogeriobosi’ at $p<0.05$, meanwhile there were no highly significant difference among them at $p>0.01$. In addition, the highest concentration of iron was found in petiole of *Colocasia esculenta* var. *antiquorum* at 0.88±0.02 mg/100g. Highest value of sodium was present in *Colocasia esculenta* var. *antiquorum* whereas the least was present in the petiole of ‘nwine’. There was no significant difference between the potassium level of *Colocasia esculenta* var. *esculenta* and ‘kochuo’ as well as between the potassium level of ‘nwine’ and ‘ogeriobosi’ at $p>0.05$ (Table 10).

Table 10: Mean mineral composition of petiole of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**P**	**Mg**	**Ca**	**Fe**	**Na**	**K**
Antiq	120.47±0.02[a]	69.28±0.04[a]	376.68±0.24[a]	0.88±0.02[a]	160.74±0.02[a]	191.11±0.03[a]
Esc	158.66±0.19[b]	75.44±0.31[b]	349.25±0.31[b]	0.79±0.05[b]	153.61±0.02[b]	192.56±0.04[b]
'Kochuo'	154.72±0.03[c]	70.84±0.02[c]	348.79±0.01[c]	0.84±0.03[a]	153.62±0.12[b]	192.71±0.11[b]
'Nwine'	154.79±0.01[c]	71.84±0.05[d]	348.42±0.10[d]	0.87±0.00[a]	149.62±0.03[c]	190.60±0.01[c]
'Ogeriobosi'	118.73±0.03[d]	74.82±0.19[e]	328.49±0.08[e]	0.87±0.01[a]	154.82±0.10[d]	190.59±0.19[c]
p>0.05	*	*	*	*	*	*
p>0.01	**	**	**	ns	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significant different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

There was no significant difference between the vitamin B_1 level of *Colocasia esculenta* var. *antiquorum* and 'nwine' at $p>0.05$; which were the greatest level (Table 11). There was significant difference between them and others at $p<0.05$ and highly significant difference at $p<0.01$. There was no significant difference between the vitamin B_2 content of *Colocasia esculenta* var. *esculenta* and 'nwine' and between 'kochuo' and 'ogeriobosi' at $p>0.05$ whereas the highest level was found in the leaf of *Colocasia esculenta* var. *antiquorum* at 0.08±0.00 mg/100g. There was significant difference among the vitamin B_3 content of all the varieties at $p<0.05$ but high significant difference did not exist between *Colocasia esculenta* var. *esculenta* and 'nwine'. The highest concentration of vitamin B_3 was found in the leaf of *Colocasia esculenta* var. *antiquorum*. There was significant difference among the vitamin C content all the varieties except that of *Colocasia esculenta* var. *antiquorum* and 'kochuo' which there was no significant difference between them and were the least value. The highest level of vitamin C was present in the leaf of 'nwine'. There was significant difference among the vitamin A concentration of all the varieties with the greatest level in the leaf of 'nwine at 18.29±0.09 mg/100g and the least in 'ogeriobosi' at 14.80±0.02 mg/100g. In addition, there was significant difference among the vitamin E value of all the varieties with the highest value present at the leaf of *Colocasia esculenta* var. *antiquorum* at 11.81±0.03 mg/100g, while the least was present in the leaf of 'nwine' at 10.33±0.05 mg/100g (Table 11).

Table 11: Mean vitamin composition of leaf of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**B_1**	**B_2**	**B_3**	**C**	**A**	**E**
Antiq	0.61±0.01[a]	0.08±0.00[a]	0.98±0.00[a]	165.78±0.03[a]	17.36±0.07[a]	11.81±0.03[a]
Esc	0.56 ±0.02[b]	0.07±0.00[b]	1.86±0.03[b]	169.34±0.09[b]	16.76±0.03[b]	11.31±0.13[b]
'Kochuo'	0.47±0.03[c]	0.06±0.01[b]	1.63±0.01[c]	165.79±0.01[a]	15.45±0.03[c]	11.66±0.04[c]
'Nwine'	0.59±0.01[a]	0.07±0.00[b]	1.85±0.02[b]	167.46±0.30[c]	18.29±0.09[d]	10.33±0.05[d]
'Ogeriobosi'	0.54±0.03[d]	0.06±0.00[b]	1.71±0.01[d]	163.83±0.03[d]	14.80±0.02[e]	11.58±0.05[c]
p>0.05	*	*	*	*	*	*
p>0.01	**	ns	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$);**highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

There was no significant difference among the levels of vitamin B_1 of all the varieties, as well as vitamins B_2 and B_3 at $p>0.05$ (Table 12). Vitamin B_2 was the least vitamin present in the root of all the varieties. There was significant difference among the vitamin C content of root in all the varieties but highly significance difference did not exist between *Colocasia esculenta* var. *antiquorum* and 'kochuo' as well as between *Colocasia esculenta* var. *esculenta* and 'nwine'. Significant difference existed among the vitamin A concentration of all the varieties but those of *Colocasia esculenta* var. *antiquorum* and 'nwine' were not highly significant. The value of vitamin E present in all the varieties were significantly different at $p<0.05$, whereas high significant difference did not exist between those of *Colocasia esculenta* var. *esculenta* and 'ogeriobosi' as well as between 'kochuo' and 'nwine' (Table 12).

Table 12: Mean vitamin composition of root of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**B_1**	**B_2**	**B_3**	**C**	**A**	**E**
Antiq	0.08±0.00[a]	0.03±0.00[a]	0.08±0.00[a]	0.79±0.01[a]	0.64±0.01[a]	0.68±0.03[a]
Esc	0.09 ±0.00[a]	0.03±0.00[a]	0.10±0.00[a]	0.86±0.03[b]	0.69±0.01[b]	0.65±0.02[b]
'Kochuo'	0.07±0.00[a]	0.03±0.00[a]	0.08±0.00[a]	0.83±0.01[a]	0.70±0.01[c]	0.88±0.02[c]
'Nwine'	0.07±0.00[a]	0.03±0.00[a]	0.08±0.00[a]	0.87±0.08[b]	0.65±0.01[a]	0.90±0.02[c]
'Ogeriobosi'	0.08±0.00[a]	0.03±0.00[a]	0.08±0.00[a]	0.93±0.02[c]	0.79±0.01[d]	0.64±0.01[b]
p> 0.05	ns	ns	ns	*	*	*
p>0.01	ns	ns	ns	ns	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$);**highly significant difference exist ($p<0.01$); ns: not significant ($p>0.05$); ($p>0.01$).

Significant difference existed among the vitamin B_1 level of all the varieties with greatest concentration in 'nwine' at 0.54±0.04 mg/100g and least in 'kochuo' at 0.43±0.01 mg/100g (Table 13). There was no significant difference among the vitamin B_2 value of *Colocasia esculenta* var. *esculenta,* 'kochuo' and 'ogeriobosi'. The highest concentration of vitamin B_2 was found in the petiole of *Colocasia esculenta* var. *antiquorum* at 0.07±0.00 mg/100g. There was significant difference among the vitamin B_3 of all the varieties at $p<0.05$ but there was no significant difference among the vitamin B_3 of *Colocasia esculenta* var. *esculenta* and 'ogeriobosi' at $p<0.01$. Highest level of vitamin B_3 was present in the petioles of *Colocasia esculenta* var. *antiquorum* at 1.88±0.02 mg/100g. There was significant difference among the vitamin C level of all the varieties with highest level in the petiole of *Colocasia esculenta* var. *antiquorum at* 160.36±0.07 mg/100g and least in *Colocasia esculenta* var. *esculenta* at 143.58±0.19 mg/100g. There was no significant difference between the vitamin A content of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* at $p>0.05$; whereas significant difference existed between them and others at both $p<0.05$ and $p<0.01$. Significant difference existed among the vitamin E value of all the varieties at $p<0.05$, but high significant difference did not exist between *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* and between 'nwine' and 'ogeriobosi' at $p>0.01$. The least level of vitamin E was present in petiole of 'kochuo' being 1.84±0.57 mg/100g (Table 13).

Table 13: Mean vitamin composition of petiole of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**B_1**	**B_2**	**B_3**	**C**	**A**	**E**
Antiq	0.52±0.01[a]	0.07±0.00[a]	1.88±0.02[a]	160.36±0.07[a]	14.34±0.10[a]	6.78±0.00[a]
Esc	0.48 ±0.01[b]	0.05±0.00[b]	1.64±0.06[b]	143.58±0.19[b]	14.30±0.04[a]	6.80±0.01[a]
'Kochuo'	0.43±0.01[c]	0.05±0.00[b]	1.60±0.02[c]	149.27±0.03[c]	14.75±0.04[b]	1.84±0.57[b]
'Nwine'	0.54±0.04[d]	0.06±0.00[c]	1.75±0.01[d]	145.45±0.34[d]	15.30±0.07[c]	6.79±0.15[c]
'Ogeriobosi'	0.49±0.01[e]	0.05±0.00[b]	1.63±0.02[b]	146.80±0.02[e]	15.14±0.04[d]	5.84±0.09[c]
p>0.05	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

There was no significant difference among the vitamin B_1 concentration of *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'ogeriobosi' (Table 14). The highest value was found in *Colocasia esculenta* var. *antiquorum* and 'nwine'. There was no significant difference among the vitamin B_2 content of all the species at $p>0.05$. The greatest level of vitamin B_3 was present in *Colocasia esculenta* var. *esculenta* at 0.91±0.02 mg/100g. There was no significant difference among the vitamin B_3 values of *Colocasia esculenta* var. *antiquorum* and 'ogeriobosi'. High level of vitamin C was present in all the varieties with greatest value in *Colocasia esculenta* var. *antiquorum* and 'nwine' while the least value was in *Colocasia esculenta* var. *esculenta*. There was significant difference among the vitamin A content of all the varieties with the highest level in 'nwine' at 9.83±0.01 mg/100g, whereas the least concentration was found in *Colocasia esculenta* var. *antiquorum* at 0.79±0.01 mg/100g. There was also significant difference among the vitamin E level of all the varieties. The greatest concentration was present in 'kochuo' at 1.89±0.04 mg/100g and least value in *Colocasia esculenta* var. *antiquorum* at 0.66±0.02 mg/100g (Table 14).

Table 14: Mean vitamin composition of cormels of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (mg/100g)					
Varieties	**B_1**	**B_2**	**B_3**	**C**	**A**	**E**
Antiq	0.28±0.03[a]	0.04±0.00[a]	0.48±0.01[a]	10.73±0.03[a]	0.79±0.01[a]	0.66±0.02[a]
Esc	0.22 ±0.01[b]	0.04±0.00[a]	0.91±0.02[b]	9.28±0.02[b]	9.73±.03[b]	0.80±0.02[b]
'Kochuo'	0.21±0.01[b]	0.04±0.00[a]	0.58±0.04[c]	10.29±0.04[c]	8.92±.01[c]	1.89±0.04[c]
'Nwine'	0.25±0.01[a]	0.04±0.00[a]	0.70±0.02[d]	10.79±0.04[a]	9.83±0.01[d]	1.65±0.01[d]
'Ogeriobosi'	0.20±0.02[b]	0.04±0.00[a]	0.48±0.03[a]	11.66±0.04[d]	9.55±0.09[e]	1.81±0.01[e]
p>0.05	*	ns	*	*	*	*
p>0.01	**	ns	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$); ns: not significant ($p>0.01$).

There was no significant difference between the moisture content of the *Colocasia esculenta* var. *antiquorum* and ‘ogeriobosi’ (Table 15). The highest value of moisture was found in the leaf of ‘kochuo’ whereas least was found in ‘nwine’. There was significant difference among the dry matter of the all varieties with highest level in ‘nwine’ and least in ‘kochuo’. There was no significant difference between ash levels of *Colocasia esculenta* var. *esculenta* and ‘ogeriobosi’ with *Colocasia esculenta* var. *antiquorum* having the greatest level whereas the least was present in ‘nwine’. There was no significant difference between crude fibre concentration of *Colocasia esculenta* var. *esculenta* and ‘ogeriobosi’; ‘kochuo’ and ‘nwine’ which was the highest value. The greatest level of fat was present in the leaf of *Colocasia esculenta* var. *esculenta.* There was no significant difference between the fat content of ‘nwine’ and ‘ogeriobosi’. There was also no significant difference among the crude protein content of *Colocasia esculenta* var. *esculenta*, ’kochuo’ and ‘nwine’. Highest level of carbohydrate was present in the leaf of ‘nwine’ and least in *Colocasia esculenta* var. *esculenta.* There was no significant difference between the carbohydrate content of ‘kochuo’ and ‘ogeriobosi’ (Table 15).

Table 15: Mean proximate composition of leaf of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

Varieties	Composition (%)						
	Moisture content	Dry matter	Ash	Crude fibre	Fat	Crude protein	CHO
Antiq	9.62±0.02[a]	90.38±0.03[a]	24.39±0.03[a]	13.46±0.02[a]	3.44±0.02[a]	22.54±0.08[a]	26.55±0.13[a]
Esc	10.24±0.01[b]	89.76±0.01[b]	22.64±0.05[b]	12.80±0.04[b]	8.49±0.06[b]	21.74±0.12[b]	24.09±0.10[b]
'Kochuo'	10.74±0.02[c]	89.28±0.02[c]	22.60±0.13[c]	13.81±0.02[c]	2.30±0.02[c]	21.66±0.04[b]	28.89±0.21[c]
'Nwine'	8.78±0.02[d]	91.23±0.03[d]	18.65±0.08[d]	13.79±0.12[c]	3.18±0.02[d]	21.78±0.04[b]	33.89±0.16[d]
'Ogeriobosi'	9.63±0.02[a]	90.38±0.03[a]	22.75±0.01[b]	12.79±0.01[b]	3.13±0.01[d]	22.82±0.02[c]	28.89±0.05[c]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*; CHO= Carbohydrate. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$);**highly significant difference exist ($p<0.01$).

There was no significant difference between the moisture content of the root of *Colocasia esculenta* var. *antiquorum* and 'nwine' (Table 16). The greatest concentration of moisture was found in the root of *Colocasia esculenta* var. *esculenta* whereas the least concentration was found in 'ogeriobosi'. There was significant difference among the dry matter of the root of all the varieties at $p<0.05$ and highly significant difference existed at $p<0.01$. There was no significant difference among the ash of *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *esculenta*, 'kochuo' and 'ogeriobosi' with 'nwine' having the least level. There was no significant difference between the crude fibre content of root of 'kochuo' and 'nwine'. The highest level of crude fibre content was present in the root of 'ogeriobosi' whereas the least level was present in *Colocasia esculenta* var. *esculenta.* There was no significant difference between the fat content of the root of 'kochuo', 'nwine' and 'ogeriobosi', which was the least concentration whereas the highest concentration was present in *Colocasia esculenta* var. *antiquorum.* There was significant difference among the crude protein content of all the varieties with the greatest level in the root of 'kochuo' and least in *Colocasia esculenta* var. *esculenta.* There was also significant difference among the carbohydrate concentration of all the varieties with the highest value in 'nwine' and least in 'kochuo' (Table 16).

Table 16: Mean proximate composition of root of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (%)						
Varieties	**Moisture content**	**Dry matter**	**Ash**	**Crude fibre**	**Fat**	**Crude protein**	**CHO**
Antiq	7.71±0.08[a]	92.30±0.08[a]	23.7±0.03[a]	9.62±0.03[a]	0.83±0.01[a]	6.77±0.04[a]	51.30±0.18[a]
Esc	10.33±0.08[b]	89.73±0.08[b]	23.7±0.03[a]	8.49±0.01[b]	0.75±0.01[b]	5.67±0.03[b]	50.99±0.12[b]
'Kochuo'	9.88±0.05[c]	90.30±0.23[c]	23.8±0.02[a]	9.85±0.01[c]	0.48±0.03[c]	6.89±0.04[c]	49.07±0.04[c]
'Nwine'	7.33±0.06[a]	92.64±0.07[d]	20.6±0.04[b]	9.80±0.03[c]	0.49±0.01[c]	6.65±0.10[d]	55.07±0.21[d]
'Ogeriobosi'	6.77±0.02[d]	93.22±0.01[e]	23.8±0.02[a]	10.35±0.03[d]	0.49±0.01[c]	5.86±0.05[e]	52.71±0.08[e]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc= *Colocasia esculenta* var. *esculenta*; CHO= Carbohydrate. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

There was significant difference among the moisture content of petioles of all the varieties with greatest level in *Colocasia esculenta* var. *esculenta* and least in 'ogeriobosi' (Table 17). Dry matter of petioles of *Colocasia esculenta* var. *esculenta* and 'nwine' was not significant different. Highest concentration of dry matter was present in the petioles of 'ogeriobosi' whereas the least value was found in *Colocasia esculenta* var. *antiquorum.* There was no significant difference between the ash content of petioles of *Colocasia esculenta* var. *esculenta* and 'kochuo'; which was the greatest value. The least value was found in petioles of 'nwine'. Highest level of crude fibre was present in the petioles of 'kochuo' whereas the least was present in 'nwine'. There was no significant difference between the fat content of petioles of *Colocasia esculenta* var. *antiquorum*, 'kochuo' and 'ogeriobosi'. There was also no significant difference between the fat content of petioles of *Colocasia esculenta* var. *esculenta* and 'nwine'. The highest level of crude protein was found in the petiole of 'kochuo' whereas the least was found in that of *Colocasia esculenta* var. *antiquorum.* Highest value of carbohydrate was present in the petioles of 'nwine' and least in the petiole of 'kochuo' (Table 17).

Table 17: Mean proximate composition of petiole of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

Varieties	Composition (%)						
	Moisture content	Dry matter	Ash	Crude fibre	Fat	Crude protein	CHO
Antiq	10.62±0.07[a]	89.13±0.72[a]	21.53±0.06[a]	10.46±0.04[a]	2.79±0.01[a]	16.35±0.02[a]	38.25±0.18[a]
Esc	10.81±0.03[b]	89.19±0.03[b]	23.69±0.21[b]	10.63±0.02[b]	3.32±0.07[b]	17.51±0.09[b]	34.04±0.37[b]
'Kochuo'	10.28±0.04[c]	89.68±0.05[c]	23.41±0.68[b]	12.77±0.03[c]	2.73±0.02[a]	19.50±0.09[c]	31.31±0.65[c]
'Nwine'	9.85±0.01[d]	90.16±0.02[b]	16.24±0.01[c]	9.71±0.04[d]	3.05±0.01[b]	16.91±0.03[d]	44.23±0.04[d]
'Ogeriobosi'	8.90±0.06[e]	91.10±0.06[d]	17.48±0.10[d]	11.89±0.01[e]	2.82±0.10[a]	18.91±0.01[e]	40.00±0.18[e]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*; CHO=Carbohydrate. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different. * Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

There was significant difference among the moisture content of cormels of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta* and 'kochuo' whereas there was no significant difference between the moisture content of 'nwine' and 'ogeriobosi' both at $p>0.05$ and $p>0.01$ (Table 18). Highest moisture content was observed in the cormels of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta*. There was no significant difference between the dry matter content of cormels of *Colocasia esculenta* var. *esculenta* and 'nwine' and significant difference existed between the dry matter content of cormels of these and others. There was significant difference among the ash of cormels of all the varieties with greatest value in 'nwine' and least in 'kochuo'. Highest level of crude fibre was present in the cormels of *Colocasia esculenta* var. *antiquorum* and least level in *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'nwine'; three of which were not significantly different. There was no significant difference between the fat content of *Colocasia esculenta* var. *antiquorum* and 'ogeriobosi' which have the greatest value; as well as between those of *Colocasia esculenta* var. *esculenta* and 'nwine'. The least level of fat was present in the cormels of 'kochuo'. Highest concentration of crude protein was found in the cormels of 'ogeriobosi' and least in 'nwine'. There was no significant difference between the crude protein content of cormels of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta*. There was significant difference among the carbohydrate content of cormels of all the varieties at both $p<0.05$ and $p<0.01$; with highest value in 'kochuo' and least in 'nwine' (Table 18).

Table 18: Mean proximate composition of cormels of *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *esculenta*, 'Kochuo', 'Nwine' and 'Ogeriobosi'

	Composition (%)						
Varieties	**Moisture content**	**Dry matter**	**Ash**	**Crude fibre**	**Fat**	**Crude protein**	**CHO**
Antiq	11.77±0.03[a]	88.21±0.01[a]	7.62±0.02[a]	0.65±0.04[a]	0.92±0.01[a]	8.31±0.01[a]	70.73±0.04[a]
Esc	11.39±0.04[b]	88.62±0.03[d]	7.34±0.06[b]	0.51±0.02[b]	0.86±0.03[b]	8.32±0.02[a]	71.59±0.14[b]
'Kochuo'	9.48±0.03[c]	90.51±0.03[c]	6.94±0.03[c]	0.50±0.02[b]	0.60±0.02[c]	7.57±0.04[b]	74.91±0.10[c]
'Nwine'	9.80±0.12[d]	90.17±0.08[d]	12.72±0.02[d]	0.52±0.01[b]	0.83±0.02[b]	7.89±0.04[c]	68.23±0.13[d]
'Ogeriobosi'	9.82±0.19[d]	90.36±0.29[c]	10.64±0.02[e]	0.61±0.06[c]	0.95±0.02[a]	8.74±0.02[d]	69.25±0.16[e]
p>0.05	*	*	*	*	*	*	*
p>0.01	**	**	**	**	**	**	**

Antiq = *Colocasia esculenta* var. *antiquorum*; Esc = *Colocasia esculenta* var. *esculenta*; CHO = Carbohydrate. Values are in mean ± Standard deviation of triplicate determinations. Columns with the same letter are not significantly different.* Significant difference exist ($p<0.05$); **highly significant difference exist ($p<0.01$).

CHAPTER 5

DISCUSSION

Morphologically, inter-relatedness of all the varieties of *C. esculenta* was suggested by their leaves surface characters. The features include sagittate, net venation and not shiny. In addition, the anterior lobes of the leaf were twice as large as the posterior lobes which were round in shape and the petiole attachment was peltate. These features can be regarded as the field characters of varieties of *C. esculenta*. In other words, they might probably be the generic characters of *Colocasia.* They can be used to distinguish *Colocasia* from *Xanthosoma.* Both genera are commonly known as cocoyam, and there has been much confusion over the use of this term. Manner (2011) stated the morphological characters of *Xanthosoma*. They reported that the leaves of *Xanthosoma* (Tannia) are large, approximately 20 cm in length, 15 cm in width, hastate (saggitate-ovate) in shape, with the anterior lobe twice as large as the posterior lobe, with distinct marginal vein, basal lobes are rounded. In addition, the petiole attachment of *Xanthosoma* is at the margin of the leaf. *Colocasia* can therefore, be easily distinguished from *Xanthosoma* by the point where the petiole is attached to the leaf as well as the shape of the basal lobes. In *Colocasia,* the petiole attachment was peltate whereas for *Xanthosoma*, the petiole attachment is at the margin of the leaf. The basal lobes of *C. esculenta* were round while for *Xanthosoma* is more or less pointed.

Interestingly, however, there were remarkable differences in some parts of the varieties. There was presence of foliaceous appendages at the veins of the abaxial surface of ‘ogeriobosi’ leaves and this could be regarded as a diagnostic character; in addition, the leaf length of ‘ogeriobosi’ was the highest at 49.9$\pm$3.55 cm; and deep purple colour of the petiole, were the most conspicuous morphological

features differentiating it from others. These features suggested that a speciation has occurred. Schilthuizen (2000) stated that, in the "isolation" theory, the evolution of reproductive isolation is a key element of speciation; natural selection is given only secondary importance while gene flow is considered prohibitive to the process. In addition, Stace (1980) stated that an ecotype is one of the stages and process of speciation. Moreover, ‘nwine’ and ‘kochuo’ also had striking morphological characters; for ‘nwine’, it was possession of yellowish-green petiole with highest leaf width at 45.27±3.66 cm, whereas that of ‘kochuo’ was location of purplish spot in the centre of the adaxial surface of the leaves as well as numerous brownish stripes on the surface of the cormels. Sharma (1993) documented that the leaf characters, such as arrangement, type, form, duration and venation are widely used in both the classification and identification. In *Ulmus* and *Betula*, the species are delimited only on the basis of leaf characters. In *Trifolium*, the species were separated on the basis of stipule morphology. *Dalbergia* species were distinguished on the basis of their leaflet size, shape and arrangement on the rachis. The leaves are important for identification in palms, *Salix* and *Populus* (Singh, 2004). Illoh *et al*. (2011) stated that leaf character has been a critical tool in the hand of taxonomists in the classification and separation of taxa.

Colocasia esculenta var. *antiquorum* and *Colocasia esculenta* var. *esculenta*, which are the known varieties of *C. esculenta* could be differentiated in terms of morphology. The colour of the base of the petiole of *Colocasia esculenta* var. *antiquorum* was deep green whereas that of *Colocasia esculenta* var. *esculenta* was deep purple. The colour of the peeled fresh tubers of all the varieties varied, but *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* had the same colour. The leaf width of *Colocasia esculenta* var. *antiquorum* at 36.90±4.25 cm, and *Colocasia esculenta* var. *esculenta* at 36.00±2.65 cm were also the same, indicating a close affinity between them. There was

no significant difference between the corm length of *Colocasia esculenta* var. *antiquorum* at 4.50±0.10 cm and 'ogeriobosi' at 4.80±0.35 cm; and between the cormel length of *Colocasia esculenta* var. *esculenta* and 'ogeriobosi' which indicated an inter group affinity among the three varieties. *Colocasia esculenta* var. *antiquorum* had large corm which was more or less orbicular (approximately, circular) in shape with numerous small round cormels whereas the corm of *Colocasia esculenta* var. *esculenta* was large and oval with few more or less cylindrical shaped cormels. Variation in the size, shape and number of corm and cormel of *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta*, therefore, were the most prominent morphological characters which distinguished them from each other; and have extensively been used by various workers (Brooks, 2001; Nyananyo, 2006; Udealor and Ezurike, 2011; Ugwuaja and Chiejina, 2011). In addition, the petiole colour can also be used as a distinguishing character. The petiole of *Colocasia esculenta* var. *antiquorum* was pale green beneath while *Colocasia esculenta* var. *esculenta* was deep purple. In addition, the leaves of *Colocasia esculenta* var. *esculenta*, 'kochuo' and 'ogeriobosi' were deep green in colour indicating a close relatedness whereas those of *Colocasia esculenta* var. *antiquorum* and 'nwine' were pale green, also suggesting a close affinity. These resemblances probably suggested a moderate affinity among them.

Moreover, the corms of 'kochuo' and 'nwine' were almost the same but can still be distiguished from each other in that 'kochuo' had a large oval shaped corm with cormels that had numerous vertical brownish stripes on the surface while 'nwine' possessed large corm with slender cormels. The corm and cormels of 'ogeriobosi' can be easily differentiated from those of other varieties of *C. esculenta* because the width of the corm was the highest, oval in shape, with largest cylindrical cormels. The size and shape of corm and cormel of 'kochuo', 'nwine' and 'ogeriobosi' might be regarded as differential

characters, with the cormel length of ‘nwine’ at 19.57±8.36 cm having the greatest value. It has been specified that underground parts, such as roots and tubers are of some taxonomic value in plants (Sharma, 1993). The tubers are helpful in the taxonomy of *Dioscorea* and Cyperaceae. Some vegetative characters that play a major role in plant taxonomy and in deducing phylogeny include growth habit, phonological characters, underground organs, stems, leaves, petiole and stipules. Take, for instance, three sesame species, namely *Sesamum alatum*, *S. radiatum* and *S. indicum* were differentiated on the basis of their vegetative and the pod characteristics (Alege *et al*., 2011). Such characteristics, because of their high taxonomic importance, could be used in constructing a taxonomic key for the purpose of easy and quick identification of the three sesame species irrespective of their growth environments. Morphological characteristics are the strongest determinants of the agronomic value and taxonomic classification of plants (Cholastova and Knotova, 2012). Although morphological characters were the major criteria for classification over the last many centuries, other characters have also contributed in specific groups of plants.

Anatomically, the vascular bundles of all the varieties were small in size, collateral and closed; which is in line with the vascular bundles of monocotyledonous stems, which are collateral and closed (Pandey, 2012). The arrangement of primary vascular tissues was polyarch pattern because they were numerous and this is a characteristic of monocotyledons. The stomata were present in both adaxial and abaxial surfaces. Esau (1977) reported that stomata occur on all aerial parts of the plant, but they are most abundant on leaves. Moreover, in leaves, stomata may occur on both sides or only on one side, usually the lower side. Anomocytic stomatal type was observed in both adaxial and abaxial surfaces of all the varieties of *C. esculenta*. Cutler (1978) stated that although occasionally some species exist which have several types of stomata on one leaf, most have one type only.

The arrangements of the mesophyll were the same in all the varieties. The mesophyll of monocotyledonous leaf is usually not differentiated into palisade and spongy parenchyma, but consists of parenchyma cells, having chloroplasts and intercellular spaces among them (Pandey, 2012). Since some leaves lack a distinction of layers and others have very well-marked layers, the mesophyll can be used as a guide in identification (Cutler, 1978). In addition, it cannot often been used as a guide to the taxonomic position of a plant, but within a group of related plants there may be close similarities of arrangement.

Anatomical characters shared by all the varieties including, presence of anomocytic stomatal type in both adaxial and abaxial surfaces; the same arrangement of the mesophyll; size and appearance of vascular bundles; presence of large and well developed pith in the centre of root, and round shape of pores/vessels of petioles in all the varieties, could be regarded as general characters shared by these varieties of *C. esculenta*, thereby suggesting an inter group affinity among them, that is, they could be the specific identity.

Moreover, the size of vessels of all the five varieties of *C. esculenta* varied. Their length and width suggested that their shapes were more or less oval, which implied a close affinity among them. Nonetheless, the smallest size of both the length and width of 'ogeriobosi' vessels could be applied as a distinctive character. Furthermore, occurrence of air-chambered cortex in the roots of *Colocasia esculenta* var. *antiquorum*, 'kochuo' and 'ogeriobosi' only, suggested a close affinity among them, and could be regarded as conspicuous distinguishable anatomical character from others. Cutler (1978) reported that the cortex is sufficiently variable to be used to assist in identification. Okeke (2004) used

anatomical parameter among others in separating the three taxa: *Dioscorea cayenensis*, *D. pruinosa and D. rotundata*. In addition, varietal status was suggested for *D. cayenensis*, on the basis of epidermal and starch grain evidence (Green and Okoli, 2007).

Histochemically, occurrence of oxalate crystals in the petioles of all the varieties of *C. esculenta,* in the form of crystal sands, druses, raphides bundle, raphides, prisms, rosettes and styloid indicated that close affinity existed among them. It was reported that oxalate crystals can be of several forms, including raphides, conglomerate, and cystoliths, in the plant tissues where they exist (Okoli, 1988; Osuji, 2013). Moreover, the shapes of CaOx crystals vary and are commonly described as raphides, druses, styloids, prisms and sandy crystals (Franceschi and Nakata, 2005; Meric, 2009). Ilarslan *et al*. (2001) suggested that crystal formation within the cell is under genetic control; hence, the type, presence or absence of crystals may be represented as a taxonomic character (Prychid and Rudall, 1999; Lersten and Horner, 2000). In addition, oxalate crystal can form in any organ or tissue within the plants, including stems, petiole, leaves, roots and tubers, and have a variety of functions including calcium storage, defense and providing structural strength (Franceschi and Horner, 1980; Nakata, 2003; Saadi and Mondal, 2011). A bar of styloid was only found in petiole of *C. esculenta* var. *antiquorum* which distinguished it from other varieties, and therefore could be referred to as a diagnostic feature. Wu and Cutler (1985) found that variation in styloid size and shape has some taxonomic application among species of *Iris*. Raphides and prismatic crystals were also found in petioles of *C. esculenta* var. *antiquorum*. Prychid and Rudall (1999) stated that raphides, styloids and intermediate forms may be present in some families. Morphology of raphides and styloids look alike, but they can still be differentiated. Raphides are bundles of narrow, elongated needle-shaped crystals, usually of similar orientation, with pointed ends at maturity, whereas styloids also known as

'pseudoraphides', are thicker than raphides and usually solitary within a cell (Prychid and Rudall, 1999). In addition, styloids may have pointed or squared ends, and may be elongated or cuboidal. High concentration of raphides which was observed in the petioles of *Colocasia esculenta* var. *esculenta* suggested that its irritating activity might be highest when compared to others. Druses and rosettes only occurred in petioles of 'kochuo', and may be species specific. This characteristic could be used to distinguish 'kochuo' from other varieties, and hence, specific status was suggested for it. Moreover, other varieties of *C. esculenta* can only be boiled before eating but 'kochuo' can also be roasted. Osuji *et al.* (1997) showed that crystals of calcium oxalate could play a taxonomic role since their quantity, frequency of occurrence and distribution could distinguish between related species and cultivars. Druses are common in dicotyledons, relatively rare in monocotyledons, where they are almost restricted to some early-branching taxa such as *Acorus*, Araceae and *Tofieldia* (Prychid and Rudall, 1999). Raphide bundle was also observed in petiole of 'kochuo'. Occurrence of both raphides and druses in Araceae has been previously reported (Genua and Hillson, 1985; Grayum, 1990; Prychid and Rudall, 1999). Moreover, both druse and raphide idioblasts were reported in leaves of *Aglaonema commutatum* Schott (Saadi and Mondal, 2012b). Araceae have numerous druses, multi-crystal druses and needle shaped raphide crystals of calcium oxalate (CaOx) present in the tissue (Franceschi and Nakata, 2005). Druses were found in leaves and bud scales of *Prunus*, *Rosa* (Lersten and Horner, 2006), *Allium*, *Vitis*, *Morus* and *Phaseolus* (Jáuregui-Zúñiga *et al*., 2003; Katayama *et al*., 2007).

Crystal sands were seen in petioles of *Colocasia esculenta* var. *esculenta* and 'nwine' with higher concentration in 'nwine', but were not seen in *Colocasia esculenta* var. *antiquorum*, 'kochuo' and 'ogeriobosi'; probably because sandy crystals were mainly seen in leaves and roots, and this implied that a close affinity existed between *Colocasia esculenta* var. *esculenta* and 'nwine'. 'Kochuo' and

‘nwine’ cultivars do not require prolong cooking before eaten. ‘Kochuo’ can only be used together with *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta* or either, for soup thickening. The corm and cormels of ‘nwine’ are not used for soup thickening, probably because of the slimy nature of the cormels, which might be as a result of higher concentration of crystal sands in the petiole. The corm is principally boiled and pounded as ‘fufu’, locally known as ‘utara-ede’ in Uga, Anambra State and is mainly eaten by the elderly. In addition, it normally grows in the wild and only few quantities are cultivated. Santha and Sree (2007) reported wild cultivars of *Colocasia* in India. In addition, Ugbajah (2013) reported wild tubers of *C. esculenta* in Dunukofia L.G.A. of Anambra State, which are locally known as ‘akonoke’. She also stated that ‘edeofe’ is the most popular cultivar grown in Dunukofia L.G.A. of Anambra State. This cultivar is commonly used as paste for soup thickening, boiled and mixed with vegetables for occasions as special delicacy. The fresh tender leaves and petioles are used as vegetables during periods of scarcity.

Generally, it was observed that all the forms of calcium oxalate were not found in all the varieties of *Colocasia esculenta*, rather some were present in some varieties and absent in others. This suggested that occurrence of different forms of calcium oxalate in plant parts could be genetically controlled. Saadi and Mondal (2011) stated that the size and appearance of calcium oxalate crystals (COC) can differ within families, genus, and species and these characteristics might be genetically controlled. Hence, some workers were of the opinion that calcium oxalate crystals may have taxonomic potential for both botanist and taxonomist (Horner and Wagner, 1995). In addition, microscopic characters of cell contents such as calcium oxalate crystals are important diagnostic tools, and at times prove extremely helpful in delineating species, genera and families. Therefore, the occurrence of specific

types and forms of crystals in the petioles of varieties of *C. esculenta* may have a taxonomic value and could be used as a dependable tool in their proper identification.

The frequency of occurrence, quantity and distribution of oxalates of calcium are important taxonomic characters, which have been clearly used to delimit cultivars as well as characterize plant germplasm (Osuji *et al.,* 1997; Osuji, 2006; Osuji, 2013). Some of these widely distributed secondary metabolites have been a source of material and information useful in the improvement of perception, value and taxonomic characterization of plants. Among the ergastic substances that belong to this widely distributed category, tannins and calcium oxalate are notable examples (Osuji, 2013). Their occurrence and distribution in the living tissues of plants have made them very useful in germplasm characterization and classification (Osuji *et al*., 1997). Raphides occur in conjunction with styloid in petiole of *Colocasia esculenta* var. *antiquorum*. Prychid and Rudall (1999) stated that raphides and styloids were reported present in the families of Agavaceae (McDougall *et al*., 1993) and Alliaceae (Arnott, 1981; Kausch and Horner, 1982).

Moreover, the chemotaxonomic studies include the investigations of the pattern of the compounds existing in plants, and in all the individual parts of the plants, such as bark, wood, leaves, and roots. The chemical constituents usually differ much in different organs. Therefore, such investigations are needed for obtaining real evidence relationships or otherwise of plants (Pandey, 1981). The least amount of flavonoid was detected in the leaf at 0.55±0.02%, petiole at 0.47±0.02% and cormel at 0.18±0.01% of *Colocasia esculenta* var. *antiquorum* as well as in the root at 0.56±0.04% and cormel at 0.19±0.01% of ‘nwine’. This suggested that these varieties of *Colocasia esculenta* synthesized small quantity of flavonoid, and this character could be used to distinguish them from others. The most

widely and effectively used compounds in chemotaxonomy are the flavonoids; take, for instance, South American species of *Eucryphia* of the family Eucryphiaceae may easily be distinguished on the basis of their flavonoid glycosides (Sharma, 1993).

Oxalate was present in the leaves, petioles, corms, cormels and roots of all the varieties examined, but in varying concentrations. The presence of oxalate in all the parts of *C. esculenta* indicated that it is being synthesized and stored in every part of the plant. However, higher concentrations were detected in the cormels and roots, which was probably because they are the underground parts. In addition, this implied that all parts of varieties of *C. esculenta* would be irritating to the mouth and oesophagus when eaten, especially when fresh or not properly cooked; which is probably the reason behind prolong cooking of the tubers before consumption. All the varieties are boiled while 'kochuo' can also be roasted to destroy the acrid calcium oxalate crystals. Bradbury and Nixon (1998) reported that this acridity is caused in part by microscopic needle-like raphides of calcium oxalate monohydrate and in part by another chemical, probably a protease.

Furthermore, the least concentration of all the phytochemicals investigated with the exception of hydrogen cyanide was found in the cormel of *Colocasia esculenta* var. *antiquorum*. This implied that *Colocasia esculenta* var. *antiquorum* accumulated low levels of chemicals in the cormels in comparison to the other parts. Finally, these strikingly differentiating chemical characters could be regarded as additional characters in identification and classification of these varieties of *C. esculenta*. Pandey (1981) reported that the science of chemical taxonomy was based on the classification of

plants on the basis of their chemical constituents which were deeply concerned with the molecular characteristics.

Highest concentration of calcium was detected in the cormel of 'kochuo' at 419.44±0.21 mg/100g, followed by 'ogeriobosi' at 416.75±0.05 mg/100g, and the two values fell within the same range and suggested a close affinity between them. Least value of calcium was found in cormel of *Colocasia esculenta* var. *esculenta* at 200.76±0.02 mg/100g. This signified that *Colocasia esculenta* var. *esculenta* accumulated very low level of calcium when compared with others. The concentrations of each of the other minerals fell within the same range, indicating an inter group affinity. Highest concentration of potassium at 208.62±0.15 mg/100g and magnesium at 103.69±0.21 mg/100g were present in the root of *Colocasia esculenta* var. *antiquorum*, whereas other minerals each fell within the same range in other varieties. In addition, highest value of magnesium was detected in the leaf of *Colocasia esculenta* var. *antiquorum* at 103.49±0.09 mg/100g indicating that this variety synthesized greatest level of magnesium in the root and leaf. Potassium contents of leaf of *Colocasia esculenta* var. *esculenta* at 206.80±0.01 mg/100g and 'nwine' at 207.62±0.02 mg/100g fell within the same range and they could be said to share a close affinity.

There was no significant difference among the levels of vitamin B_1 of the root of all the varieties, as well as vitamins B_2 and B_3 at $p>0.05$. This indicated that these vitamins are synthesized in low concentrations in these varieties of *C. esculenta*. Besides, vitamin B_2 was the least vitamin present in the root of all the varieties, signifying that it was least synthesized vitamin in the root of these varieties of *C. esculenta*. *Colocasia esculenta* var. *antiquorum* at 0.98±0.00 mg/100g contained the least vitamin B_3 in the leaf of all the varieties. This signified that leaf of *Colocasia esculenta* var.

antiquorum synthesized the lowest vitamin B_3 when compared with others. The highest concentration of vitamin B_2, B_3 and C were found in the petiole of *Colocasia esculenta* var. *antiquorum* at 0.07±0.00 mg/100g, 1.88±0.02 mg/100g and 160.36±0.07 mg/100 g, respectively. The least vitamin E level was present in petiole of 'kochuo' at 1.84±0.57 mg/100g whereas the values of *Colocasia esculenta* var. *antiquorum* at 6.78±0.00 mg/100g, *Colocasia esculenta* var. *esculenta* at 6.80±0.01 mg/100g and 'nwine' at 6.79±0.15 mg/100g fell within the same range. The least value of vitamin A was found in cormel of *Colocasia esculenta* var. *antiquorum* at 0.79±0.01 mg/100g. Concentration of vitamin B_2 was small in the cormel of all varieties. This probably meant that cormels of these varieties of *C. esculenta* synthesized low quantity of this vitamin, hence indicating an inter group affinity among them. The least value of vitamin C was detected in cormel of *Colocasia esculenta* var. *esculenta* at 9.28±0.02 mg/100g. Levels of vitamin E were small in the cormel of *Colocasia esculenta* var. *antiquorum* at 0.66±0.02 mg/100g and *Colocasia esculenta* var. *esculenta* at 0.80±0.02 mg/100g suggesting a close affinity between the two varieties.

The highest value of moisture in the leaves was found in 'kochuo' while the least was detected in 'nwine'.This indicated that moisture retaining capacity was greatest in the leaf of 'kochuo' and least in 'nwine'. The highest concentration of moisture in the petioles and roots was present in *Colocasia esculenta* var. *esculenta* whereas the least was found in 'ogeriobosi'. In the cormels, the highest moisture content was detected in *Colocasia esculenta* var. *antiquorum*. Level of ash in the petioles was highest in 'kochuo' and *Colocasia esculenta* var. *esculenta* whereas the least was found in 'nwine'. Highest content of ash in cormels of all the varieties was highest in 'nwine' and least in 'kochuo' while in the leaves and roots, the least level occurred in 'nwine'. Ash content depicts the mineral composition in food. Highest level of carbohydrate was present in the leaves, petioles and roots of

‘nwine’ and least in leaves of *Colocasia esculenta* var. *esculenta*, petioles and roots of ‘kochuo’ while in the cormels the highest value was found in ‘kochuo’ and least ‘nwine’. There was no significant difference between the crude fibre content of leaf and root of ‘kochuo’ and ‘nwine’. In the petioles, the highest level of crude fibre was present in ‘kochuo’ whereas the least was present in ‘nwine’. In the roots and petioles, ‘kochuo’ had the highest crude protein content while *Colocasia esculenta* var. *esculenta* had the least level in root and *Colocasia esculenta* var. *antiquorum* had the least concentration in the petiole. In the cormels, highest quantity of crude protein was found in‘ogeriobosi’ and least in ‘nwine’. Greatest percentages of most of the nutrients were found in parts of ‘kochuo’ while least compositions were detected in parts of ‘nwine’. This enviced that ‘kochuo’ synthesized and accumulated highest levels of nutrients whereas ‘nwine’ synthesized and accumulated smallest levels.

Morphological, anatomical, histochemical, phytochemical and proximate data, alone, may not be sufficient to obtain a clear affinities existing among the various varieties, when a species with many varieties is considered. Chromosome count of 2n=24 was observed in *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *esculenta*, ‘nwine’ and ‘kochuo’ whereas 2n=28 was found in ‘ogeriobosi’. In previous studies, chromosome count of 2n = 24 was reported in four cultivars of *C. esculenta* in Nigeria, namely: ‘Coco India’, ‘Ede Ukpong’, ‘Ede Ghana’ and ‘Ede Ofe’ (Ekanem and Osuji, 2006). The similarity in the chromosome number of *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *esculenta,* ‘nwine’ and ‘kochuo’ suggested a close affinity among them, which could be the specific identity. Asenge (2007) reported that cytology deals with number, structure and behaviour of chromosome and is, therefore, of immense value in understanding many intricate of hereditary characters. Although, *Colocasia esculenta* var. *antiquorum, Colocasia esculenta* var. *esculenta,* ‘nwine’ and ‘kochuo’ had the same chromosome number, specific status can still be

proposed for ‘kochuo’. Take, for instance, it has extensively been reported that *Citrus* species have the same chromosome number of n = 9 or 2n = 18 (Guerra *et al*., 1997; Yamamoto and Tominaga, 2004; Silva *et al*., 2010; Hynniewta *et al.*, 2011); yet, they are regarded as different species. Moreover, chromosome number of 2n=28 was observed in ‘ogeriobosi’. This chromosome number has previously been reported for some varieties of *C. esculenta* (Coates *et al*., 1988; Parvin *et al.*, 2008). The same number of chromosome was also reported for other species of *Colocasia* namely: *Colocasia gaoligongensi*, *C. gigantea* and *C. gongii* in previous research (Yang *et al*., 2003). Therefore, the difference in the chromosome number of ‘ogeriobosi’ suggested a species level for it. Chromosome counts are helpful in taxonomy and may suggest evolutionary processes (Arora *et al*., 1985; Udengwu and Arukwe, 2010).

Moreover, time of visibility of chromosome in these varieties of *C. esculenta* varied. The chromosomes were visible in ‘nwine’ at 12.00 noon, *Colocasia esculenta* var. *antiquorum* at 1.00 pm, ‘kochuo’ at 2.00 pm, *Colocasia esculenta* var. *esculenta* and ‘ogeriobosi’ at 3.00 pm. The visibility of chromosomes in *Colocasia esculenta* var. *esculenta* and ‘ogeriobosi’ at the same hour indicated that they had the peak of metaphase at the same time. This also suggested a close affinity between the two varieties. Moreover, it showed that the ideal time to collect root stock of *C. esculenta* for chromosome studies is from 12.00 noon to 3.00 pm, because the metaphase is probably at its peak within these hours of the day. This partly agrees with the work of Ekanem and Osuji (2006); who reported that the mitotic process in *Colocasia* increased as from noon, and the peak of metaphase for most of the cultivars was between 12.00 noon and 4.00 pm. This is because the time of visibility of the chromosomes in this study lagged by an hour.

Morphological, anatomical, histochemical, phytochemical, proximate and cytological characteristics of *C. esculenta* were enumerated for constructing a taxonomic key for the purpose of easy and quick identification.

Diagnostic Features of *C. esculenta* var. *antiquorum*

1. **Morphological Characters**

i. *Colocasia esculenta* var. *antiquorum* had the smallest corm being 4.10±0.10 cm in length, which was more or less orbicular (approximately circular) in shape with numerous small round cormels being 3.70±0.96 cm in length

2. **Histochemical Character**

i. A bar of styloid was only found in petiole of *C. esculenta* var. *antiquorum*.

3. **Cytological Character**

i. The chromosomes were visible at 1.00 pm

Diagnostic Features of *C. esculenta* var. *esculenta*

i. The corm of *C. esculenta* var. *esculenta* was larger being 6.17±0.50 cm in length and oval with few more or less bigger cylindrical shaped cormels being 5.00±1.35 cm in length.

4. **Cytological Character**

i. Chromosomes were visible at 3.00 pm.

Diagnostic Characters Observed in 'Kochuo'

1. Morphological Features

i. Presence of a purplish dot on the centre of the adaxial surface of the leaf.

ii. Numerous vertical brownish stripes on the surface of the cormels.

ii. The colour of peeled fresh tuber was purple.

3. Histochemical Character

Druses and rosettes were only present in petioles of 'kochuo'.

ii. **Cytological Character**

i. The chromosomes were visible at 2.00 pm.

Diagnostic Features Observed in 'Nwine'

1. Morphological Characters

i. Possession of large corm with slender cormels with 6.63±1.42 cm in length.

ii. The petiole was yellowish-green.

iii. Colour of peeled fresh tuber was pink.

2. Histochemical Characters

i. Crystal sands were seen in petioles of *C. esculenta* var *esculenta* and 'nwine' only.

ii. Absence of raphides in the petiole

3. Cytological Character

i. The chromosomes were visible at 12.00 noon.

Diagnostic Characters Observed in 'Ogeriobosi'

1. Morphological Features

i. Presence of foliaceous appendages at the veins of the abaxial surface of the leaves.

ii. Presence of purplish colour at the point of attachment to the leaf of the abaxial leaf surface.

iv. Colour of peeled fresh tuber was milk.

2. Anatomical Feature

i. Pore arrangements of petiole were in clusters.

3. Cytological Characters

i. Chromosome count of 2n=28 was found.

ii. The chromosomes were visible at 3.00 pm.

Therefore, in addition to the two known varieties of *C. esculenta*; morphological, anatomical, histochemical, phytochemical, proximate and cytological investigations suggested a new variety as well as two new species.

The two known varieties of *Colocasia esculenta* are:

Colocasia esculenta var. *antiquorum* (‘ede ofe green’, ‘ugwuta’)

Colocasia esculenta var. *esculenta* (‘ede ofe purple’, ‘nkashi’, ‘nachi’)

Suggested scientific names for the new variety of *C. esculenta* with local name and proposed two new species of *Colocasia* are as follows:

1. *Colocasia ugensis* for ‘kochuo’ (‘coco India’)

2. *Colocasia ezeabarae* for ‘ogeriobosi’ (‘nworoko’)

3. *Colocasia esculenta* var. *nwine* for ‘nwine’ (‘akonoke’, ‘nkpong’)

Bracketed Dichotomous Key to *Colocasia* Cultivated in Anambra State

1. Cormels which are numerous and small round shaped.....2
1. Cormels which are few and more or less cylindrical shaped..... *C. esculenta* var. *esculenta*
2. Petiole with bar of styloid; chromosomes visible at 1.00..... *C. esculenta* var. *antiquorum*
2. Petiole with druses and rosettes; chromosomes visible at 2.00…….....3
3. Cormels with slender shape; chromosomes visible at 12.00; petiole with yellowish-green colour; pink peeled fresh tuber …… *C. esclenta* var. *nwine*
3. Cormels with numerous vertical brownish stripes on the surface; purple peeled fresh tuber …4
4. Abaxial leaf surface with purplish colour at the point of attachment to the petiole; purplish dot at the centre of adaxial leaf surface …… *C. ugensis*
4. Abaxial leaf surface with foliaceous appendages at the veins; milk peeled fresh tuber; purple petiole; pores arranged in clusters; chromosome count of 2n=28. ……. …….*C. ezeabarae*

CONCLUSION AND RECOMMENDATION

This work provided information on the morphology, anatomy, histochemistry, phytochemistry, proximate, and cytology of five varieties of *C. esculenta* present in Anambra State; which was previously lacking. Secondly, the overwhelming evidence from morphology suggested closely relatedness between *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta*, and provided diagnostic characters for 'kochuo' and 'ogeriobosi'. Evidence from anatomy indicated a close affinity between 'kochuo' and 'ogeriobosi'; as well as between *Colocasia esculenta* var. *antiquorum* and *Colocasia esculenta* var. *esculenta*. Evidence from histochemistry implied a close affinity between *Colocasia esculenta* var. *esculenta* and 'nwine'. Phytochemistry and proximate

evidence suggested a close affinity among all the varieties. In addition, evidence from cytology indicated the same with the exception of ‘ogeriobosi’.

Conspicuous diagnostic characters observed in ‘ogeriobosi’ include presence of foliaceous appendages at the veins of the abaxial surface of the leaves and presence of purplish colour at the point of attachment to the leaf of the abaxial leaf surface. For ‘kochuo’, they were presence of a purplish dot on the centre of the adaxial surface of the leaf and numerous vertical brownish stripes on the surface of the cormels. For ‘nwine’, it was possession of large corm with elongated cormels. The differential characters possessed by the varieties include: *Colocasia esculenta* var. *antiquorum* had large corm which was more or less orbicular (approximately circular) in shape with numerous small round cormels; the corm of *Colocasia esculenta* var. *esculenta* was large and oval with few more or less cylindrical shaped cormels; and ‘ogeriobosi’ had the longest petiole and leaf as well as the largest oval shaped corm with largest cylindrical cormels.

This study established that forms of calcium oxalate crystals differed within varieties. Presence of druses and rosettes in petioles of ‘kochuo’ only, presence of crystal sands in petioles of *Colocasia esculenta* var. *esculenta* and ‘nwine’, and the absence in *Colocasia esculenta* var. *antiquorum*, ‘‘kochuo’’ and ‘‘ogeriobosi’ could probably be used as diagnostic characters. The chromosomes of these varieties were relatively small in size, therefore, making the microscopic observations hard. The time for visibility of the chromosomes varied in the varieties. Generally, the peak of metaphase in varieties of *C. esculenta* was indicated to be between 12:00 noon and 3:00 pm. This information is very important and provided a platform for further cytological studies of *C. esculenta.*

Plants are generally grouped by their relationship to one another, according to their similarities and differences; which is based on the characters they possess. This study, therefore, supplied additional information which might be helpful in resolving the on-going controversy in the taxonomy of

Colocasia; which would, in turn, probably lead to possible adoption of scientific names for 'kochuo', 'nwine', and 'ogeriobosi' thereby instituting an order in the genus.

Furthermore, the morphological, anatomical, histochemical, phytochemical and proximate characters of these varieties of *C. esculenta* could aid in the current research towards finding a lasting solution to the devastating leaf blight disease of *C. esculenta* in Southeastern Nigeria. The rich bioactive and nutritional values also suggested the medicinal and nutritive potentials of *C. esculenta* and, hence could increase its acceptance in Anambra State, other than regarding it as ordinary woman's crop; thereby enhancing its production for both food security and industrial application.

Contributions to Knowledge

1. Varieties of *C. esculenta* had been taxonomically analysed.
2. Diagnostic characters of 'kochuo', 'nwine' and 'ogeriobosi' were provided.
3. Scientific names were suggested for 'nwine' as well as the two proposed new species.
4. The time for chromosome visibility of each of the varieties and the chromosome number of 'ogeriobosi' were supplied.
5. The study established that forms of calcium oxalate crystals differed within the varieties of *C. esculenta*.
6. A taxonomic key was formulated for easy identification of the varieties as well as the proposed new species of *C. esculenta.*
7. The medicinal and nutritive qualities of *C. esculenta* were provided.

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Appendix

Anti Mineral

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
P	Leaf	3	167.4267	.02517	.01453	167.3642	167.4892	167.40	167.45
	Root	3	162.3167	.02082	.01202	162.2650	162.3684	162.30	162.34
	Petiole	3	158.6633	.18502	.10682	158.2037	159.1230	158.45	158.78
	Cormels	3	223.5100	.02646	.01528	223.4443	223.5757	223.48	223.53
	Total	12	177.9792	27.64804	7.98130	160.4124	195.5459	158.45	223.53
Mg	Leaf	3	96.3367	.09815	.05667	96.0928	96.5805	96.28	96.45
	Root	3	78.4600	.03606	.02082	78.3704	78.5496	78.43	78.50
	Petiole	3	75.4400	.31241	.18037	74.6639	76.2161	75.24	75.80
	Cormels	3	63.4800	.02646	.01528	63.4143	63.5457	63.45	63.50
	Total	12	78.4292	12.28264	3.54569	70.6252	86.2332	63.45	96.45
Ca	Leaf	3	452.7900	.02646	.01528	452.7243	452.8557	452.76	452.81
	Root	3	128.2033	.03512	.02028	128.1161	128.2906	128.17	128.24
	Petiole	3	349.2467	.30616	.17676	348.4861	350.0072	349.06	349.60
	Cormels	3	2.7600	.02000	.01155	2.7103	2.8097	2.74	2.78
	Total	12	233.2500	185.23710	53.47335	115.5560	350.9440	2.74	452.81
Fe	Leaf	3	1.0733	.02082	.01202	1.0216	1.1250	1.05	1.09
	Root	3	.8367	.04726	.02728	.7193	.9541	.80	.89
	Petiole	3	.7933	.04509	.02603	.6813	.9053	.75	.84
	Cormels	3	.6867	.01528	.00882	.6487	.7246	.67	.70
	Total	12	.8475	.15064	.04349	.7518	.9432	.67	1.09
Na	Leaf	3	185.7833	.03786	.02186	185.6893	185.8774	185.74	185.81
	Root	3	135.8167	.02082	.01202	135.7650	135.8684	135.80	135.84
	Petiole	3	153.6067	.01528	.00882	153.5687	153.6446	153.59	153.62
	Cormels	3	98.4200	.02646	.01528	98.3543	98.4857	98.40	98.45
	Total	12	143.4067	32.95134	9.51223	122.4704	164.3429	98.40	185.81
K	Leaf	3	206.7967	.00577	.00333	206.7823	206.8110	206.79	206.80
	Root	3	149.5733	.06429	.03712	149.4136	149.7330	149.50	149.62
	Petiole	3	192.5567	.04041	.02333	192.4563	192.6571	192.52	192.60
	Cormels	3	162.7967	.08386	.04842	162.5883	163.0050	162.70	162.85
	Total	12	177.9308	23.81950	6.87610	162.7966	193.0650	149.50	206.80

Nwine Mineral

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
P	Leaf	3	172.5800	.19157	.11060	172.1041	173.0559	172.45	172.80
	Root	3	160.6200	.16371	.09452	160.2133	161.0267	160.48	160.80
	Petiole	3	154.7900	.01000	.00577	154.7652	154.8148	154.78	154.80
	Cormels	3	226.7500	.07810	.04509	226.5560	226.9440	226.70	226.84
	Total	12	178.6850	29.74839	8.58762	159.7838	197.5862	154.78	226.84
Mg	Leaf	3	92.6167	.18824	.10868	92.1491	93.0843	92.40	92.74
	Root	3	81.6567	.04041	.02333	81.5563	81.7571	81.62	81.70
	Petiole	3	71.8433	.04509	.02603	71.7313	71.9553	71.80	71.89
	Cormels	3	61.6000	.14526	.08386	61.2392	61.9608	61.45	61.74
	Total	12	76.9292	12.01517	3.46848	69.2951	84.5632	61.45	92.74
Ca	Leaf	3	445.5567	.23965	.13836	444.9613	446.1520	445.28	445.70
	Root	3	131.7533	.04163	.02404	131.6499	131.8568	131.72	131.80
	Petiole	3	348.4167	.10408	.06009	348.1581	348.6752	348.30	348.50
	Cormels	3	228.8100	.11790	.06807	228.5171	229.1029	228.71	228.94
	Total	12	288.6342	124.01158	35.79906	209.8410	367.4274	131.72	445.70
Fe	Leaf	3	.9300	.01732	.01000	.8870	.9730	.92	.95
	Root	3	.6867	.00577	.00333	.6723	.7010	.68	.69
	Petiole	3	.8667	.02082	.01202	.8150	.9184	.85	.89
	Cormels	3	.7100	.01000	.00577	.6852	.7348	.70	.72
	Total	12	.7983	.10811	.03121	.7296	.8670	.68	.95
Na	Leaf	3	192.6833	.20207	.11667	192.1814	193.1853	192.45	192.80
	Root	3	129.6567	.04041	.02333	129.5563	129.7571	129.62	129.70
	Petiole	3	149.6233	.02517	.01453	149.5608	149.6858	149.60	149.65
	Cormels	3	92.7500	.04583	.02646	92.6362	92.8638	92.71	92.80
	Total	12	141.1783	37.66660	10.87341	117.2461	165.1105	92.71	192.80
K	Leaf	3	207.6233	.02082	.01202	207.5716	207.6750	207.60	207.64
	Root	3	153.6867	.20502	.11837	153.1774	154.1960	153.45	153.81
	Petiole	3	190.5967	.01528	.00882	190.5587	190.6346	190.58	190.61
	Cormels	3	165.6300	.30315	.17502	164.8769	166.3831	165.28	165.81
	Total	12	179.3842	21.98842	6.34751	165.4134	193.3549	153.45	207.64

Ogeri Mineral

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
P	Leaf	3	174.6167	.01528	.00882	174.5787	174.6546	174.60	174.63
	Root	3	159.2967	.01528	.00882	159.2587	159.3346	159.28	159.31
	Petiole	3	118.7300	.02646	.01528	118.6643	118.7957	118.70	118.75
	Cormels	3	226.7900	.04583	.02646	226.6762	226.9038	226.74	226.83
	Total	12	169.8583	40.41663	11.66728	144.1788	195.5378	118.70	226.83
Mg	Leaf	3	91.4500	.05000	.02887	91.3258	91.5742	91.40	91.50
	Root	3	82.7667	.03055	.01764	82.6908	82.8426	82.74	82.80
	Petiole	3	74.8167	.18771	.10837	74.3504	75.2830	74.60	74.93
	Cormels	3	59.5200	.08888	.05132	59.2992	59.7408	59.45	59.62
	Total	12	77.1383	12.27335	3.54301	69.3402	84.9364	59.45	91.50
Ca	Leaf	3	459.3100	.03606	.02082	459.2204	459.3996	459.28	459.35
	Root	3	132.6533	.04509	.02603	132.5413	132.7653	132.61	132.70
	Petiole	3	328.4933	.08083	.04667	328.2925	328.6941	328.40	328.54
	Cormels	3	416.7500	.04583	.02646	416.6362	416.8638	416.71	416.80
	Total	12	334.3017	131.20484	37.87558	250.9381	417.6652	132.61	459.35
Fe	Leaf	3	1.0867	.02517	.01453	1.0242	1.1492	1.06	1.11
	Root	3	.7700	.01732	.01000	.7270	.8130	.75	.78
	Petiole	3	.8667	.01155	.00667	.8380	.8954	.86	.88
	Cormels	3	.7033	.02517	.01453	.6408	.7658	.68	.73
	Total	12	.8567	.15240	.04399	.7598	.9535	.68	1.11
Na	Leaf	3	190.5267	.08622	.04978	190.3125	190.7408	190.45	190.62
	Root	3	147.6167	.01528	.00882	147.5787	147.6546	147.60	147.63
	Petiole	3	154.8167	.10017	.05783	154.5678	155.0655	154.72	154.92
	Cormels	3	95.3300	.06245	.03606	95.1749	95.4851	95.28	95.40
	Total	12	147.0725	35.51888	10.25342	124.5049	169.6401	95.28	190.62
K	Leaf	3	194.7433	.10693	.06173	194.4777	195.0090	194.62	194.81
	Root	3	152.8267	.02309	.01333	152.7693	152.8840	152.80	152.84
	Petiole	3	190.5900	.18520	.10693	190.1299	191.0501	190.45	190.80
	Cormels	3	161.7633	.03215	.01856	161.6835	161.8432	161.74	161.80
	Total	12	174.9808	18.82752	5.43504	163.0184	186.9433	152.80	194.81

Anti vitamins

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.5580	.02307	.01332	.5007	.6153	.54	.58
	Root	3	.0887	.00058	.00033	.0872	.0901	.09	.09
B1	Petiole	3	.4833	.01155	.00667	.4546	.5120	.47	.49
	Cormels	3	.2167	.01155	.00667	.1880	.2454	.21	.23
	Total	12	.3367	.20018	.05779	.2095	.4639	.09	.58
	Leaf	3	.0683	.00058	.00033	.0669	.0698	.07	.07
	Root	3	.0297	.00153	.00088	.0259	.0335	.03	.03
B2	Petiole	3	.0537	.00153	.00088	.0499	.0575	.05	.06
	Cormels	3	.0357	.00208	.00120	.0305	.0408	.03	.04
	Total	12	.0468	.01596	.00461	.0367	.0570	.03	.07
	Leaf	3	1.8600	.02646	.01528	1.7943	1.9257	1.84	1.89
	Root	3	.0950	.00100	.00058	.0925	.0975	.09	.10
B3	Petiole	3	1.6367	.05686	.03283	1.4954	1.7779	1.59	1.70
	Cormels	3	.9067	.01528	.00882	.8687	.9446	.89	.92
	Total	12	1.1246	.72238	.20853	.6656	1.5836	.09	1.89
	Leaf	3	169.3433	.09292	.05364	169.1125	169.5741	169.28	169.45
	Root	3	.8600	.02646	.01528	.7943	.9257	.84	.89
C	Petiole	3	143.5833	.18930	.10929	143.1131	144.0536	143.45	143.80
	Cormels	3	9.2833	.01528	.00882	9.2454	9.3213	9.27	9.30
	Total	12	80.7675	79.69270	23.00530	30.1332	131.4018	.84	169.45
	Leaf	3	16.7633	.03215	.01856	16.6835	16.8432	16.74	16.80
	Root	3	.8267	.02309	.01333	.7693	.8840	.80	.84
A	Petiole	3	14.3000	.03464	.02000	14.2139	14.3861	14.28	14.34
	Cormels	3	9.7267	.03215	.01856	9.6468	9.8065	9.69	9.75
	Total	12	10.4042	6.34909	1.83282	6.3702	14.4382	.80	16.80
	Leaf	3	11.3067	.12897	.07446	10.9863	11.6270	11.20	11.45
	Root	3	.6467	.01528	.00882	.6087	.6846	.63	.66
E	Petiole	3	6.7967	.01528	.00882	6.7587	6.8346	6.78	6.81
	Cormels	3	.7967	.01528	.00882	.7587	.8346	.78	.81
	Total	12	4.8867	4.65877	1.34487	1.9266	7.8467	.63	11.45

Nwine Vitamin

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.5933	.00577	.00333	.5790	.6077	.59	.60
	Root	3	.0747	.00115	.00067	.0718	.0775	.07	.08
B1	Petiole	3	.5400	.03464	.02000	.4539	.6261	.52	.58
	Cormels	3	.2533	.00577	.00333	.2390	.2677	.25	.26
	Total	12	.3653	.22179	.06403	.2244	.5063	.07	.60
	Leaf	3	.0733	.00115	.00067	.0705	.0762	.07	.07
	Root	3	.0317	.00058	.00033	.0302	.0331	.03	.03
B2	Petiole	3	.0630	.00000	.00000	.0630	.0630	.06	.06
	Cormels	3	.0387	.00058	.00033	.0372	.0401	.04	.04
	Total	12	.0517	.01785	.00515	.0403	.0630	.03	.07
	Leaf	3	1.8500	.02000	.01155	1.8003	1.8997	1.83	1.87
	Root	3	.0837	.00115	.00067	.0808	.0865	.08	.09
B3	Petiole	3	1.7533	.01155	.00667	1.7246	1.7820	1.74	1.76
	Cormels	3	.6967	.01528	.00882	.6587	.7346	.68	.71
	Total	12	1.0959	.77202	.22286	.6054	1.5864	.08	1.87
	Leaf	3	167.4600	.29462	.17010	166.7281	168.1919	167.28	167.80
	Root	3	.8667	.07572	.04372	.6786	1.0548	.78	.92
C	Petiole	3	145.4467	.34429	.19877	144.5914	146.3019	145.20	145.84
	Cormels	3	10.7867	.04163	.02404	10.6832	10.8901	10.74	10.82
	Total	12	81.1400	79.16616	22.85330	30.8402	131.4398	.78	167.80
	Leaf	3	18.2933	.09292	.05364	18.0625	18.5241	18.23	18.40
	Root	3	.6467	.00577	.00333	.6323	.6610	.64	.65
A	Petiole	3	15.2967	.06506	.03756	15.1350	15.4583	15.23	15.36
	Cormels	3	9.8300	.01000	.00577	9.8052	9.8548	9.82	9.84
	Total	12	11.0167	7.01081	2.02385	6.5622	15.4711	.64	18.40
	Leaf	3	10.3333	.04726	.02728	10.2159	10.4507	10.28	10.37
	Root	3	.8967	.02082	.01202	.8450	.9484	.88	.92
E	Petiole	3	6.7900	.15133	.08737	6.4141	7.1659	6.62	6.91
	Cormels	3	1.6533	.00577	.00333	1.6390	1.6677	1.65	1.66
	Total	12	4.9183	4.03428	1.16460	2.3551	7.4816	.88	10.37

Ogeri Vitamins

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.5400	.03000	.01732	.4655	.6145	.51	.57
	Root	3	.0767	.00416	.00240	.0663	.0870	.07	.08
B1	Petiole	3	.4933	.00577	.00333	.4790	.5077	.49	.50
	Cormels	3	.1967	.01528	.00882	.1587	.2346	.18	.21
	Total	12	.3267	.20459	.05906	.1967	.4567	.07	.57
	Leaf	3	.0640	.00173	.00100	.0597	.0683	.06	.07
	Root	3	.0343	.00208	.00120	.0292	.0395	.03	.04
B2	Petiole	3	.0527	.00115	.00067	.0498	.0555	.05	.05
	Cormels	3	.0350	.00361	.00208	.0260	.0440	.03	.04
	Total	12	.0465	.01320	.00381	.0381	.0549	.03	.07
	Leaf	3	1.7067	.01155	.00667	1.6780	1.7354	1.70	1.72
	Root	3	.0847	.00115	.00067	.0818	.0875	.08	.09
B3	Petiole	3	1.6300	.02000	.01155	1.5803	1.6797	1.61	1.65
	Cormels	3	.4800	.02646	.01528	.4143	.5457	.45	.50
	Total	12	.9753	.73908	.21336	.5057	1.4449	.08	1.72
	Leaf	3	163.8300	.02646	.01528	163.7643	163.8957	163.80	163.85
	Root	3	.9300	.01732	.01000	.8870	.9730	.92	.95
C	Petiole	3	146.8000	.02000	.01155	146.7503	146.8497	146.78	146.82
	Cormels	3	11.6567	.04041	.02333	11.5563	11.7571	11.62	11.70
	Total	12	80.8042	78.17812	22.56808	31.1322	130.4762	.92	163.85
	Leaf	3	14.8000	.02000	.01155	14.7503	14.8497	14.78	14.82
	Root	3	.7900	.01000	.00577	.7652	.8148	.78	.80
A	Petiole	3	15.1433	.04041	.02333	15.0429	15.2437	15.12	15.19
	Cormels	3	9.5533	.08963	.05175	9.3307	9.7760	9.45	9.61
	Total	12	10.0717	6.05664	1.74840	6.2235	13.9199	.78	15.19
	Leaf	3	11.5767	.04933	.02848	11.4541	11.6992	11.52	11.61
	Root	3	.6433	.00577	.00333	.6290	.6577	.64	.65
E	Petiole	3	5.8400	.09000	.05196	5.6164	6.0636	5.75	5.93
	Cormels	3	1.8067	.01155	.00667	1.7780	1.8354	1.80	1.82
	Total	12	4.9667	4.46620	1.28928	2.1290	7.8044	.64	11.61

ESC Vitamins

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.6100	.01000	.00577	.5852	.6348	.60	.62
	Root	3	.0757	.00208	.00120	.0705	.0808	.07	.08
B1	Petiole	3	.5167	.00577	.00333	.5023	.5310	.51	.52
	Cormels	3	.2833	.03055	.01764	.2074	.3592	.25	.31
	Total	12	.3714	.21782	.06288	.2330	.5098	.07	.62
	Leaf	3	.0830	.00265	.00153	.0764	.0896	.08	.09
	Root	3	.0340	.00346	.00200	.0254	.0426	.03	.04
B2	Petiole	3	.0727	.00153	.00088	.0689	.0765	.07	.07
	Cormels	3	.0383	.00208	.00120	.0332	.0435	.04	.04
	Total	12	.0570	.02225	.00642	.0429	.0711	.03	.09
	Leaf	3	.9800	.00000	.00000	.9800	.9800	.98	.98
	Root	3	.0840	.00173	.00100	.0797	.0883	.08	.09
B3	Petiole	3	1.8767	.02309	.01333	1.8193	1.9340	1.85	1.89
	Cormels	3	.4833	.00577	.00333	.4690	.4977	.48	.49
	Total	12	.8560	.69916	.20183	.4118	1.3002	.08	1.89
	Leaf	3	165.7800	.02646	.01528	165.7143	165.8457	165.75	165.80
	Root	3	.7933	.01155	.00667	.7646	.8220	.78	.80
C	Petiole	3	160.3567	.06658	.03844	160.1913	160.5221	160.28	160.40
	Cormels	3	10.7300	.02646	.01528	10.6643	10.7957	10.70	10.75
	Total	12	84.4150	82.25702	23.74556	32.1514	136.6786	.78	165.80
	Leaf	3	17.3633	.07234	.04177	17.1836	17.5430	17.28	17.41
	Root	3	.6367	.01155	.00667	.6080	.6654	.63	.65
A	Petiole	3	14.3400	.09539	.05508	14.1030	14.5770	14.28	14.45
	Cormels	3	.7933	.01155	.00667	.7646	.8220	.78	.80
	Total	12	8.2833	7.98369	2.30469	3.2107	13.3559	.63	17.41
	Leaf	3	11.8100	.02646	.01528	11.7443	11.8757	11.79	11.84
	Root	3	.6800	.02646	.01528	.6143	.7457	.65	.70
E	Petiole	3	6.7800	.00000	.00000	6.7800	6.7800	6.78	6.78
	Cormels	3	.6633	.02309	.01333	.6060	.7207	.65	.69
	Total	12	4.9833	4.87145	1.40627	1.8882	8.0785	.65	11.84

Kochi Vitamin

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.4667	.02887	.01667	.3950	.5384	.45	.50
	Root	3	.0677	.00058	.00033	.0662	.0691	.07	.07
B1	Petiole	3	.4267	.00577	.00333	.4123	.4410	.42	.43
	Cormels	3	.2100	.01000	.00577	.1852	.2348	.20	.22
	Total	12	.2928	.17030	.04916	.1845	.4010	.07	.50
	Leaf	3	.0623	.00577	.00333	.0480	.0767	.06	.07
	Root	3	.0253	.00058	.00033	.0239	.0268	.03	.03
B2	Petiole	3	.0503	.00153	.00088	.0465	.0541	.05	.05
	Cormels	3	.0357	.00115	.00067	.0328	.0385	.04	.04
	Total	12	.0434	.01493	.00431	.0339	.0529	.03	.07
	Leaf	3	1.6267	.01155	.00667	1.5980	1.6554	1.62	1.64
	Root	3	.0857	.00153	.00088	.0819	.0895	.08	.09
B3	Petiole	3	1.6000	.01732	.01000	1.5570	1.6430	1.59	1.62
	Cormels	3	.5767	.04933	.02848	.4541	.6992	.52	.61
	Total	12	.9723	.69415	.20038	.5312	1.4133	.08	1.64
	Leaf	3	165.7933	.01155	.00667	165.7646	165.8220	165.78	165.80
	Root	3	.8333	.01155	.00667	.8046	.8620	.82	.84
C	Petiole	3	149.2667	.03055	.01764	149.1908	149.3426	149.24	149.30
	Cormels	3	10.2867	.04163	.02404	10.1832	10.3901	10.24	10.32
	Total	12	81.5450	79.67456	23.00006	30.9222	132.1678	.82	165.80
	Leaf	3	15.4500	.02646	.01528	15.3843	15.5157	15.42	15.47
	Root	3	.7000	.01000	.00577	.6752	.7248	.69	.71
A	Petiole	3	14.7533	.04163	.02404	14.6499	14.8568	14.72	14.80
	Cormels	3	8.9167	.00577	.00333	8.9023	8.9310	8.91	8.92
	Total	12	9.9550	6.17813	1.78347	6.0296	13.8804	.69	15.47
	Leaf	3	11.6600	.03606	.02082	11.5704	11.7496	11.63	11.70
	Root	3	.8767	.01528	.00882	.8387	.9146	.86	.89
E	Petiole	3	1.8433	.56589	.32672	.4376	3.2491	1.19	2.18
	Cormels	3	1.8867	.03512	.02028	1.7994	1.9739	1.85	1.92
	Total	12	4.0667	4.60471	1.32927	1.1410	6.9924	.86	11.70

Anti Phytochemical

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Tannin	Leaf	3	.5233	.00577	.00333	.5090	.5377	.52	.53
	Root	3	.4433	.01155	.00667	.4146	.4720	.43	.45
	Petiole	3	.3567	.01155	.00667	.3280	.3854	.35	.37
	Cormels	3	.1867	.00577	.00333	.1723	.2010	.18	.19
	Total	12	.3775	.13074	.03774	.2944	.4606	.18	.53
Flavonoid	Leaf	3	.7067	.01528	.00882	.6687	.7446	.69	.72
	Root	3	.6067	.01155	.00667	.5780	.6354	.60	.62
	Petiole	3	.5300	.01000	.00577	.5052	.5548	.52	.54
	Cormels	3	.2367	.01155	.00667	.2080	.2654	.23	.25
	Total	12	.5200	.18325	.05290	.4036	.6364	.23	.72
Alkaloid	Leaf	3	.9133	.02309	.01333	.8560	.9707	.90	.94
	Root	3	.8433	.01528	.00882	.8054	.8813	.83	.86
	Petiole	3	.7600	.01732	.01000	.7170	.8030	.75	.78
	Cormels	3	.1767	.01528	.00882	.1387	.2146	.16	.19
	Total	12	.6733	.30521	.08811	.4794	.8673	.16	.94
Saponin	Leaf	3	1.3500	.01732	.01000	1.3070	1.3930	1.34	1.37
	Root	3	1.3100	.01732	.01000	1.2670	1.3530	1.29	1.32
	Petiole	3	1.1733	.01155	.00667	1.1446	1.2020	1.16	1.18
	Cormels	3	.6233	.01155	.00667	.5946	.6520	.61	.63
	Total	12	1.1142	.30405	.08777	.9210	1.3073	.61	1.37
HCN	Leaf	3	5.8133	.01155	.00667	5.7846	5.8420	5.80	5.82
	Root	3	4.7833	.02082	.01202	4.7316	4.8350	4.76	4.80
	Petiole	3	2.3367	.00577	.00333	2.3223	2.3510	2.33	2.34
	Cormels	3	1.7467	.00577	.00333	1.7323	1.7610	1.74	1.75
	Total	12	3.6700	1.75635	.50701	2.5541	4.7859	1.74	5.82
Oxalate	Leaf	3	.5400	.02000	.01155	.4903	.5897	.52	.56
	Root	3	1.7900	.01000	.00577	1.7652	1.8148	1.78	1.80
	Petiole	3	.5333	.01155	.00667	.5046	.5620	.52	.54
	Cormels	3	2.1633	.00577	.00333	2.1490	2.1777	2.16	2.17
	Total	12	1.2567	.76463	.22073	.7708	1.7425	.52	2.17
Phytate	Leaf	3	.5867	.04163	.02404	.4832	.6901	.54	.62
	Root	3	.4933	.00577	.00333	.4790	.5077	.49	.50
	Petiole	3	.4767	.02309	.01333	.4193	.5340	.45	.49
	Cormels	3	.8167	.02082	.01202	.7650	.8684	.80	.84
	Total	12	.5933	.14336	.04138	.5022	.6844	.45	.84

Nwine Phytochemical

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Tannin	Leaf	3	.5667	.01155	.00667	.5380	.5954	.56	.58
	Root	3	.3833	.02082	.01202	.3316	.4350	.36	.40
	Petiole	3	.3333	.02309	.01333	.2760	.3907	.32	.36
	Cormels	3	.1600	.01732	.01000	.1170	.2030	.15	.18
	Total	12	.3608	.15216	.04393	.2642	.4575	.15	.58
Flavonoid	Leaf	3	.7300	.01000	.00577	.7052	.7548	.72	.74
	Root	3	.5633	.03512	.02028	.4761	.6506	.53	.60
	Petiole	3	.5267	.04041	.02333	.4263	.6271	.49	.57
	Cormels	3	.1833	.01155	.00667	.1546	.2120	.17	.19
	Total	12	.5008	.20887	.06030	.3681	.6335	.17	.74
Alkaloid	Leaf	3	.8567	.01155	.00667	.8280	.8854	.85	.87
	Root	3	.8500	.01732	.01000	.8070	.8930	.84	.87
	Petiole	3	.7333	.01155	.00667	.7046	.7620	.72	.74
	Cormels	3	.1233	.00577	.00333	.1090	.1377	.12	.13
	Total	12	.6408	.31641	.09134	.4398	.8419	.12	.87
Saponin	Leaf	3	1.3067	.01155	.00667	1.2780	1.3354	1.30	1.32
	Root	3	1.2400	.01732	.01000	1.1970	1.2830	1.22	1.25
	Petiole	3	1.1000	.01732	.01000	1.0570	1.1430	1.09	1.12
	Cormels	3	.5600	.03464	.02000	.4739	.6461	.54	.60
	Total	12	1.0517	.30712	.08866	.8565	1.2468	.54	1.32
HCN	Leaf	3	5.3267	.06429	.03712	5.1670	5.4864	5.28	5.40
	Root	3	4.9300	.03606	.02082	4.8404	5.0196	4.90	4.97
	Petiole	3	1.9233	.03055	.01764	1.8474	1.9992	1.89	1.95
	Cormels	3	1.2767	.02517	.01453	1.2142	1.3392	1.25	1.30
	Total	12	3.3642	1.86413	.53813	2.1798	4.5486	1.25	5.40
Oxalate	Leaf	3	.5667	.03512	.02028	.4794	.6539	.53	.60
	Root	3	1.6633	.02309	.01333	1.6060	1.7207	1.65	1.69
	Petiole	3	.6233	.02517	.01453	.5608	.6858	.60	.65
	Cormels	3	2.2667	.02517	.01453	2.2042	2.3292	2.24	2.29
	Total	12	1.2800	.75001	.21651	.8035	1.7565	.53	2.29
Phytate	Leaf	3	.5100	.01000	.00577	.4852	.5348	.50	.52
	Root	3	.4700	.03464	.02000	.3839	.5561	.45	.51
	Petiole	3	.4033	.02517	.01453	.3408	.4658	.38	.43
	Cormels	3	.7533	.02309	.01333	.6960	.8107	.74	.78
	Total	12	.5342	.13964	.04031	.4454	.6229	.38	.78

Ogeri Phytochemicals

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.6467	.02517	.01453	.5842	.7092	.62	.67
	Root	3	.4600	.01732	.01000	.4170	.5030	.45	.48
Tannin	Petiole	3	.3833	.00577	.00333	.3690	.3977	.38	.39
	Cormels	3	.2133	.00577	.00333	.1990	.2277	.21	.22
	Total	12	.4258	.16312	.04709	.3222	.5295	.21	.67
	Leaf	3	.7767	.00577	.00333	.7623	.7910	.77	.78
	Root	3	.6100	.01000	.00577	.5852	.6348	.60	.62
Flavonoid	Petiole	3	.5733	.01528	.00882	.5354	.6113	.56	.59
	Cormels	3	.3067	.01528	.00882	.2687	.3446	.29	.32
	Total	12	.5667	.17634	.05091	.4546	.6787	.29	.78
	Leaf	3	.9400	.01732	.01000	.8870	.9730	.92	.95
	Root	3	.9300	.02000	.01155	.8803	.9797	.91	.95
Alkaloid	Petiole	3	.8367	.01528	.00882	.7987	.8746	.82	.85
	Cormels	3	.1867	.00577	.00333	.1723	.2010	.18	.19
	Total	12	.7208	.32483	.09377	.5144	.9272	.18	.95
	Leaf	3	1.4133	.01155	.00667	1.3846	1.4420	1.40	1.42
	Root	3	1.3267	.02082	.01202	1.2750	1.3784	1.31	1.35
Saponin	Petiole	3	.8633	.56580	.32667	-.5422	2.2689	.21	1.19
	Cormels	3	.6333	.01528	.00882	.5954	.6713	.62	.65
	Total	12	1.0592	.41472	.11972	.7957	1.3227	.21	1.42
	Leaf	3	5.2767	.03215	.01856	5.1968	5.3565	5.24	5.30
	Root	3	4.7967	.01528	.00882	4.7587	4.8346	4.78	4.81
HCN	Petiole	3	1.7063	.27523	.15890	1.0226	2.3900	1.39	1.88
	Cormels	3	1.3500	.01732	.01000	1.3070	1.3930	1.34	1.37
	Total	12	3.2824	1.84931	.53385	2.1074	4.4574	1.34	5.30
	Leaf	3	.7667	.03055	.01764	.6908	.8426	.74	.80
	Root	3	1.6233	.02517	.01453	1.5608	1.6858	1.60	1.65
Oxalate	Petiole	3	.6200	.02000	.01155	.5703	.6697	.60	.64
	Cormels	3	2.1867	.02517	.01453	2.1242	2.2492	2.16	2.21
	Total	12	1.2992	.66864	.19302	.8743	1.7240	.60	2.21
	Leaf	3	.5033	.01528	.00882	.4654	.5413	.49	.52
	Root	3	.3967	.00577	.00333	.3823	.4110	.39	.40
Phytate	Petiole	3	.4033	.01528	.00882	.3654	.4413	.39	.42
	Cormels	3	.7667	.04163	.02404	.6632	.8701	.72	.80
	Total	12	.5175	.15789	.04558	.4172	.6178	.39	.80

ESC Phytochemical

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.4133	.01155	.00667	.3846	.4420	.40	.42
	Root	3	.3433	.00577	.00333	.3290	.3577	.34	.35
Tannin	Petiole	3	.2933	.00577	.00333	.2790	.3077	.29	.30
	Cormels	3	.1233	.00577	.00333	.1090	.1377	.12	.13
	Total	12	.2933	.11195	.03232	.2222	.3645	.12	.42
	Leaf	3	.5467	.01528	.00882	.5087	.5846	.53	.56
	Root	3	.5767	.02082	.01202	.5250	.6284	.56	.60
Flavonoid	Petiole	3	.4667	.01528	.00882	.4287	.5046	.45	.48
	Cormels	3	.1833	.01155	.00667	.1546	.2120	.17	.19
	Total	12	.4433	.16289	.04702	.3398	.5468	.17	.60
	Leaf	3	.7000	.05292	.03055	.5686	.8314	.64	.74
	Root	3	.8000	.15620	.09018	.4120	1.1880	.70	.98
Alkaloid	Petiole	3	.6600	.05196	.03000	.5309	.7891	.63	.72
	Cormels	3	.0900	.01000	.00577	.0652	.1148	.08	.10
	Total	12	.5625	.29912	.08635	.3724	.7526	.08	.98
	Leaf	3	1.2467	.01528	.00882	1.2087	1.2846	1.23	1.26
	Root	3	1.1867	.00577	.00333	1.1723	1.2010	1.18	1.19
Saponin	Petiole	3	1.0600	.01000	.00577	1.0352	1.0848	1.05	1.07
	Cormels	3	.4933	.01155	.00667	.4646	.5220	.48	.50
	Total	12	.9967	.31172	.08999	.7986	1.1947	.48	1.26
	Leaf	3	4.5100	.01000	.00577	4.4852	4.5348	4.50	4.52
	Root	3	3.4600	.01732	.01000	3.4170	3.5030	3.45	3.48
HCN	Petiole	3	1.7433	.00577	.00333	1.7290	1.7577	1.74	1.75
	Cormels	3	1.6367	.01155	.00667	1.6080	1.6654	1.63	1.65
	Total	12	2.8375	1.26034	.36383	2.0367	3.6383	1.63	4.52
	Leaf	3	.4733	.01155	.00667	.4446	.5020	.46	.48
	Root	3	1.5333	.02309	.01333	1.4760	1.5907	1.52	1.56
Oxalate	Petiole	3	.5467	.05033	.02906	.4216	.6717	.50	.60
	Cormels	3	1.6900	.03606	.02082	1.6004	1.7796	1.65	1.72
	Total	12	1.0608	.57957	.16731	.6926	1.4291	.46	1.72
	Leaf	3	.3800	.02000	.01155	.3303	.4297	.36	.40
	Root	3	.3700	.01732	.01000	.3270	.4130	.35	.38
Phytate	Petiole	3	.3200	.05292	.03055	.1886	.4514	.28	.38
	Cormels	3	.6333	.01528	.00882	.5954	.6713	.62	.65
	Total	12	.4258	.13000	.03753	.3432	.5084	.28	.65

Kochi Phytochemical

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	.6067	.02887	.01667	.5350	.6784	.59	.64
	Root	3	.4433	.02082	.01202	.3916	.4950	.42	.46
Tannin	Petiole	3	.3767	.02517	.01453	.3142	.4392	.35	.40
	Cormels	3	.1967	.01155	.00667	.1680	.2254	.19	.21
	Total	12	.4058	.15465	.04464	.3076	.5041	.19	.64
	Leaf	3	.7133	.04041	.02333	.6129	.8137	.67	.75
	Root	3	.5900	.02000	.01155	.5403	.6397	.57	.61
Flavonoid	Petiole	3	.6000	.06000	.03464	.4510	.7490	.54	.66
	Cormels	3	.2567	.03055	.01764	.1808	.3326	.23	.29
	Total	12	.5400	.18151	.05240	.4247	.6553	.23	.75
	Leaf	3	.9033	.03215	.01856	.8235	.9832	.88	.94
	Root	3	.9200	.02000	.01155	.8703	.9697	.90	.94
Alkaloid	Petiole	3	.7667	.02082	.01202	.7150	.8184	.75	.79
	Cormels	3	.1767	.01528	.00882	.1387	.2146	.16	.19
	Total	12	.6917	.31731	.09160	.4901	.8933	.16	.94
	Leaf	3	1.3500	.03000	.01732	1.2755	1.4245	1.32	1.38
	Root	3	1.3133	.01155	.00667	1.2846	1.3420	1.30	1.32
Saponin	Petiole	3	1.1800	.02000	.01155	1.1303	1.2297	1.16	1.20
	Cormels	3	.6233	.04933	.02848	.5008	.7459	.59	.68
	Total	12	1.1167	.30589	.08830	.9223	1.3110	.59	1.38
	Leaf	3	5.5100	.09000	.05196	5.2864	5.7336	5.42	5.60
	Root	3	4.7867	.05132	.02963	4.6592	4.9141	4.73	4.83
HCN	Petiole	3	1.9300	.01732	.01000	1.8870	1.9730	1.92	1.95
	Cormels	3	1.3500	.01732	.01000	1.3070	1.3930	1.34	1.37
	Total	12	3.3942	1.86443	.53822	2.2096	4.5788	1.34	5.60
	Leaf	3	.6633	.02309	.01333	.6060	.7207	.65	.69
	Root	3	1.5867	.01155	.00667	1.5580	1.6154	1.58	1.60
Oxalate	Petiole	3	.6500	.01732	.01000	.6070	.6930	.64	.67
	Cormels	3	2.3233	.02517	.01453	2.2608	2.3858	2.30	2.35
	Total	12	1.3058	.73078	.21096	.8415	1.7702	.64	2.35
	Leaf	3	.4833	.01155	.00667	.4546	.5120	.47	.49
	Root	3	.3833	.02082	.01202	.3316	.4350	.36	.40
Phytate	Petiole	3	.3533	.01155	.00667	.3246	.3820	.34	.36
	Cormels	3	.7033	.01528	.00882	.6654	.7413	.69	.72
	Total	12	.4808	.14387	.04153	.3894	.5722	.34	.72

Anti proximate

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	10.2433	.01155	.00667	10.2146	10.2720	10.23	10.25
	Root	3	10.3333	.08327	.04807	10.1265	10.5402	10.24	10.40
MC	Petiole	3	10.8133	.03055	.01764	10.7374	10.8892	10.78	10.84
	Cormels	3	11.3867	.03512	.02028	11.2994	11.4739	11.35	11.42
	Total	12	10.6942	.47674	.13762	10.3913	10.9971	10.23	11.42
	Leaf	3	89.7633	.01155	.00667	89.7346	89.7920	89.75	89.77
	Root	3	89.7267	.07767	.04485	89.5337	89.9196	89.64	89.79
DM	Petiole	3	89.1933	.03055	.01764	89.1174	89.2692	89.16	89.22
	Cormels	3	88.6167	.03512	.02028	88.5294	88.7039	88.58	88.65
	Total	12	89.3250	.48937	.14127	89.0141	89.6359	88.58	89.79
	Leaf	3	22.6433	.05132	.02963	22.5159	22.7708	22.60	22.70
	Root	3	23.7667	.03055	.01764	23.6908	23.8426	23.74	23.80
ASH	Petiole	3	23.6867	.20502	.11837	23.1774	24.1960	23.45	23.81
	Cormels	3	7.3400	.06000	.03464	7.1910	7.4890	7.28	7.40
	Total	12	19.3592	7.26321	2.09671	14.7443	23.9740	7.28	23.81
	Leaf	3	12.7967	.03512	.02028	12.7094	12.8839	12.76	12.83
	Root	3	8.4900	.01000	.00577	8.4652	8.5148	8.48	8.50
CF	Petiole	3	10.6333	.01528	.00882	10.5954	10.6713	10.62	10.65
	Cormels	3	.5100	.01732	.01000	.4670	.5530	.49	.52
	Total	12	8.1075	4.84968	1.39998	5.0262	11.1888	.49	12.83
	Leaf	3	8.4867	.06110	.03528	8.3349	8.6384	8.42	8.54
	Root	3	.7533	.01155	.00667	.7246	.7820	.74	.76
EE	Petiole	3	3.3233	.06658	.03844	3.1579	3.4887	3.28	3.40
	Cormels	3	.8600	.03000	.01732	.7855	.9345	.83	.89
	Total	12	3.3558	3.27531	.94550	1.2748	5.4369	.74	8.54
	Leaf	3	21.7400	.12166	.07024	21.4378	22.0422	21.60	21.82
	Root	3	5.6700	.03000	.01732	5.5955	5.7445	5.64	5.70
CP	Petiole	3	17.5100	.09000	.05196	17.2864	17.7336	17.42	17.60
	Cormels	3	8.3167	.01528	.00882	8.2787	8.3546	8.30	8.33
	Total	12	13.3092	6.84950	1.97728	8.9572	17.6611	5.64	21.82
	Leaf	3	24.0900	.09539	.05508	23.8530	24.3270	23.98	24.15
	Root	3	50.9867	.11719	.06766	50.6956	51.2778	50.90	51.12
CHO	Petiole	3	34.0367	.37448	.21620	33.1064	34.9669	33.72	34.45
	Cormels	3	71.5867	.13503	.07796	71.2512	71.9221	71.45	71.72
	Total	12	45.1750	18.83019	5.43581	33.2109	57.1391	23.98	71.72

Nwine Proximate

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MC	Leaf	3	8.7767	.02082	.01202	8.7250	8.8284	8.76	8.80
	Root	3	7.3267	.06429	.03712	7.1670	7.4864	7.28	7.40
	Petiole	3	9.8500	.01000	.00577	9.8252	9.8748	9.84	9.86
	Cormels	3	9.8000	.11533	.06658	9.5135	10.0865	9.69	9.92
	Total	12	8.9383	1.07143	.30929	8.2576	9.6191	7.28	9.92
DM	Leaf	3	91.2300	.02646	.01528	91.1643	91.2957	91.20	91.25
	Root	3	92.6433	.06658	.03844	92.4779	92.8087	92.60	92.72
	Petiole	3	90.1600	.02000	.01155	90.1103	90.2097	90.14	90.18
	Cormels	3	90.1700	.07810	.04509	89.9760	90.3640	90.08	90.22
	Total	12	91.0508	1.06328	.30694	90.3753	91.7264	90.08	92.72
ASH	Leaf	3	18.6500	.07810	.04509	18.4560	18.8440	18.60	18.74
	Root	3	20.6600	.03606	.02082	20.5704	20.7496	20.63	20.70
	Petiole	3	16.2433	.00577	.00333	16.2290	16.2577	16.24	16.25
	Cormels	3	12.7233	.02309	.01333	12.6660	12.7807	12.71	12.75
	Total	12	17.0692	3.08810	.89146	15.1071	19.0313	12.71	20.70
CF	Leaf	3	13.7900	.12288	.07095	13.4847	14.0953	13.70	13.93
	Root	3	9.8033	.02517	.01453	9.7408	9.8658	9.78	9.83
	Petiole	3	9.7133	.04163	.02404	9.6099	9.8168	9.68	9.76
	Cormels	3	.5233	.00577	.00333	.5090	.5377	.52	.53
	Total	12	8.4575	5.08438	1.46773	5.2270	11.6880	.52	13.93
EE	Leaf	3	3.1767	.01528	.00882	3.1387	3.2146	3.16	3.19
	Root	3	.4900	.01000	.00577	.4652	.5148	.48	.50
	Petiole	3	3.0467	.01155	.00667	3.0180	3.0754	3.04	3.06
	Cormels	3	.8300	.01732	.01000	.7870	.8730	.81	.84
	Total	12	1.8858	1.28743	.37165	1.0678	2.7038	.48	3.19
CP	Leaf	3	21.7800	.04359	.02517	21.6717	21.8883	21.73	21.81
	Root	3	6.6467	.10116	.05840	6.3954	6.8980	6.53	6.71
	Petiole	3	16.9133	.03215	.01856	16.8335	16.9932	16.89	16.95
	Cormels	3	7.8900	.04359	.02517	7.7817	7.9983	7.84	7.92
	Total	12	13.3075	6.57498	1.89803	9.1300	17.4850	6.53	21.81
CHO	Leaf	3	33.8933	.15503	.08950	33.5082	34.2784	33.74	34.05
	Root	3	55.0733	.20526	.11851	54.5634	55.5832	54.90	55.30
	Petiole	3	44.2333	.03786	.02186	44.1393	44.3274	44.19	44.26
	Cormels	3	68.2333	.13204	.07623	67.9053	68.5613	68.09	68.35
	Total	12	50.3583	13.31863	3.84476	41.8961	58.8206	33.74	68.35

Ogeri Phytochemical

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MC	Leaf	3	9.6333	.01528	.00882	9.5954	9.6713	9.62	9.65
	Root	3	6.7700	.01732	.01000	6.7270	6.8130	6.75	6.78
	Petiole	3	8.9033	.05508	.03180	8.7665	9.0401	8.84	8.94
	Cormels	3	9.8167	.18824	.10868	9.3491	10.2843	9.60	9.94
	Total	12	8.7808	1.26680	.36569	7.9759	9.5857	6.75	9.94
DM	Leaf	3	90.3767	.02517	.01453	90.3142	90.4392	90.35	90.40
	Root	3	93.2167	.00577	.00333	93.2023	93.2310	93.21	93.22
	Petiole	3	91.0967	.05508	.03180	90.9599	91.2335	91.06	91.16
	Cormels	3	90.3633	.28676	.16556	89.6510	91.0757	90.06	90.63
	Total	12	91.2633	1.22438	.35345	90.4854	92.0413	90.06	93.22
ASH	Leaf	3	22.7467	.01155	.00667	22.7180	22.7754	22.74	22.76
	Root	3	23.8200	.02000	.01155	23.7703	23.8697	23.80	23.84
	Petiole	3	17.4833	.10116	.05840	17.2320	17.7346	17.42	17.60
	Cormels	3	10.6367	.01528	.00882	10.5987	10.6746	10.62	10.65
	Total	12	18.6717	5.45456	1.57460	15.2060	22.1373	10.62	23.84
CF	Leaf	3	12.7867	.01155	.00667	12.7580	12.8154	12.78	12.80
	Root	3	10.3467	.10116	.05840	10.0954	10.5980	10.23	10.41
	Petiole	3	11.8867	.03055	.01764	11.8108	11.9626	11.86	11.92
	Cormels	3	.6100	.01000	.00577	.5852	.6348	.60	.62
	Total	12	8.9075	5.08610	1.46823	5.6759	12.1391	.60	12.80
EE	Leaf	3	3.1267	.01155	.00667	3.0980	3.1554	3.12	3.14
	Root	3	.4933	.01155	.00667	.4646	.5220	.48	.50
	Petiole	3	2.8200	.09849	.05686	2.5753	3.0647	2.71	2.90
	Cormels	3	.9500	.01732	.01000	.9070	.9930	.94	.97
	Total	12	1.8475	1.19409	.34470	1.0888	2.6062	.48	3.14
CP	Leaf	3	22.8167	.02082	.01202	22.7650	22.8684	22.80	22.84
	Root	3	5.8633	.04933	.02848	5.7408	5.9859	5.83	5.92
	Petiole	3	18.9100	.01000	.00577	18.8852	18.9348	18.90	18.92
	Cormels	3	8.7400	.02000	.01155	8.6903	8.7897	8.72	8.76
	Total	12	14.0825	7.30547	2.10891	9.4408	18.7242	5.83	22.84
CHO	Leaf	3	28.8900	.04583	.02646	28.7762	29.0038	28.85	28.94
	Root	3	52.7067	.07638	.04410	52.5169	52.8964	52.64	52.79
	Petiole	3	39.9967	.17616	.10171	39.5591	40.4343	39.80	40.14
	Cormels	3	69.2467	.15885	.09171	68.8521	69.6413	69.15	69.43
	Total	12	47.7100	15.68895	4.52901	37.7417	57.6783	28.85	69.43

ESC proximate

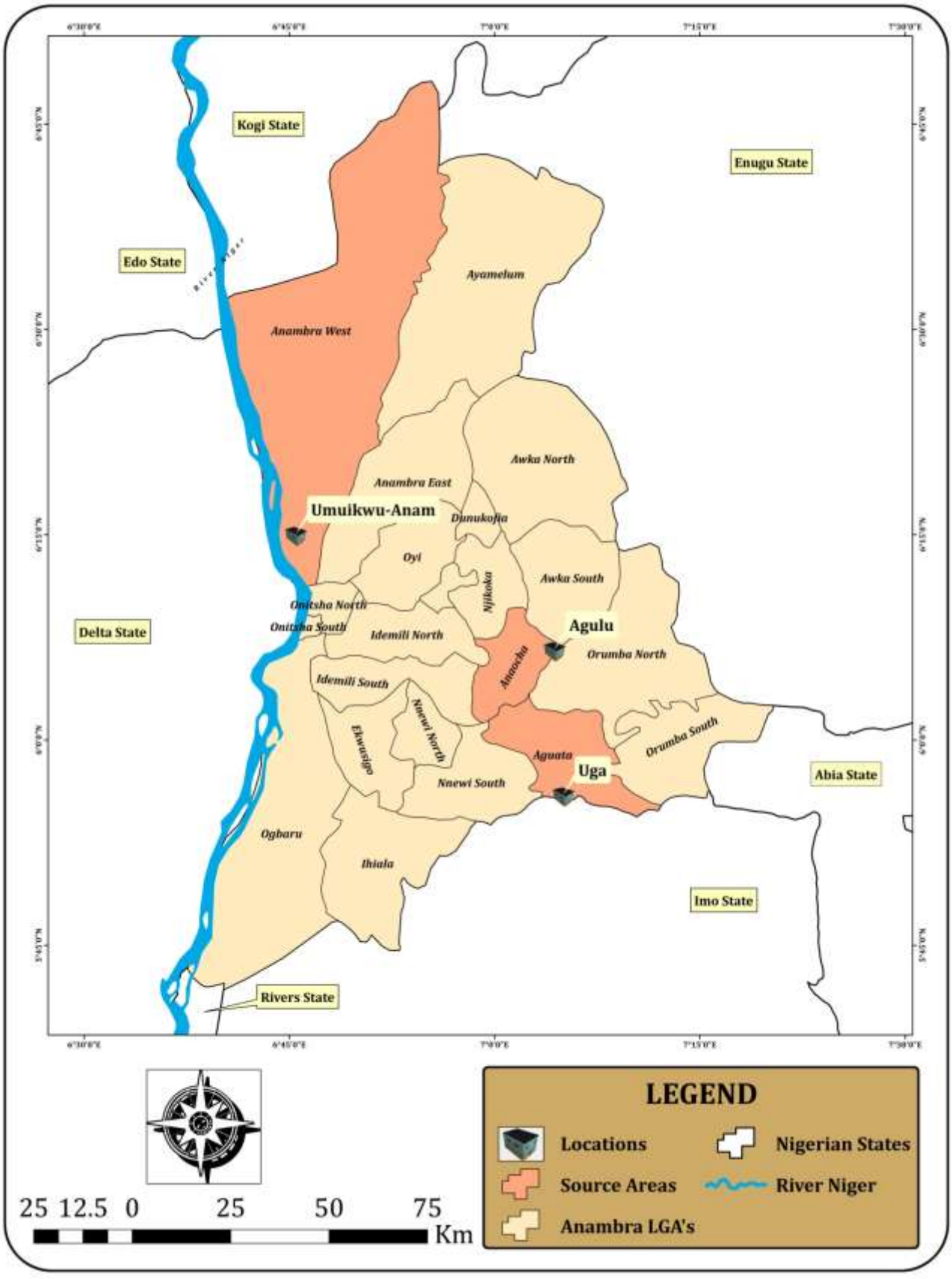
Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	Leaf	3	9.6167	.01528	.00882	9.5787	9.6546	9.60	9.63
	Root	3	7.7133	.08327	.04807	7.5065	7.9202	7.62	7.78
MC	Petiole	3	10.6233	.07095	.04096	10.4471	10.7996	10.56	10.70
	Cormels	3	11.7733	.03055	.01764	11.6974	11.8492	11.74	11.80
	Total	12	9.9317	1.55789	.44973	8.9418	10.9215	7.62	11.80
	Leaf	3	90.3767	.02517	.01453	90.3142	90.4392	90.35	90.40
	Root	3	92.3033	.08021	.04631	92.1041	92.5026	92.22	92.38
DM	Petiole	3	89.1333	.72176	.41671	87.3404	90.9263	88.30	89.56
	Cormels	3	88.2067	.01155	.00667	88.1780	88.2354	88.20	88.22
	Total	12	90.0050	1.63204	.47113	88.9681	91.0419	88.20	92.38
	Leaf	3	24.3900	.02646	.01528	24.3243	24.4557	24.36	24.41
	Root	3	23.7667	.03055	.01764	23.6908	23.8426	23.74	23.80
ASH	Petiole	3	21.5300	.06245	.03606	21.3749	21.6851	21.48	21.60
	Cormels	3	7.6200	.02000	.01155	7.5703	7.6697	7.60	7.64
	Total	12	19.3267	7.14631	2.06296	14.7861	23.8672	7.60	24.41
	Leaf	3	13.4600	.01732	.01000	13.4170	13.5030	13.45	13.48
	Root	3	9.6233	.02517	.01453	9.5608	9.6858	9.60	9.65
CF	Petiole	3	10.4567	.04041	.02333	10.3563	10.5571	10.42	10.50
	Cormels	3	.6500	.03464	.02000	.5639	.7361	.63	.69
	Total	12	8.5475	4.99021	1.44055	5.3769	11.7181	.63	13.48
	Leaf	3	3.4433	.02082	.01202	3.3916	3.4950	3.42	3.46
	Root	3	.8333	.01155	.00667	.8046	.8620	.82	.84
EE	Petiole	3	2.7900	.01000	.00577	2.7652	2.8148	2.78	2.80
	Cormels	3	.9233	.00577	.00333	.9090	.9377	.92	.93
	Total	12	1.9975	1.19408	.34470	1.2388	2.7562	.82	3.46
	Leaf	3	22.5400	.07937	.04583	22.3428	22.7372	22.45	22.60
	Root	3	6.7667	.04163	.02404	6.6632	6.8701	6.72	6.80
CP	Petiole	3	16.3500	.01732	.01000	16.3070	16.3930	16.34	16.37
	Cormels	3	8.3133	.01155	.00667	8.2846	8.3420	8.30	8.32
	Total	12	13.4925	6.64876	1.91933	9.2681	17.7169	6.72	22.60
	Leaf	3	26.5500	.13000	.07506	26.2271	26.8729	26.47	26.70
	Root	3	51.2967	.17954	.10366	50.8507	51.7427	51.16	51.50
CHO	Petiole	3	38.2500	.18083	.10440	37.8008	38.6992	38.06	38.42
	Cormels	3	70.7267	.03786	.02186	70.6326	70.8207	70.70	70.77
	Total	12	46.7058	17.12964	4.94490	35.8222	57.5895	26.47	70.77

Kochi Proximate

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MC	Leaf	3	10.7367	.01528	.00882	10.6987	10.7746	10.72	10.75
	Root	3	9.8833	.04933	.02848	9.7608	10.0059	9.85	9.94
	Petiole	3	10.2833	.03512	.02028	10.1961	10.3706	10.25	10.32
	Cormels	3	9.4800	.02646	.01528	9.4143	9.5457	9.45	9.50
	Total	12	10.0958	.48803	.14088	9.7858	10.4059	9.45	10.75
DM	Leaf	3	89.2800	.02000	.01155	89.2303	89.3297	89.26	89.30
	Root	3	90.3000	.22716	.13115	89.7357	90.8643	90.14	90.56
	Petiole	3	89.6767	.04509	.02603	89.5647	89.7887	89.63	89.72
	Cormels	3	90.5133	.03215	.01856	90.4335	90.5932	90.49	90.55
	Total	12	89.9425	.52222	.15075	89.6107	90.2743	89.26	90.56
ASH	Leaf	3	22.6000	.13229	.07638	22.2714	22.9286	22.45	22.70
	Root	3	23.8167	.02082	.01202	23.7650	23.8684	23.80	23.84
	Petiole	3	23.4100	.68418	.39501	21.7104	25.1096	22.62	23.81
	Cormels	3	6.9367	.02517	.01453	6.8742	6.9992	6.91	6.96
	Total	12	19.1908	7.40966	2.13898	14.4830	23.8987	6.91	23.84
CF	Leaf	3	13.8133	.02309	.01333	13.7560	13.8707	13.80	13.84
	Root	3	9.8533	.01155	.00667	9.8246	9.8820	9.84	9.86
	Petiole	3	12.7667	.03055	.01764	12.6908	12.8426	12.74	12.80
	Cormels	3	.5033	.01528	.00882	.4654	.5413	.49	.52
	Total	12	9.2342	5.47870	1.58157	5.7532	12.7152	.49	13.84
EE	Leaf	3	2.2967	.01528	.00882	2.2587	2.3346	2.28	2.31
	Root	3	.4800	.02646	.01528	.4143	.5457	.45	.50
	Petiole	3	2.7267	.02309	.01333	2.6693	2.7840	2.70	2.74
	Cormels	3	.6033	.01528	.00882	.5654	.6413	.59	.62
	Total	12	1.5267	1.04212	.30084	.8645	2.1888	.45	2.74
CP	Leaf	3	21.6600	.03606	.02082	21.5704	21.7496	21.63	21.70
	Root	3	6.8933	.04163	.02404	6.7899	6.9968	6.86	6.94
	Petiole	3	19.5000	.08660	.05000	19.2849	19.7151	19.45	19.60
	Cormels	3	7.5700	.04359	.02517	7.4617	7.6783	7.52	7.60
	Total	12	13.9058	7.02103	2.02680	9.4449	18.3668	6.86	21.70
CHO	Leaf	3	28.8933	.20526	.11851	28.3834	29.4032	28.72	29.12
	Root	3	49.0733	.03786	.02186	48.9793	49.1674	49.03	49.10
	Petiole	3	31.3133	.64856	.37445	29.7022	32.9245	30.89	32.06
	Cormels	3	74.9067	.09609	.05548	74.6680	75.1454	74.82	75.01
	Total	12	46.0467	19.21423	5.54667	33.8385	58.2548	28.72	75.01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