CHAPTHER ONE

INTRODUCTION

1.1 Background of the Study

Plants are important in our everyday existence. They provide our foods, produce the oxygen we breathe, and serve as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our buildings and in the manufacture of biofuels, dyes, perfumes, pesticides, adsorbents and drugs. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development (Ajibesin, 2011). Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques. The phytoconstituents which are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. Plants are now occupying important position in allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Medicinal plants are the sources of many important drugs of the modern world. Many of these indigenous medicinal plants are used as spices and food plants; they are also sometimes added to foods meant for pregnant mothers for medicinal purposes (Akinpela and Onakoya, 2006). Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for

their worldwide attention and use. The medicinal properties of some plants have been documented by some researchers (Akinpelu and Onukoya, 2006). Medicinal plants are of great importance to the health of individuals and communities. It was the advent of antibiotics in the 1950s that led to the decline of the use of plant derivatives as antimicrobials (Marjorie, 1999). Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments (Ajibesin, 2011). A relatively small percentage of less than 10% of all the plants on earth is believed to serve as sources of medicine (Marjorie, 1999).

In an effort to find alternative sources of feedstuffs to replace some or all of the maize in the diet of pigs and other non-ruminant farm animals, several studies have been conducted to determine the suitability of some agro-industrial wastes as feed ingredients. These include cocoa pod husks, brewers spent grains, rice bran, maize bran, groundnut skins, and wheat bran. However, one by-product that requires consideration is cashew nut testa, a by-product obtained from the processing of cashew nuts. Its utilization as animal feed even at relatively low dosage formulations will minimize its disposal problem as well as reduce the cost of animal feeding.

1.2 Statement of the Problem

It is now known that agricultural materials are used as animal feeds and that they contain phytochemicals. These phytochemicals serve as antibiotic principles of plants.

The need for a cheap, renewable, easily available and nutritive source of material as feed supplements has therefore attracted me to investigate African pear leaf, (APL) as an alternative.

1.3 Objectives of the Study

Broadly stated, the purpose of this work is to investigate/assess the nutritive and medicinal values of African pear leaf as an effective replacement in animal diets. Specifically, this work investigated:

(i) the proximate constituents of African pear leaf; and

(ii) the qualitative and quantitative phytochemicals of African pear leaf.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Dacryodes edulis*

Dacryodes edulis is an oliferous fruit tree found in equatorial and humid tropic climates and originates from Central Africa and Gulf of Guinea area (Ayuk et al, 1999). Its actual geographical area spreads from nearly all over the western coast of Africa across to Uganda. It is an evergreen tree attaining a height of 18–40 m in the forest but not exceeding 12 m in plantations. It has a relatively short trunk and a deep, dense crown. The bark is pale gray and rough with droplets of resin. The leaves are a compound with 5-8 pairs of leaflets. The upper surface of the leaves is glossy. The flowers are yellow and about 5 mm across. They are arranged in a large inflorescence. The fruit is an ellipsoidal drupe which varies in length from 4 to 12 cm. The skin of the fruit is dark blue or violet, whereas the flesh is pale to light green. The tree flowers at the beginning of the rainy season and bears fruits during 2 to 5 months after flowering. There are two variants of Dacryodes edulis: D. e. var. edulis and D. e. var. parvicarpa. The fruit of D. e. var. edulis is larger and the tree has stout, ascending branches. D. e. var. parvicarpa has smaller fruit and slender, drooping branches.(Kola, et al, 2011)

The preferential habitat of D. edulis is a shady, humid tropical forest. However, it adapts well to variations in soil type, humidity, temperature and day length. The natural range extends from Angola in the South, Nigeria in the North, Sierra Leone in the West and Uganda in the East. It is also cultivated in Malaysia.

A traditional food plant in Africa, this little-known fruit has potential to improve nutrition, boost food security, foster rural development and support sustainable land care. The main use of D. edulis is its fruit, which can be eaten raw, cooked in warm salt water or roasted. Cooked flesh of the fruit has a texture similar to butter. The pulp contains 48% oil and a plantation can produce 7-8 tons of oil per hectare. It is also rich in vitamins. The kernel can be used as fodder for sheep or goats. The flowers are useful in apiculture. Shade tolerant traditional crops, such as Xanthosoma sagittifolium and taro can be co-cultivated with D. edulis.

The wood of D. edulis is elastic, greyish-white to pinkish. The wood has general use for tool handles, and occasionally for mortars, and is suitable for carpentry. The resin is sometimes burnt for lighting or used as glue. The tree is used as an ornamental plant and is known to improve soil quality by providing large quantities of biomass. The tree is also a source of many herbal medicines.

The plant has long been used in the traditional medicine of some African countries to treat various ailments such as wound, skin diseases, dysentery and fever. The extracts and secondary metabolites have been found to show biological activities such as antimicrobial, antioxidant and anti sickle-cell disease [Ajibesin, 2011]. A wide range of chemical constituents such as terpenes, flavonoids, tannins, alkaloids and saponins have been isolated from the plant [Ajibesin, 2011]. The bark of the plant has long been used to cicatrize wound in Gabon [Walker and Silans, 1961]. In Democratic Republic of Congo, the plant is employed for the treatment of diver’s ailments. The decoction of the bark is taken orally to treat leprosy. It is used as gargle and mouth wash to treat tonsillitis

[Bouquet, 1969]. The bark is comminuted with meleguetta pepper to cure dysentery, anemia, spitting blood and as an emmenagogue; when mixed with palm oil, it is applied topically to relieve pains, debility, stiffness and skin diseases [Bouquet, 1969]. The leaves are chewed with kola nut as an antilmentic. The leaf sap is used as ear drop to treat ear trouble, while a leaf decoction is prepared to produce vapour that treats fever and headache [Bouquet, 1969; Bouet, 1980]. In Congo Brazzaville, the leaves are boiled with those of Lantana camara, Cymbopogon citratus and Persea Americana in water to form a decoction for treating malaria. A steam bath can also be taken from the decoction to treat the same ailment. Boiling the leaves with those of P. Americana alone can be used to treat headache, antalgic and cephalgy [Diafouka, 1997]. Recently, Jiofack et al [2010] reported that the leaves are made into plaster to treat snakebite in southwest Cameroon. The bark resin is used in Nigeria to treat parasitic skin diseases and jiggers [Dalziel, 1937; Hutchinson et al, 1963]. When applied in lotions and creams, the resin smoothens and protects the skin [Ekpa, 1993]. The aroma of the resin when liberated through burning is believed to ward off evil spirit in Nigeria [Sofowora, 2008]. The leaves are often crushed and the juice released to treat generalized skin diseases such as scabies, ringworm, rash and wound, while the stem or stem twigs are employed as chewing sticks for oral hygiene [Igoli et al, 2005; Ajibesin et al, 2008].

2.2 Empirical studies on Dacryodes Edulis

The oil from edible African pear (Dacryodes edulis) was extracted with chloroform. The oil were characterized for melting point, refractive index, relative viscosity, free fatty acids, saponification value, iodine value, acid value and percentage unsaponifable matter. The percent oil content in the fruit pulp was determined. The oil content of African pear was 23.2%. The fatty acid content determined in % are: palmitic (9.06), stearic (15.46), stearic isomer (18.00), oleic (26.63) and linoleic (30.85). Results on physical characteristics are: average melting point (80^{o}C), refractive index (1.456), viscosity (0.33 poise). Results on chemical characteristics are: free fatty acid (1.100%), saponification value (143.760), iodine value (44.079), acid value (15.280), ester value (128.480), unsaponifiable matter (53.920%). The physico-chemical characteristics and fatty acid composition of these oils, suggest some industrial potentials [Ikhuoria and Maliki, 2007].

The phytochemical contents and medicinal values of Dacryodes edulis exudates were investigated. Phytochemical screening of the plant showed that it contain the presence of bioactive compounds comprising saponins (2.08–3.98mg 100g^{-1}), alkaloids (0.28–0.49 mg 100g^{-1}), tannins (0.47–0.72 mg 100g^{-1}), flavonoids (0.26–0.39 mg 100g^{-1}), and phenolic compounds (0.01–0.05 mg 100g^{-1}). The carbohydrates, lipids and protein content were 77.42–78.90%, 2.02–4.185% and 16.63–18.38% respectively. The exudate is a good source of water soluble vitamins; ascorbic acid (7.04–26.40 mg 100g^{-1}), niacin (3.12–4.00 mg 100g^{-1}), riboflavin (0.14–0.54 mg 100g^{-1}) and thiamine (0.15–0.22 mg 100g^{-1}),). The plant exudate is a good source of minerals such as Ca, Mg, P, Fe, Zn, Cu and Mn while Cr and Co were in trace values. These results indicate that

exudates can be potential sources of feedstock for the pharmaceutical industry [Okwu and Nnamdi, 2008].

Some physical and chemical properties of African pear (Dacryodes edulis) samples from nine different trees were analysed in order to determine the level of differences that exist between trees. The length of the individual fruits ranged from 39.86 mm to 80.76 mm while the weight ranged from 15.97 g to 39.36 g. Significant differences ($p \geq 0.05$) were observed between the samples for all the parameters measured. Fruit density showed a negative correlation ($r = -0.86$) with the pulp/seed ratio. Significant differences were also observed in the proximate composition of the African pear pulp. The major components of the pulp were moisture (36.5% to 53.82%), oil (18.81% to 38.36%) and protein (11.09% to 19.19%). The pulp acidity ranged from 0.92% to 1.69% expressed as citric acid [Onuegbu and Ihediohanma, 2008].

CHAPTHER THREE

METHODOLOGY

3.1 Proximate Analyses

The leaves of Dacryodes edulis were collected (plucked) from the tree at Obukpa, Nsukka, shade dried for three (3) weeks, then grinded them with industrial grinder. There were then collected and used for the analyses.

3.1.1 Determination of percentage ash content

2.0 g of the grinded APL sample was weighed into a pre-weighed crucible and burnt over a Bunsen burner flame until there was no more smoke. The sample was then placed in the muffle furnace at 600^{o}C until it turned grey-white. This was cooled in a dissector and weighed to a constant weight. The following expression was used to calculate ash content [Ani et al, 2012]:

$$\text{Ash content (\%)} = \frac{w_{ash}}{w_{sample}} \times 100 \qquad 1$$

Where w_{ash} is weight of ash and

W s_{ample} is weight of sample.

3.1.2 Determination of percentage oil/lipid content

2.0 g of powdered APL were weighed into a filter paper and wrapped; the filter paper was placed inside the inner part of the Soxhlet extractor. The apparatus was then fitted to a round bottom flask, which contained 200 mL of hexane solvent. It was then

attached to a reflux condenser. The set-up was clamped and heated in a water bath such that extraction is considered completed by the extracting solution becoming clear. The solvent was distilled off in the distillation set. The oil was then poured into a bottle and left for 5 days for the remaining solvent to evaporate. The oil was then weighed and the percentage oil content determined using the following expression [Ani et al, 2012]:

$$\text{Percentage of oil/lipid yield} = \frac{w_{oil}}{w_{sample}} \times 100 \qquad 2$$

Where w_{oil} is the weight of oil and

Wsample is the weight of sample.

3.1.3 Percentage moisture content

2 g of the grinded APL were weighed into pre-weighed crucible. The crucible and the content were weighed again. This was then put in the oven at 150^{o}C for 5 h after which it was removed, cooled and weighed until a constant weight was obtained. The following expression was used to calculate the moisture content [Ani et al, 2012]:

$$\text{Moisture content (\%)} = \frac{w_{sample} - w_{dry}}{w_{sample}} \times 100 \qquad 3$$

Where w_{sample} is the weight of sample before drying,

w_{dry} is weight of sample after drying.

3.1.4 Determination of percentage protein

2 g of the grinded APL were transferred to a Kjeldahl digestion flask and 8 g of the catalyst (96% anhydrous Na_2SO_4, 3.5% $CuSO_4 \cdot 5H_2O$, 0.5% Selenium dioxide) were added. 20 mL of conc. H_2SO_4 were added in an inclined position and shaken occasionally

for 2 h. The liquid formed was cooled and washed into the distilling flask with distilled water. 50 mL of boric acid (2%) solution and screened methyl red indicator were added to the receiving flask. The distillation apparatus was collected with the delivery tube Deeping below the boric acid solution. The diluted digest was made alkaline by the addition of 50% NaOH solution. About 50 mL of the distillate were collected and titrated with 0.1 M H_2SO_4. A blank was also titrated under the same condition.

$$\text{Percentage nitrogen (\% nitrogen)} = V_{0.1MH_2SO_4} \times 0.28 \quad (4)$$

$$\text{Protein content (\%)} = \%nitrogen \times 6.25 \quad (5)$$

Where $V_{0.1MH_2SO_4}$ represents volume of H_2SO_4.

3.1.5 Determination of carbohydrates

The carbohydrate value is the difference between 100 and the sum of all other values (protein, fiber, ash, fats, and moisture contents) present in the work.

3.2 Qualitative Phytochemical Analyses

Phytochemical tests were conducted on the extracts of African pear leaf using standard methods as reported elsewhere [Edoga et al, 2005; Krishnaiah et al, 2009; Egwaikhide, 2007].

3.2.1 Test for Tannins

0.5 g of powdered sample of APL was boiled in 20 mL of distilled water in a test tube and filtered using a conical flask and filter paper. 0.1 % $FeCl_3$ was added to the

filterate and observed for brownish green or blue black colouration which indicates the presence of tannins.

3.2.2 Test for Saponins

2 g of powdered sample of APL was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filterate was mixed with 5 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion which indicates the presence of saponins.

3.2.3 Test for Flavonoids

A few (three) drops of 1 % ammonia solution was added to 10 mL aqueous extract of APL in a test tube. A yellow colouration observed indicates the presence of flavonoid compounds.

3.2.4 Test for Terpenoids

5 mL of APL aqueous extract was mixed with 2 mL of $CHCl_3$ in a test tube. 3 mL of conc. H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown colouration formed indicates the presence of terpenoid constituents.

3.2.5 Test for (Cardiac) Glycosides

1 ml (a drop) of conc. H_2SO_4 was added to a mixture of glacial acetic acid (2 mL) containing a drop of $FeCl_3$ to give an interface (with the conc. H_2SO_4 underneath the mixture). The presence of glycosides is confirmed by the formation of a brown ring.

3.2.6 Test for Carbonyls (Aldehydes)

To 2 mL of APL aqueous extract was added 2, 4- dinitrophenyl hydrazine solution and shaken. Formations of yellow crystals indicate the presence of aldehydes.

3.2.7 Test for Steroids

2 mL of acetic acid and 2 mL methanol was added to 0.5g extract of APL containing 2 mL H_2SO_4. The presence of steroids was confirmed when a violet coloration that changes to blue or green was obtained.

3.2.8 Test for Alkaloids

0.2 g of APL aqueous extract was warmed with 2 % H_2SO_4 for two minutes, filtered and three drops of Dragendoff's reagent was added. Formation of orange-red precipitate indicates the presence of alkaloids.

3.3 Quantitative Phytochemical Analyses

The analyses for the quantitative phytochemicals of African pear leaf were done by Mr. C. C. Chukwu of the Analytical Laboratory, Department of Home Science Nutrition and Dietetics, University of Nigeria N

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Qualitative Analyses

Table 1 presents the qualitative analyses carried out on the various extracts of APL and shows the presence of phytochemical constituents. It shows that tannins, saponins, flavonoids, glycosides and alkaloids are present in APL. From the amount of precipitate formed and degree of colour change, it was deduced that APL contained tannins, saponins, glycosides and alkaloids in small/low concentrations.

Table 1: Qualitative Phytochemical Tests on African pear leaf

Phytochemicals	Inference
Tannins	+
Aldehydes	-
Saponins	+
Terpenoids	-
Flavonoids	++
Glycosides	+
Alkaloids	+
Steroids	-

Key: + (present), ++ (moderately present), +++ (adequately present) and – (absent).

4.1.2 Quantitative Analyses

The results of quantitative analyses on seven groups of phytochemical constituents in APL are presented in table 2. APL has the highest yield of flavonoids (3.660%).

Table 2: Quantitative Phytochemical Analyses on African pear leaf (mg/100gx100%)

Phytochemicals	Inference
Tannins (mg/100 g)	93.030
Saponins (%)	1.680
Flavonoids (%)	3.660
Glycosides (%)	0.179
Alkaloids (%)	1.225

4.1.3 Proximate Composition

The proximate composition of APL is given in table 3. Our results show that the moisture content (3.77%) is very low compared to the value of 89.00% obtained and reported by Okaraonye and Ikewuchi (2009) for Penniselum purpureum.

Lipid content of 0.393% for APL is much lower than 17.1%, 37.03% and 1.29% respectively for Moringa oleifera, Persea Americana and Carica papaya [Dike, 2010; Oloyode, 2005].

The energy value of APL (292.137 kcal) is lower than 1086 kJ/100 g for Pterocarpus mildbraedi [Akinyeye et al, 2010].

Table 3: Proximate Analyses on African pear leaf

Parameter	Value
Protein (%)	6.720
Ash (%)	12.881
Fats (Oils/Lipids) (%)	0.393
Moisture (%)	3.770
Carbohydrates (%)	65.430

4.2 Discussions

4.2.1 Phytochemical Constituents

Phytochemicals such as saponins, terpenoids, flavonoids and alkaloids have been shown to have anti-inflammatory effects [Cherian and Augustine, 1995]. Although terpenoids were absent, the presence of alkaloids, flavonoids and saponins in APL (this work) therefore supports the use of APL in treatments of ear troubles, head aches and snakebites [Ajibesin, 2011].

APL contains tannins, as presented in table 1, which has been reported by Akinpelu and Onakoya [2006] as the main component for treating intestinal disorders like diarrhea and dysentery.

The presence of flavonoids [Stauth, 2007] and tannins [Karthishwaran et al, 2010] have been reported to be antioxidants used to neutralize highly unstable and extremely reactive molecules like free radicals that attack the cells of human body. The presence of these phytochemicals in APL explains why it is used in the treatments of diarrhea, scabies, ringworms, rashes and wounds [Ajibesin, 2011].

Infact, the aqueous and ethanol extracts of APL were discovered to normalize SS (sickle cell) blood erythrocytes, following the deoxygenation of haemoglobin in anaerobic condition. This validates its uses in traditional medicine [Mpiana et al, 2007].

4.2.2 Proximate Analyses

This results show that moisture content is low, as revealed in table 3. The moisture content of any food is an index of its water activity [Frazier and Westoff, 1978] and is used as a measure of stability and the susceptibility of microbial contamination [Scott, 1980]. This implies that APL will very likely to have a long shelf life because of its low moisture content.

The protein content of APL is not appreciably high so as to meet the required daily protein of 23-55 g [Chaney, 2006]. The use of APL a protein source is therefore not encouraged, however, in extreme conditions of protein deficiency; APL may be used as a protein source.

Ash content of 12.881% for APL [this work] suggests good level compared to levels found in other leaves- A. Africana (4.03%) and R. glabra (4.34%). Ash content of APL indicates it contains good level of mineral contents because low ash content suggests high mineral composition [Egharevba and Kunle, 2010].

Carbohydrate level of 65.430% for APL indicates that APL is a rich carbohydrate source and has potentials to provide fuel and energy for daily activities [Yisa et al, 2010].

4.3 Conclusions

From qualitative phytochemical analyses, APL contains tannins, flavonoids, saponins, alkaloids and glycosides. Quantitative phytochemical analyses reveal flavonoids as the most abundant component of APL. These phytochemicals present have justified the use of APL in traditional medicine for the treatments of ear troubles, headaches, wounds, and so on. It is hoped that these information on the phytochemical constituents and their ethno-medicinal properties would be useful in agriculture as food supplement and for evaluation of the plant in medicine which may lead to drug discovery.

Information from the proximate analyses reveal that APL has long shelf life, good mineral composition (ash level) and a good source of energy (carbohydrate). Therefore, this work provides some information on the nutritional value of APL and because it is not toxic [Ajibesin, 2011], it can be eaten as vegetables.

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