PHARMACOGNOSTIC AND PRO-FERTILITY EVALUATIONS OF *DRACAENA ARBOREA* (WILLD) LINN. (DRACAENACEAE)

BY

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JANUARY, 2015

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B.Sc. APPLIED BIOLOGY (BUK)
M.Sc./PHARM SCI/00846/2010-2011

BEING A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACOGNOSY

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA

JANUARY, 2015

DECLARATION

I JAMILAH OIZA ISAH solemly declare that this project titled **Pharmacognostic and Pro-fertility Evaluations of *Dracaena arborea* (Willd) Linn. (Dracaenaceae)** is a record of my research under the supervision of Prof. M. S. Abubakar and Dr. G. Ibrahim in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. It has never been published or presented for any degree. All references therein have been dully acknowledged.

_____ _____

JAMILAH OIZA ISAH Date

CERTIFICATION

This thesis entitled **Pharmacognostic and Pro-fertility evaluations of *Dracaena arborea* (Willd) Linn. (Dracaenaceae)** by Jamilah Oiza Isah, meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to God Almighty.

ACKNOWLEDGEMENT

With special gratitude to God, the Creator of the Universe and its entirety, I wish to sincerely convey my thanks to all those that have contributed to the accomplishment of this work.

I sincerely thank my supervisors, Prof. M. S. Abubakar and Dr. G. Ibrahim, who in spite of their very tight schedules gave their consent and untiredly continued to guide and supervised me from the commencement of this work to its final stage.

I gratefully acknowledge the valuable and essential assistance of Dr. Shittu of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine for his help in the biological aspect of this work.

My gratitude also goes to all the lecturers and the laboratory staff in the Department for their assistance.

I am most grateful to my husband, Dr. A.O. Yakubu, parents, Engr. and Mrs J. O. Isah, siblings, Muazu, Ibrahim, Nimah, and Abdulrahman for all their assistance, support, understanding and prayers, which contributed greatly towards the realization of this work.

ABSTRACT

The plant *Dracaena arborea* (Willd) Linn is a member of the Dracaenaceae family. It grows abundantly in western Nigeria where its leaves are used as traditional herbal treatment for male infertility and as aphrodisiacs and also in the treatment of gonorrhoea, viral ailments like small pox, chicken pox, and measles, epilepsy and stomach troubles. This work was aimed at providing some pharmacognostic standards for the leaf of *Dracaena arborea* (Willd) and also to provide a scientific rationale for the traditional use of the plant in treatment of male infertility. Standard Pharmacognostic methods were used to conduct microscopic studies of the leaf and also biological activities of the leaves were evaluated. The physicochemical characters such as the ash values, extractive values, moisture content tannins content, swelling index, foaming index, microbial contaminants of the leaf of *D. arborea* were evaluated according to the methods in the WHO manual (1998). Acute toxicity studies was carried out using the method of Lorke (1983) and the Pro-fertility evaluations for sperm count, motility, viability and abnormal morphology of the methanol extract on male wistar rats was done according to the protocols of Saalu et al (2007) and WHO (1999).The leaf is amphistomatic with anomocytic stomata measuring 36.00 µm in length and 24.20 µm in width with no trichomes. The stomatal number was (10.40-**12.20**-14.00) at the upper epidermis and (46.30-**54.50**-62.70) at the lower epidermis. The stomatal index was (7.80-**9.20**-10.60) at the lower epidermis and (1.30-**1.50**-1.70) at the upper epidermis. The palisade ratio was (5.60-**6.60**-7.60). The powdered leaves had a moisture content of 3.89 ± 0.61 %. The total ash, acid insoluble ash and water soluble ash values were 14.78 ± 0.61 %, 0.53 ± 0.23 %, and 11.22 ± 0.48 % respectively. The alcohol extractive value was 1.13 ± 0.35 % and water extractive value was 1.29 ± 0.13 %.The swelling index was 5.60 ± 0.13 ml and foaming index was less

than 100(<100) and the quantity of tannins present in the plant was 4.42 %. The total aerobic count was 6,100cfu/ml for bacteria and 14,800cfu/ml for fungi. The aqueous methanol extract was relatively safe (LD_{50} >5000 mg/kg) after oral administration to adult male wistar rats. The sperm function analysis conducted with the extracts on adult male rats showed a significant ($p<0.05$) increase in sperm count after 28 days of treatment and an increase in sperm motility after 14 and 28 days of treatment. There was also a significant increase in sperm viability and there was no significant difference in sperm morphology as compared with the controls. This study has therefore been able to provide pharmacognostic standards for the leaf of *D. arborea* and that the leaves have pro-fertility activity in male wistar rats which suggests the scientific evidence for the traditional use of the leaves of the plant in the improvement of male fertility.

TABLE OF CONTENTS

Title **Page**

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF PLATES

Plate **Page**

LIST OF APPENDICES

Appendix **Page**

LIST OF ABBREVIATIONS AND ACRONYMS

ABU	–	Ahmadu Bello University
et al	-	And others
Aq	-	Aqueous
cm	-	Centimetre
Fig	-	Figure
H	-	Hour
Kg	-	Kilograms
mg	-	Milligrams
ml	-	Millilitres
w/w	-	Weight by weight
˚C	-	Degree Celsius
µl	-	Microlitres
g	-	Grams
SEM	-	Standard error of mean
Cfu	-	Colony forming units
TS	-	Transverse section
TBO	-	Toluidine blue O
IKI	-	Iodine potassium iodide

TLC	-	Thin layer chromatography
R_f	-	Retardation factor

CHAPTER ONE

1.0 INTRODUCTION

Plants have been used in traditional medicine for thousands of years by humans (Watcho *et al*., 2009). It is estimated that 80% of the population in developing countries continue to use medicinal plant and plant products in handling primary medical problems due to their accessibility, availability and affordability (Fonge *et al*., 2012).

Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of health and the search for new plant-derived drugs as a potential alternative to combat the problems of drug resistance by micro-organisms and as a cheaper and safer alternative to chemical drugs (Soetan and Aiyelaagbe, 2009).

Infertility is a common problem affecting perhaps one in six couples and a large proportion of childless couples are confronted with social stigmatization and personal frustration (Nantia *et al.*, 2009).

Male infertility represents the commonest single defined cause of infertility and amongst the methods used to treat male infertility, medicinal plants have been used. These medicinal plants are used in the treatment of dysfunction of the libido, erection and sperm disorders (Nantia *et al.*, 2009).

A variety of plants are claimed to be used as male fertility enhancers in traditional medicine and biological activities of some of these plants have been shown in some animal and human studies, some of these plants include *Sesamum radiatum* (Schum. and Thonn.), *Cissus populneas* L., *Tribulus terretris* L., *Asparagus recemosus*Willd., *Gingko biloba* L., *Moringa oleifera* Lam., *Withania somnifera* (L.) Dunal, *Mucuna prureins* (L.) DC., *Lophira lanceolata* van Tiegh., *Phoenix dactylifera* L.etc. However

a great proportion of medicinal plants used to treat male reproductive disorders have not yet been scientifically evaluated.

Dracaena arborea (Willd) Linn. Dracaenaceae is one of these plants (Etuk and Mohammed, 2009; Watcho *et al.*, 2009). Therefore this study is aimed at providing scientific rationale for the use of this plant in the treatment of male infertility and providing pharmacognostic standards for the leaf of the plant for the assurance of its identity.

1.1 Background Knowledge

Infertility is a private, social and economic problem. It is defined as the inability to conceive a pregnancy within one year after regular sexual intercourse. It is estimated that there are 60 to 80 million infertile couples world-wide. Infertility has a serious impact on the mental and social wellbeing of infertile couples and has a detrimental effect on the life supporting system (Etuk and Mohammed, 2009).

Infertility has become rampant in sub-Saharan Africa and is an urgent problem that needs to be addressed. In Nigeria, the major causes of infertility are infections, environmental factors such as diet and toxic chemicals, socio-demographic factors and hormonal dysfunction (Akinloye and Truter, 2011).

Male factor infertility is contributory in at least 50% of infertile couples. More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both (Crimmel *et al.*, 2001).

Other causes of male infertility include congenital and acquired urogenital abnormalities, increased scrotal temperature (varicocele),endocrine disturbances, infection and exposure to toxins and radiations (Estakhr and Javdan, 2011).

Among the methods used to treat male infertility problems, medicinal plants have been used as extracts, decoctions, fractions or semi-purified compounds. Several plants have shown positive effects in the treatment of male infertility, some of which include *Astragalus membranaceus* L.and *Acanthopanax senticosus* (Rupr. Et Maxim) which increased the motility and viability of infertile male sperm at a concentration of 10mg/ml. Ethanol extract of *Croton zambesicus* Mull., at a dose of 10mg/kg increased sperm count and motility. The powder of *Mucuna pruriens* administered to infertile men increased male fertility at a dose of 5 g/day. *Nigella sativa* L.oil administered orally at a dose of 0.5 ml per day for two months increased sperm functions. Pollen suspensions from *Phoenix dactylifera* at a dose of 120 mg/kg improved sperm functions in male rats. The juice of *Punica granatum* L. given at a dose of 1 ml/day for seven weeks to normal rats increased sperm functions. Treatment of patients with a formulation of *Tribulus terrestris* and *Withania somniferea* increased semen volume, sperm count and motility (Singh *et al.*, 2013).

Dracaena arborea is a plant native to West Africa and is used in traditional medicines for the treatment of venereal diseases and also used as aphrodisiacs and genital stimulants (Watcho *et al,* 2009).It is also used to treat viral and bacterial infections (Burkill, 1985). The traditional uses of this plant as aphrodisiacs and in treating infections can point to its use in the treatment of male infertility.

1.2 Justification of Study

Male factor infertility is contributory in at least 50% of infertile couples. More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both.

D. arborea is a tall tree native to West Africa and is widely used as herbal remedies for various ailments such as epilepsy, convulsions, spasms, small pox, chicken pox,

measles, stomach troubles and other venereal diseases and has been reported to possess genital stimulant and aphrodisiac properties.

The widespread use of this plant particularly in treating infertility makes it imperative to evaluate its pharmacognostic properties so as to ensure its quality, safety and efficacy and also to justify its biological activity in the improvement of male infertility. There is therefore a need to evaluate the pharmacognostic properties of *D. arborea* and also the biological activities of *D. arborea* in the improvement of male infertility which is of growing societal concern.

1.3 Aim and Objectives of the Study

The overall aim of the study is to evaluate the pharmacognostic characters and pro-fertility properties of *D. arborea*.

The specific objectives for the study are:

i. To establish the pharmacognostic characteristics of the leaves of *D. arborea*.
ii. To determine the margin of safety of the leaves of the plant
iii. To evaluate the pro-fertility effect of the leaves of the plant in male wistar rats.

1.4 Hypothesis

The leaves of *Dracaena arborea* contain phytochemical constituents that are responsible for its pro-fertility effects.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General Description of the Family Dracaenaceae

The Family was first described in 1866 by Salisbury. The *Dracaenaceae* are a group of about 160 species of tropical trees, shrubs and herbs in the monocotyledonous genera *Dracaena* and *Sansevieria* (Klimko and Szymanska, 2008).

They were formerly included in *Agavaceae* family due to their secondary thickening meristem and also the presence of xylem vessels in the root. However, some characters recognised to be present in the *Dracaena* and *Sanseviera* such as superior or inferior ovaries and type of fruit which are absent in the *Agave* and *Yucca* made the separation of *Dracaena* and *Sansevieria* into their own distinct family. More recently, molecular studies have established the number of chromosomes to be 18-20 and hence, strong evidence for its removal from the *Agavaceae* family, as the Agaves has 30 chromosomes (Bos, 1986).

Chemical compounds commonly found in the family include flavonoids and saponins/sapogenins (Watson and Dallwitz, 1992).

The geographical range of the family is contained in the tropical zone with the exception of South America. They are distributed in the tropics and subtropics, preferred habitats are semi-deserts (shrubby Dracaenas), rain forests and savannah (Dragon tree). South-western parts of America and Africa (Klimko and Szymanska, 2008).

Due to their ornamental leaves, *Dracaena* species are amongst the most important ornamental foliage plants in Europe, North America, Asia and Africa (Pennisi and McConnel, 2001).

Plants in the family usually have linear, parallel-veined fibrous, semi-succulent leaves arranged in rosettes with a pachycaul, woody supporting trunk (*Dracaena*) or with a short stoloniferous stem (*Sanseviera)*. The leaves are alternate with parallel or palmate venation, the leaves are leathery or fleshy in texture,lanceolate or ovate in shape and the attachment of the leaf to the stem is petiolate to sessile (Klimko and Szymanska, 2008). The leaves of many species contain mucilage cells (with raphides), containing irritant calcium oxalate crystals and xylem vessels which are end wall scalariform vessels (Klimko and Szymanska, 2008).

The stem has a secondary thickening meristem (which develops typically in scattered bundles) on the trunk known as Dracaenoid thickening. Trunks reach more than two meters in diameter in some species (Pennisi and McConnel, 2001).The roots contain xylem vessels which are very unusual and diagnostic. These vessels are end wall scalariform and are simple (Watson and Dallwitz, 1992). Flowers are aggregated in inflorescence, racemes, or in panicles. They are minute to large in size. Fruits are usually fleshy or non-fleshy, indehiscent berry (usually red or orange) or sometimes a woody capsule. Seeds are endospermic and monocotyledonous (Watson and Dallwitz, 1992).

2.1.1 Description of the Genus *Dracaena*

Dracaena, a genus of ornamental foliage plants in the family *Dracaenaceae* consisting of about 100 species native primarily to the old world tropics. Most species have short stalks and narrow, sword-shaped leaves, but some have taller stalks and resemble trees

with crown of leaves; the small flowers are red, yellow or green. The berry like fruit contains one to three seeds (Bos, 1986).

Species of *Dracaena* have a secondary thickening meristem in their trunk. This monocotyledonous secondary thickening meristem is quite different from the thickening meristem found in dicotyledonous plants and is termed Dracaenoid thickening (Hodgkiss, 2012).

Commonly known dragon trees, *D. americana*, *D. arborea*, *D. cinnabari*, *D. draco*, *D. ombet*, and *D. tamaranae* grow in arid semi-desert areas. They are tree-sized with stout trunks and stiff, broad-based leaves. The remaining species are known collectively as shrubby dracaenas. They are smaller and shrub-like, with slender stems and flexible strap-shaped leaves, and grow as under storey plants in rain forests (Hodgkiss, 2012).

The stem of *Dracaena* trees especially *D. cinnabari* from Socotra and *D. draco* from the Canary Islands, exude a reddish sap (Dragon's Blood) containing spirit-soluble resins, from cracks in the bark of the trunk (Bos, 1986).

Dracaena is distributed throughout the Canary Islands, Madeira, Cape Verde islands and Morocco, tropical Africa, a few species are found in Asia and a specie is found in South America (Hodgkiss, 2012).

2.1.2 Description of *Dracaena arborea*

The trees of *D. arborea* grow up to 20 meters tall, and trunk to 20 to 30 cm in diameter; they are often planted in boundaries or as ornamentals. Leaves are narrowly oblanceolate to sword-shaped, 50–120–150 cm x 4–6–10cm in size, tapering to both ends, the widest part distinctly above the middle, tip acute, fresh leaves are shiny bright

to dark green, they have a parallel venation and sometimes irregular transverse venation visible in leaves (Bos, 1986).

Flowers are white, 17–20–22 mm long, receptacle extended for about 2 to 3 mm below the ovary into a cone, perianth tube 5 to 8 mm long, lobes up to twice as long, 10 to 13 mm, up to 3 mm wide, showing a single median vein, filaments inflated up to 1 mm diameter, ovary cylindrical to bottle-shaped, up to 3 mm x 2 mm, style up to ¼ mm in diameter., reaching the top of the perianth, stigma about 1 mm in diameter (Hodgkiss, 2012).

Fruits are bright orange, depressed globose and more or less distinctly lobed when more than 1 seeded, 12 to 24 mm long, 12 to 27 mm in diameter, and the persistent receptacle 3 to 6 mm long. Seeds are bony white to pale brown, and globular (Hodgkiss, 2012).

The plant *D. arborea* is also known as Tree Dracaena in English, ‘Peregun’ in Yoruba, ‘Odo’ in Igbo, ‘Okono’ in Efik and ‘Okumagba’ in Ebira.

Plate I: The picture of *D. arborea* growing in its natural habitat

2.1.3 Ethnobotanical and Medicinal uses of *Dracaena arborea*

D. arborea has a wide range of uses. It is used in Agri-horticulture as hedges and boundary markers that define property and provide evidence of rights in judicial disputes. *D. arborea* is traditionally considered as a symbol of peace and is therefore used to mark property boundaries (Sheridan, 2008). *D. arborea* is planted at the entrance of shrines and cemeteries as a sacred plant throughout West Africa (Obiri and Addai, 2007).

D. arborea is often regarded as an ancient cultural plant of many village settlements. The plant is important for social practices which range from initiation ceremonies to rites of land tenure and land ownership. *D. arborea* is also grown in compounds as they are believed to serve as a protection from witchcraft. They are also used to mark the graves of political elites in some African countries like Burundi and Cameroon (Sheridan, 2008).

The leaf of *D. arborea* is used as fish poisons, reptile-repellents; pesticides, soaps, hunting and fishing apparatus. *D. arborea* has many uses in traditional medicine. They have been used as genital stimulants, aphrodisiacs and in the treatment of venereal diseases (Watcho *et al.*, 2009).The plant is also used to treat some viral infections like small pox, chicken pox, and measles. It is also used to treat epilepsy, convulsions, stomach troubles, heart and kidney troubles, it is used as diuretics and sedatives and the decoction is taken orally to treat liver disorders (Burkill, 1985).

2.1.4 The Traditional Uses of *Dracaena arborea* in Male Infertility

It is used in West Africa to treat gonorrhoea, small pox, malaria and leishmaniasis. In the western part of Cameroon, the mixture of the roots of this plant with palm wine is used as a male aphrodisiac (Watcho *et al.*, 2009).

According to the Nai wulomo culture of the Ghanians, *D. arborea* is made into a concoction with six other herbs including *Mormodica charantia* and *Portulaca olearacae*. These herbs are carefully mixed with sea water, schnapps and eggs in a special kind of bowl known as *tsese*, and this concoction is believed to treat male infertility (Nortey and Kwame, 2012).

The dried leaf of *Dracaena arborea* is ground to a powder and mixed with local rum for two weeks and the solution is drunk: one spoonful in half a glass of water thrice a day in Foumban Cameroon (Emmanuel, 2010).

It is used for the promotion of pregnancy, sexual impulse and fertility in animals. The ground leaves of *Basella alba*, *D. arborea* and fruits of *Solanum symbriifolium* and *Phoenix reclinata* are rubbed on the hand to introduce the preparation into the animal vagina every morning. Also, vaginal administration of the decoction and oral administration of the maceration prepared from the stem barks of *D. arborea* is used in the treatment of male infertility in the Bushi area of congo (Chifundera, 1998).

Ethanol extract of *D. arborea* showed a significant ($p < 0.05$)increase in male erectile functions of normal castrated rats in animals receiving 100 mg/kg of b/w of the extract after 7 and 14 days respectively (Watcho *et al.*, 2007).

Wankeu-Nya *et al.*, (2013) showed that the aqueous and ethanol extract of *D. arborea* at 500 mg/kg improved testes morphology and reversed the impairment of spermatogenesis in rats with chemically induced diabetes (treated with the drug streptozotocin), although the effect observed was more pronounced in animals treated with the aqueous extract.

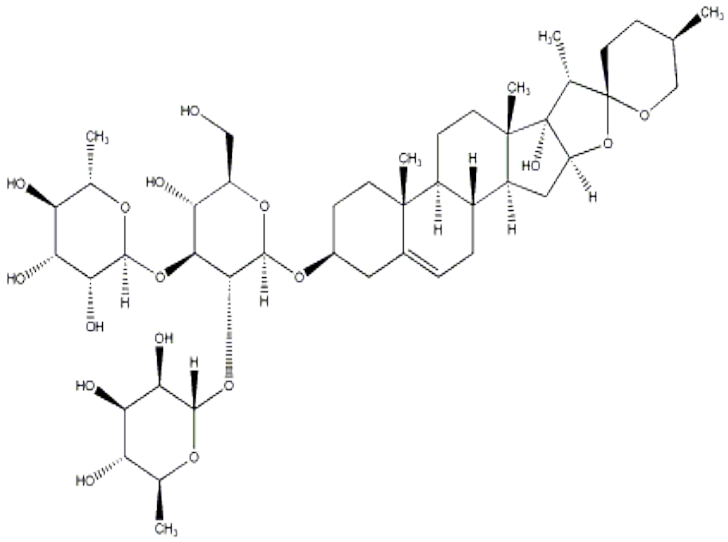
2.1.5 Chemical Constituents and Biological Activities of *D.arborea* Extracts

Some of the chemical constituents present in this plant include flavonoids and steroidal saponins (Watson and Dallwitz, 1992).Qualitative phytochemical evaluation of aqueous and ethanolic extracts of *D. arborea* showed the presence of saponins, flavonoids, tannins and sterols (Nnamani *et al.,* 2008,Wankeu-Nya *et al.,* 2013,Watcho *et al.,* 2007).

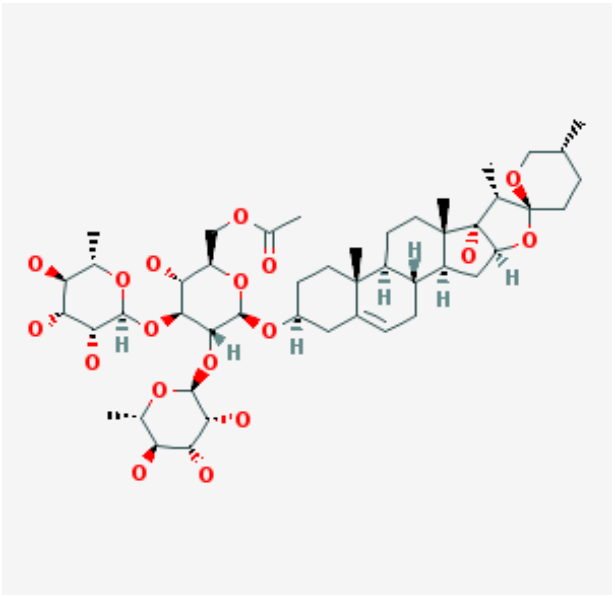
Two steroidal saponins were isolated from the stem bark of *D. arborea*, arboreasaponin A and arboreasaponin B and they showed cytotoxic activity when evaluated against the HT-29 and HCT 116 human colon cancer cell lines (Kougan *et al*., 2010).

Okunji *et al.,* (1996), evaluated the biological activities of saponins from extracts of *D. arborea* and spiroconazole Awas isolated, which exhibited pronounced antileishmanial, antimalarial, and molluscicidal activities. It also showed fungistatic, fungicidal and bacteriostatic activity against some species of fungi and bacteria.

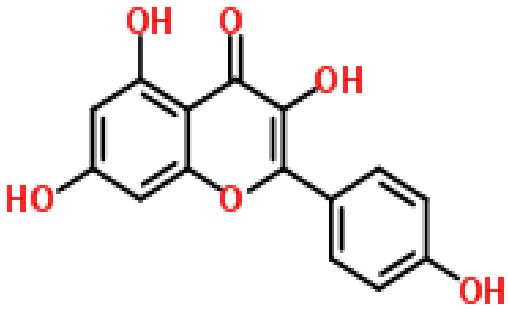
Epidi and Njoku, (2009), conducted a field bioassay to evaluate the biocidal potential of *D. arborea* against millipede specie *Tribolium castaneum* in stored groundnuts after 28 days of application; *D. arborea* was as efficacious as photoxin (used as a positive control) in protecting the ground nut seed against damage by *T. castaneum.*



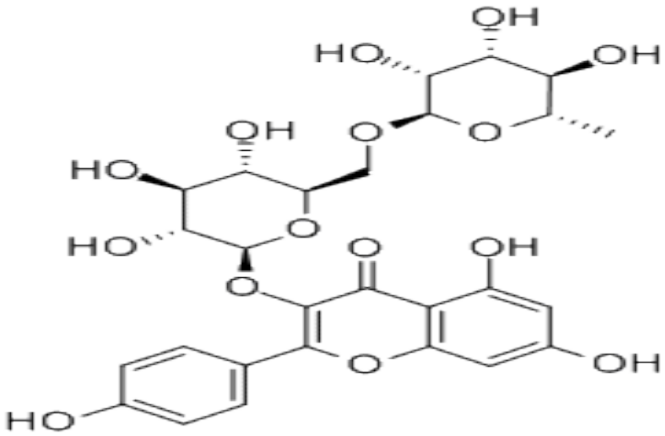
a) Spiroconazole A



b) Arboreasaponin A



c) Kaempferol



d) Kaempferol-3-rutinoside

Figure 2.1(a-d): Structures of Some Chemical Compounds Isolated from *Dracaena arborea* (Kougan *et al*., 2010) and (Okunji *et al*., 1996)

2.2 Male Infertility

An Estimated 40 to 90% of male infertility is due to deficient sperm production either due to lack of sperm (Azoo-spermia) or too little sperm (Oligozoospermia). Some cases of male infertility are due to anatomical abnormalities such as varicoceles, ductal obstructions or ejaculatory disorders (Emmanuel, 2010).

Infertility may also be due to abnormal sperm morphology (teratozoospermia) and insufficient sperm motility (athenozoospermia), Infections of the male genito-urinary tract which include infections of the epididymis, seminal vesicles, prostate, bladder and urethra. Chlamydial infections and gonorrhoea are the most common and critical infections of the male genito-urinary tract. Other factors include heavy metals poisoning, cigarette smoking, exposure to radiation (Emmanuel, 2010).

2.2.1 Traditional Method of Treating Male Infertility

For centuries, there have been different traditional methods used in the treatment of male infertility the most common of which is the natural and holistic treatments such as herbal and homeopathic remedies. Herbal remedies provide powerful but safe substances that stimulate reproductive hormones, detoxify and nourish the body. These herbs have fertility enhancing properties which help promote male potency and libido while also acting as a natural aphrodisiac and increasing sperm functions (Crimmel *et al.*, 2001).

Acupuncture is among the oldest healing practices in the world and is another method commonly used in Chinese traditional medicine to treat male infertility. It has been used successfully in treating low sperm count and sperm motility, ejaculatory dysfunction, varicocoeles and abnormal sperm morphology (Crimmel *et al*, 2001).

2.2.2 Plants Used in Improving Male Fertility

Fertility enhancing properties of many plants have been evaluated by many researchers. Some plants may promote fertility by treating some underlying diseases which may impair fertility. One of the main causes of male infertility is venereal disease (Noumi *et al.*, 2011) and some medicinal plants used to improve male infertility target such infections thereby indirectly improving male fertility. *Sesamum radiatum* belongs to the *Pedaliaceae* family. The decoction of combined roots and leaves has been reported to exhibit anti-viral and anti-fungal activity. The decoction of the leaves is also used for the treatment of catarrh, eye pains, bruises and erupted skin (Ogunlesi *et al.*, 2010).

Cissus populnea which belongs to the *Vitaceae* family is used extensively in medicinal preparations in West Africa and the aqueous extract of the stem bark is used as a fertility enhancer for males in South-Western Nigeria (Ogunlesi *et al.*, 2010).

Sperm functions improving effect of the plant *Myristica fragrans* (nutmeg) which belongs to the family *Myristicaceae,* was established in a study by Tajuddin *et al.*,(2005).Similarly, alcohol extract of *Argyreia nervosa* (Subramoniam *et al*., 2007) from the family *Convolvulaceae*, have been shown to have aphrodisiac effects in normal mice.

Where hormonal disturbances are involved in the causation of infertility, some other plants improve sperm function due to their steroidal constituents by directly influencing hormonal function (Rajeshwar *et al*., 2005). Several plant extract have shown positive effects on sperm functions, Singh *et al,* (2013) reported that ethanol extract of *Croton zambesicus* at 5 and 10 mg/kg increased sperm functions after five days of treatment. Oral administration of *Nigella sativa* oil to male rats increased sperm count and motility and decreased sperm abnormalities after two months of treatment (Al-Sa'aidi

et al., 2009). Also extracts of *Phoenix dactylifera* at 120 mg/kg for 35 days improved sperm functions in healthy rats. In humans, a three months treatment of patients suffering from idiopathic infertility by a formulation of plants made up of *Tribulus terrestris, Asparagus recemosus* and *Withania somniferea* increased semen volume, sperm count and motility (Singh *et al.,* 2013).

Some other plants used in the treatment of male infertility have aphrodisiac properties and this has been related to their androgenic effects (Watcho *et al*., 2007). Malviya *et al*, (2011) reported on some plants that increase sexual potency which include *Tribulus terrestris* the extract of which showed aphrodisiac properties on male rats at 5mg/kg for 8 weeks of treatment, extract of *Eurycoma longifolia* given to male rats at 200, 400 and 800 mg/kg for 10 days increased erection and mounting frequencies. *Ginkgo biloba* at a dose of 0 mg/kg given to male rats for a period of 14 to 18 days increased ejaculatory frequency.

Dioscorea spp.(Muanya and Odukoya, 2008), *Mucuna prureins, Withania somnifera* and *Psoralea cordifolia* have also been reported to have aphrodisiac properties (Rajeshwar *et al*., 2005).In a similar manner, male fertility enhancing effect has been established in plants like *Moringa oleifera* (Cajuday and Pocsidio, 2010), *Lophira lanceolata* (Etuk and Mohammed, 2009), *Abelmoschus manihot* (Rewatkar *et al*., 2010).

2.2.3 Nigerian Plants used in Male Infertility

There are several plants used in herbal medicine to promote fertility in Nigeria, some of which include the leaves and roots of *Annona senegalensis*, the leaves and roots of *Costus afer*, the stem of *Cissus populnea*, the seeds of *Garcinia kola*, the whole plant of

Momordica charantia, the leaves and roots of both *Newbouldea laevis* and *Rawolfia vomitora* (Ogunlesi *et al.*, 2010).

A. senegalensis has several medicinal properties. The roots, bark, leaves and seeds are used as herbal medication for treating cancer, dysentery, gastroenteritis, snake bite, guinea worms, cough, venereal diseases, tooth-ache, eye infections, respiratory infections and tumour growth. Venereal diseases and intestinal disorders are treated with preparations of the roots (Sofowora, 1993).

The aqueous extract of the stem bark of *C. populnea* is used as a fertility enhancer in males in south western Nigeria. Phytochemistry of the stem bark of the plant showed the presence of tannins, flavonoids, saponins and steroids, these compounds are associated with functions related to fertility enhancement potentials (Ogunlesi *et al*., 2010).

C. afer is used for the treatment of cough, diabetes, rheumatic swellings, cuts, wounds, malaria, jaundice, gonorrhoea and bilharzias. The pounded fruit is used as a relief for cough, while the sap is taken for malaria and also used to clear urine. An infusion of the roots is taken for stomach ache and is considered to be a genital stimulant and an aphrodisiac (Ogunlesi *et al.*, 2010).

G. kola is used in medicinal preparations for treating dysentery, chest pain, body weakness, liver disorders and headaches. It is also used as anti-cancer medication and as an aid to boost sperm count (Ogunlesi *et al.*, 2010).

The leaves of *N. laevis* are used as aphrodisiacs. Other parts of the plant are used for treating several ailments like convulsions, malaria, migraine, yellow fever, ear-ache, and infertility. Preliminary phytochemical screening of the leaf extract revealed the

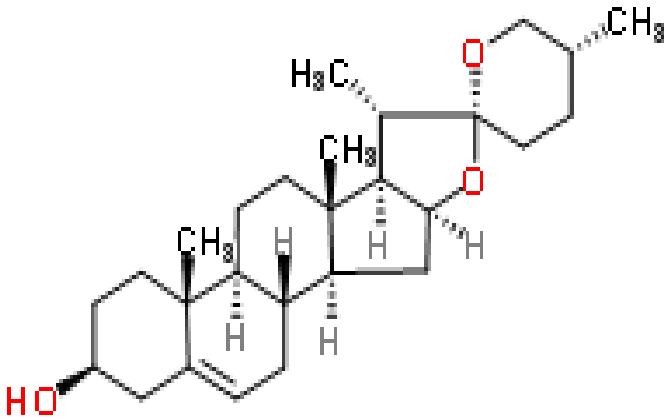
presence of flavonoids, tannins, terpenes, steroidal and cardiac glycosides (Ogunlesi *et al.*, 2010).

Rawolfia. vomitora leaves, roots and stem bark have been reported to be useful in the treatment of hypertension, insomnia, nervous disorder, diarrhoea and malaria (Ogunlesi *et al.*, 2010). Folk medicinal uses of the roots are extensive, particularly for their aphrodisiac, emetic, purgative, abortive and insecticidal properties. The stem bark extract of *Lophira lanceolata* is commonly used by herbalists in Sokoto state for the treatment of male infertility (Etuk and Mohammed, 2009).

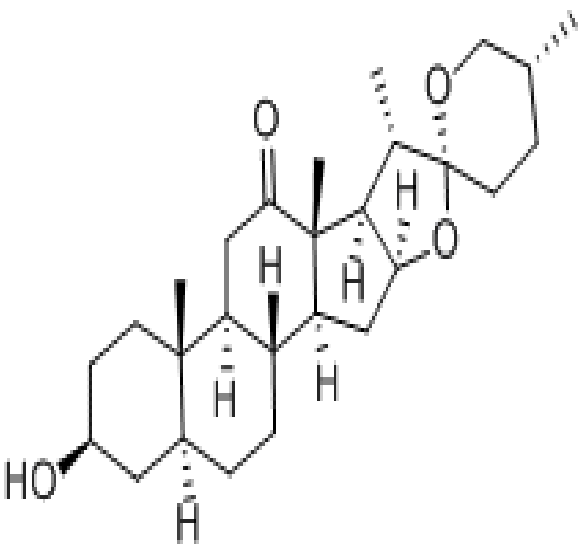
2.2.4 Chemical Constituents Isolated from Some Plants Used In Male Infertility Treatment

A lot of chemical compounds have been implicated as the bioactive constituents in the treatment of male infertility especially steroidal saponins. These steroidal saponins have the ability to influence the entire immune system of the body as they have been shown to have anti-bacterial and anti-viral effects and are also responsible for spermatogenic and aphrodisiac effects; they enable the body to produce more testosterone by raising the levels of the leutenizing hormone (LH) which is the hormone responsible for testosterone production (Abirami and Rajendran, 2011).

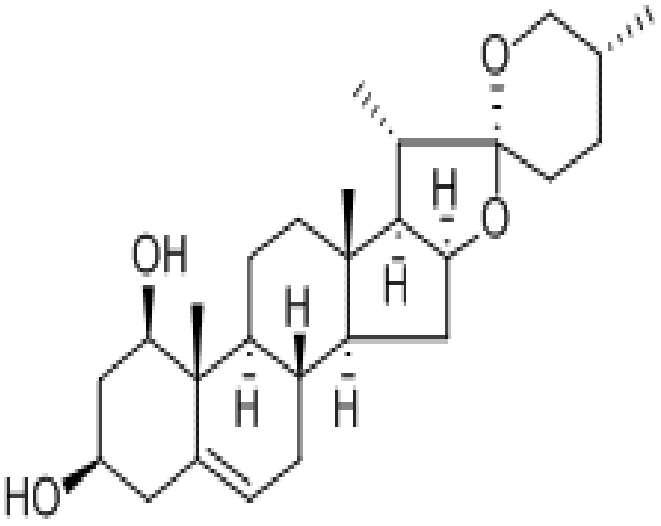
Many chemical constituents have been isolated from plants used in male infertility treatment. *Tribulus terrestris* is a plant used to treat male infertility and is also known for its aphrodisiac properties contain a lot of steroidal saponins. Diosgenin **(1)**, Hecogenin **(2)**,Gitogenin, Ruscogenin **(3)**, 25 D-spirosta-3, 5-diene **(4)**, Desgalactotigonin. Some flavonoids like Kaempferol **(5)**, Kaempferol-3-glucoside **(6)** and Kaempferol-3-rutinoside have also been isolated from the leaves and fruits of this plant (Abirami and Rajendran, 2011).



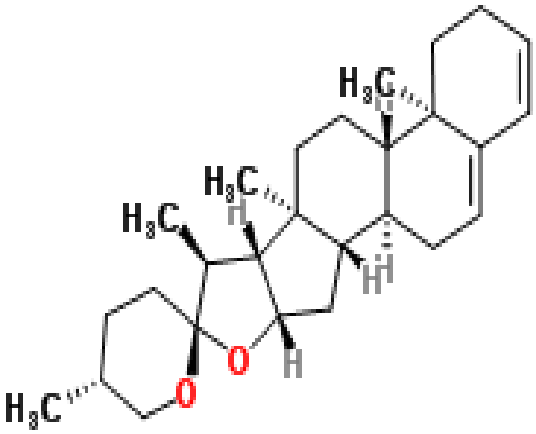
1) Diosgenin



2) Hecogenin

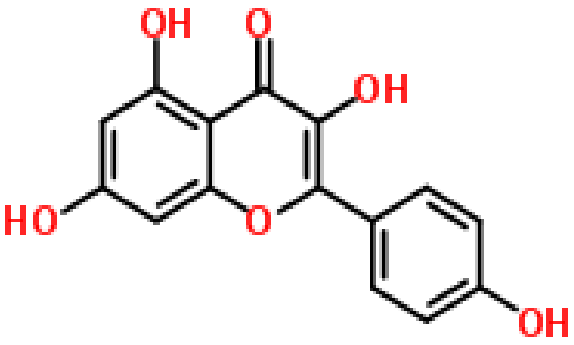


3) Ruscogenin

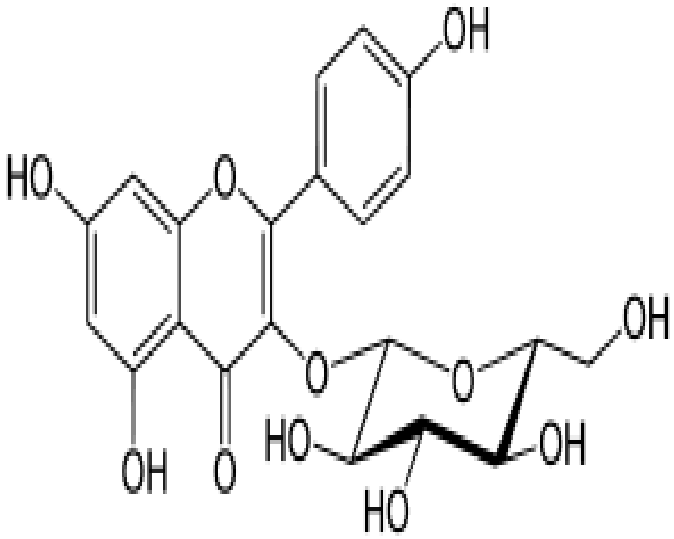


4) (25R)-Spirosta-3, 5-diene

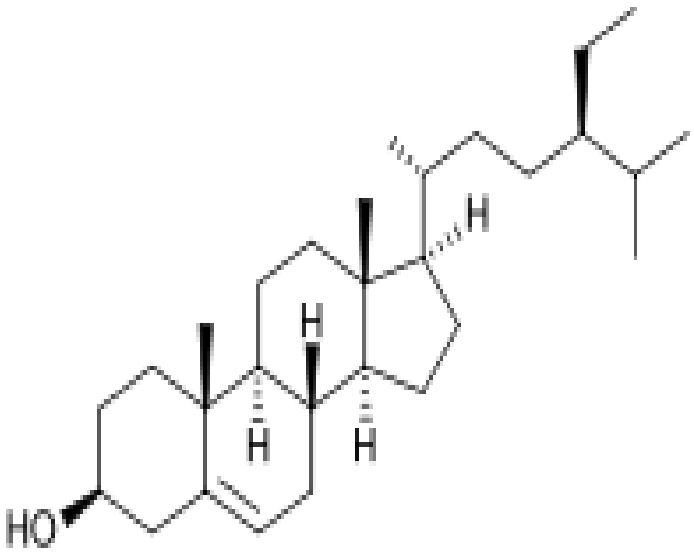
Figure 2.2a (1-4): Structure of Some Chemical Compounds Isolated from Plants Used in Male Infertility Treatment (Abirami and Rajendran, 2011)



5) Kaempferol



6) Kaempferol-3-glucoside



7) β-Sitosterol

Figure 2.2 continued

Ogunlesi *et al,* (2010) isolated fatty acids and phenolic compounds from the dried leaves of *Sesamum radiatum,* some of which include eugenol, caryophyllene, ascorbic acid, oleic acid, hexadecanoic acid, hetradecanoic acid, dodecanoic acid, asteroid Estra-

1, 3, 5(10) - trien- 17- ol which is similar to estradiol was also isolated and this may be responsible for the estrogenic properties of the plant. The presence of some of these constituents in the essential oil provides scientific evidence for the curative properties of the plant in male infertility.

Two flavonoid glycosides, carthamin and carthamidin have been isolated from the flowers of *Carthamus oxycantha* which is used to treat male infertility. Two glycosides, 2-0-methylglycopyranosyl carthamoside and beta-D-fructofuranosyl carthamoside along with a known flavonoid glycoside 3', 4', 5, 7-Tetrahydroxyflavanone was isolated from this plant (Ahmad *et al.*, 2010).

Some chemical constituents have also been isolated from *Myristica fragrans*which include myristin and myristic acid. Nutmeg yields 3-15% of volatile oil which contains pinene, sabinene, camphene, myristicin, elemicin, isoelemian, eugenol, lignans and neolignans. These constituents possess aphrodisiac properties, increasing both libido and potency which thus provides a scientific rationale for the traditional use of nutmeg in the management of male sexual disorders (Malviya *et al.*, 2011).

The study of the chemical composition of *Chione venosa,*a plant used as a male fertility enhancer yielded acetophenone derivatives along with other compounds; α-morroniside, sweroside, diderroside, daucosterol and β-sistosterol **(7)**. All of which contribute to the aphrodisiac potential of the plant (Malviya *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 List of Equipment and Glassware

Equipment and Glassware used in this study includes the following:

i. Electronic Balance (Mettler, Griefensee, Switzerland)
ii. Hot Air Oven (600 Series, Fisher Scientific, Waltham, Massachusetts, USA)
iii. Water Bath (Hh-S6)
iv. Binocular Microscope(S90009 Series, Fisher Scientific)
v. Stage And Eyepiece Micrometers (Fisher Scientific, Waltham, Massachusetts, USA)
vi. Swift-Ives Camera Lucida (Fisher Scientific, Waltham, Massachusetts, USA)
vii. Improved Newbaeur Hemocytometer (Gallenkamp, Germany)
viii. Glass Wares-TLC Tank (Pyrex), Beakers, Measuring cylinders, Test tubes, Flasks
ix. Precoated TLC Plates
x. RBC Pipette.

3.2 List of Chemicals, Reagents, Solvents and Bacteriological Media

Chemicals, reagents, solvents and bacteriological media used in the study all of which were of standard grade are as follows:

a) Chemicals

i. Sulphuric Acid
ii. Hydrochloric Acid
iii. Sodium Hydroxide
iv. Ferric Chloride

v. Acetic Acid

vi. Phloroglucinol

vii. Iodine Potassium Iodide

b) Reagents

i. Chloral Hydrate

ii. Glycerol

iii. Iodine Solution

iv. Sudan Red

v. Ruthenium Red

vi. Millon's Reagent

vii. Hide Powder

viii. 1-naphthol

ix. Normal saline

x. Formol saline

xi. Eosin-Nigrosin Stain

xii. Toluidine Blue Stain

c) Solvents

i. N-Butanol (Sigma-Aldrich, St.Louis, USA)

ii. Methanol (Sigma-Aldrich, St.Louis, USA)

iii. Chloroform (Sigma-Aldrich, St.Louis, USA)

iv. Ethyl Acetate (Sigma-Aldrich, St.Louis, USA)

v. Ethanol (Sigma-Aldrich, St.Louis, USA)

d) Bacteriological Media

i. Baird-Parker Agar

ii. Cetrimide Agar

iii. MacConkey Agar

iv. Sabouraud glucose agar

v. Soybean digest agar

vi. Salmonella-Shigella agar

3.3 Collection, Identification and Preparation of Plant Materials.

The plant was collected on 20th March 2012, fresh in the wild at Ajaokuta, Ajaokuta Local Government Area of Kogi State. It was identified and authenticated in the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria by the Taxonomist Mallam Umar Gallah and where specimen with Voucher number 9501 has been deposited. The leaves were plucked and air-dried for six days and later powdered using a mortar and pestle.

3.4 Evaluation of Pharmacognostic Characters of the Leaves of *D. arborea.*

Microscopic characters, ash values, extractive values, swelling index, foaming index, level of microbial contamination and the amount of tannins in the leaf of *D. arborea* were determined using the following procedures.

3.4.1 Evaluation of Microscopic Properties of *D. arborea*

The quantitative and qualitative leaf microscopy of the plant was carried out according to the methods described in the WHO (1998) manual and Kokate (1994).

3.4.1.1 Quantitative Leaf Microscopy of *D. arborea*

The stomatal number, stomatal index, and palisade ratio were determined as described below:

Sections of the leaf midway between the midrib and the margin were prepared by cutting with a razor blade as thinly and evenly as possible. The sections were then cleared with chloral hydrate solution and then mounted on a clean glass slide and covered with a coverslip. The Parameters were evaluated with the aid of Camera Lucida.

a. Stomatal Number

The micrometres were used to measure a square of 1mm, the numbers of stomata within 1 mm square were counted and the average of 5 determinations was taken as the stomatal number.

b. Stomatal Index

Epidermal cells and stomata within the square were counted, the epidermal cells were marked (√) and stomata were marked (X). The stomatal index was then calculated as:

$$\text{Stomatal index} = \frac{S}{E+S} x\ 100$$

Where S = the total number of stomata per square millimetre of leaf

E = the total number of epidermal cells in the same leaf area

This was repeated five times and average value was calculated.

c. Palisade Ratio

The number of palisade cells within four epidermal cells was calculated as follows

$$\text{Palisade ratio} = \frac{numberofpalisadecells}{4}$$

This was repeated five times and average value was calculated.

3.4.1.2 Qualitative Leaf Microscopy of *D. arborea*

The evaluation of the anatomical sections and powdered sample of the leaf was carried out to identify features, namely, fibres, phloem, cork cells, sclereids, calcium oxalate crystals etc.

Transverse sections of the leaf were prepared by cutting with a razor blade as thinly and evenly as possible and the sections were cleared with chloral hydrate, mounted in glycerol and observed under the microscope.

a) Chemomicroscopic Evaluation of leaf of *D. arborea*

The chemomicroscopy of the powdered drug was carried out for the phytochemical detection of the cell wall materials and cell contents.

I. Cell Wall Materials

a) Cellulose

Two drops of iodine was added to the plant powder on a glass slide and allowed to stand for one minute and then one drop of sulphuric acid was added and observed under the microscope for the presence or absence of blue or violet colouration.

b) Lignin

The plant powder on a glass slide was moistened with phloroglucinol and allowed to stand for two minutes and then one drop of hydrochloric acid was added and then mounted in glycerol and observed under the microscope for the presence or absence of pink colouration.

c) Suberin/Cutin

Two drops of Sudan red was added to the plant powder on a glass slide and allowed to stand for a minute and then observed under the microscope for the presence or absence of orange to red colouration.

d) Mucilage

One drop of ruthenium red was added to the plant powder on a glass slide and allowed to stand for a few minutes and then observed under the microscope for the presence or absence of pink colouration.

II. Cell Contents

a) Starch

One drop of iodine was added to the plant powder on a glass slide and then observed under the microscope for the presence or absence of blue colouration.

b) Hydrooxyanthraquinones

One drop of potassium hydroxide was added to the plant powder on a glass slide and then observed under the microscope.

c) Inulin

One drop each of 1-napthol and sulphuric acid was added to the plant powder on a glass slide and then observed under the microscope for the presence or absence of brownish red crystals.

d) Calcium oxalate crystals

The plant powder was cleared with chloral hydrate then hydrochloric acid was added to the plant powder on a glass slide and observed under the microscope for the presence or absence of calcium oxalate crystals.

e) Calcium Carbonate

One drop of acetic acid was added to the plant powder on a glass slide and then observed under the microscope for the presence or absence of effervescence on the dissolution of the crystals.

f) Proteins

One drop of million's reagent was added to the plant powder on a glass slide and then observed under the microscope for the presence or absence of red colouration.

3.4.2 Evaluation of Physicochemical Parameters in Powdered Leaf of *D. arborea*

a) Moisture Content

The powdered leaf (3 g) was placed in a crucible of known weight and dried in an oven at 105 ^{0}C until a constant weight was achieved and the loss of weight was calculated as:

Moisture content = $\frac{weightofwaterloss}{initialweightofsample} x100$

it was determined three times according to the methods described in the WHO (1998) manual.

b) Ash Values

The total ash, acid insoluble ash, and water soluble ash were determined using methods described in the WHO (1998) manual. The determinations were done three times.

i. Total Ash

The powdered leaf sample (3 g) was placed in a crucible an ignited in a furnace at 450^{0}C until it was completely charred and turned white; it was then cooled in a desiccator and weighed. Total ash = $\frac{weight\ of\ residual\ ash}{weight\ of\ original\ plant\ sample}\ x\ 100$

ii. Acid-insoluble Ash

Hydrochloric acid (25 ml) was added to the crucible containing the total ash, it was then covered with a watch glass and boiled gently for 5 minutes, and the insoluble matter was collected in an ash-less filter paper and washed with water until it was

neutral. The filter paper was then transferred back into the crucible, and ignited to a constant weight, allowed to cool and weighed.

Acid-insoluble ash = $\frac{weight\ of\ residual\ ash}{initial\ weight\ of\ sample}\ x\ 100$

iii. Water Soluble Ash

Water (25ml) was added to the crucible containing the total ash and boiled for five minutes. The insoluble matter was collected in an ash-less filter paper, transferred to the crucible after washing with hot water and ignited to a constant weight; ash was then allowed to cool and weighed.

$$Water\ soluble\ ash = Total\ ash - Water\ insoluble\ ash$$

c) Extractive Values

The alcohol and water soluble extractive values were determined using the methods described in the WHO (1998) manual.

i. Alcohol Soluble Extractive

The powdered plant sample (6 g) was placed in a stoppered conical flask and 100ml of ethanol was added, it was shaken for 6 hours and then allowed to stand for 18 hours. It was then filtered rapidly and 25ml of the filtrate was transferred to an evaporating dish of known weight and evaporated to dryness using a water bath. It was then allowed to cool and weighed.

Extractive value = $\frac{weight\ of\ residue\ in\ 25\ ml}{weight\ of\ powdered\ sample}\ x\ 100$

ii. Water Soluble Extractive

The powdered plant sample (6 g) was placed in a stoppered conical flask and 100ml of ethanol was added, it was shaken for 6 hours and then allowed to stand for 18 hours. It

was then filtered rapidly and 25ml of the filtrate was transferred to an evaporating dish and evaporated to dryness using a water bath.

Water Extractive value= $\frac{weight\ of\ residue\ in\ 25\ ml}{weight\ of\ powdered\ sample}\ x\ 100$

d) Determination of Tannins in Leaf of *D. arborea*

Tannins in the leaf of the plant were determined using methods described in the WHO (1998) manual.

The powdered plant sample (60 g) was weighed into a conical flask and 150ml of water was added and it was heated over a boiling water bath for 30 minutes. It was then cooled and transferred to a 250ml flask and diluted to 250 ml with water. It was then filtered.

The total amount of material that is extractable into water was determined by evaporating 50 ml of the plant aqueous extract to dryness and weighed as (T_1).

The amount of plant extract not bound to hide powder that is extractable into water was determined by taking 80ml of the plant extract and 6g of hide powder was added to it and shaken for 60 minutes. It was then filtered and 50ml of the filtrate evaporated to dryness and weighed (T_2).

The solubility of hide powder was determined by taking 6g of hide powder and adding 80ml of water and shaken for 60 minutes. It was then filtered and 50ml of the filtrate was evaporated to dryness and weighed as (T_0).

The quantity of tannins was then calculated using the formula:

$$\frac{T1 - (T2 - T0)}{W}\ x\ 100$$

Where w = weight of the plant material in grams.

T_0 = solubility of hide powder

T_1 = total amount of material extractible into water

T_2 = the amount of plant extract not bound to hide powder

e) Determination of Swelling Index in Leaf of *D. arborea*

The swelling index of the leaf of the plant was determined using the methods described in the WHO (1998) manual.

The powdered plant (1 g) was weighed into a 25 ml measuring cylinder, and 25 ml of water was added and the mixture was shaken for 1 hour and allowed to stand for 3 hours. The volume in ml occupied by the plant material was measured. This was repeated 3 times and the mean value was calculated.

f) Determination of Foaming Index in Leaf of *D. arborea*

The foaming index of the leaf of the plant was determined using the methods described in the WHO (1998) manual.

The powdered plant (1 g) was transferred into a 500 ml conical flask containing 100ml of boiled water, it was then allowed to cool and filtered into a 100 ml volumetric flask and the decoction was then poured into 10 test tubes in successive portions of 1 ml, 2 ml, 3 ml etc. up to 10 ml. The test tubes were then shaken in a length wise motion for 15 seconds and allowed to stand for 15 minutes and the height of the foam measured. The results obtained were assessed as; if the height of the foam in every tube is less than 1 cm the foaming index is less than 100, if a height of foam of 1 cm is measured in any tube the volume of the plant material decoction in this tube is used to determine the index, if the height of the foam is more than 1 cm in every tube the foaming index is over 1000.

3.4.4 Determination of Microbial contaminants in the Leaves of *D. arborea*

a. Total Viable Aerobic Count for Bacteria and Fungi

The plant powder (1 g) was diluted in 10ml of distilled water. Liquefied casein-soybean digest agar was poured into two petri-dishes and allowed to solidify. The plant-powder mixture (1 ml) was spread on the solidified medium and incubated for 24 hours days at 30-35˚C. The number of bacterial colonies formed was counted.

The same procedure was used for fungi but liquefied Sabouraud glucose agar was used as the growth medium and incubation temperature was 20-25˚C for 5 days (WHO, 1998).

b. Test for the presence of specific micro-organisms

i) Escherichia coli

The plant powder mixture (1 ml) was transferred into two petri-dishes containing MacConkey agar and incubated at 35-37˚C for 24 hours and then observed for presence or absence of growth of red, non-mucoid colonies of *Escherichia coli* (WHO, 1998).

ii) Salmonella species

1ml of the plant powder-mixture was transferred into two petri-dishes containing *Salmonella-Shigella* agar and incubated at 35-37˚C for 24 hours then observed for presence or absence of well-developed colourless colonies of *Salmonella spp* (WHO, 1998).

iii) Pseudomonas aeruginosa

1ml of the plant powder-mixture was transferred into two petri-dishes containing cetrimide agar and incubated at35-37 ˚C for 24 hours then observed for presence or

absence of growth of well-developed colonies with greenish fluorescence of gram-negative rods of *Pseudomonas aeruginosa* (WHO, 1998).

iv) Staphylococcus aureus

1ml of the plant powder-mixture was transferred into two petri-dishes containing Baird-parker agar and incubated at35-37˚C for 18-24 hours then observed for presence or absence of black colonies of gram-positive cocci of *Staphylococcus aureus* (WHO, 1998).

3.5 Phytochemical Studies of the leaves of *D. arborea*

Phytochemical studies of the methanol extract of the leaves of *D. arborea* were carried out using the methods described in the WHO (1998) manual.

3.5.1 Extraction of Powdered Leaves of *D. arborea*

Plant powder (500 g) was macerated using 3.0 litres of 70% aqueous methanol for 48 hours. The extract obtained was evaporated to dryness on a water bath, and then stored in a container for use. The percentage yield was calculated using the formula:

$$\text{Percentage yield} = \frac{weight\ of\ extract}{weight\ of\ powdered\ drug}\ x\ 100$$

3.5.2 Thin Layer Chromatographic Studies of the extract

This was carried out on the aqueous methanol extract of the plant using pre-coated silica gel plates; the solvent system used was chloroform: ethyl acetate (3:1).The chromatogram was then developed and plate was removed from the chamber.

Different spray reagents (Dragendoff spray, 10% sulphuric acid in methanol, vannilin in sulphuric acid and ferric chloride sprays) were used in detecting the presence or absence of alkaloids, steroids and phenolic compounds on several plates.

3.6 Biological Studies of Aqueous Methanol Leaf Extract of *D. arborea.*

Acute toxicity studies and sperm function analysis of the methanol extract of the leaves of *D. arborea* were carried out

3.6.1 Acute toxicity studies of Aqueous Methanol Leaf Extract of *D. arborea*

The acute toxicity study was performed using Lorke's (1983) method. Adult male rats weighing (200-250 g) were used. This was done in two phases.

Phase 1: Nine rats were divided into 3 groups of 3 rats each. The 3 groups were administered orally 10 mg/kg, 100 mg/kg and 1000 mg/kg of the methanol extract respectively.

Phase 2: three rats divided into 3 groups of 1 rat per group each group received orally, 1600, 2900 and 5000 mg/kg of the extract respectively after which the animals were kept for days with unhindered access to food and water.

3.6.2 Evaluation of Pro-fertility effects of Aqueous Methanol leaf Extract of *D. arborea*

3.6.2.1 Experimental Animals

Adult male wistar rats (200-250 g) were used and housed in a well-ventilated animal house under laboratory conditions of humidity and temperature and were fed with standard pellet diet and water was made available to them, *ad libitum*.

3.6.2.2 Experimental Design

Eighteen (18) adult male rats were randomly divided into 3 groups of 6 animals each. Group 1(control group) was administered with distilled water while groups 2 and 3 were administered with250 mg/kg and 500 mg/kg methanol extract respectively. Treatment was done daily using oral dozing needles. Three rats from each group were sacrificed after 14 days of daily administration of the extract and the remaining rats

were dozed up to 28 days and then sacrificed. Animals were sacrificed 24 hours after last administration of the extract (Morakinyo *et al*., 2008)

3.6.2.3 Sperm Function Analysis

The spermatozoa was obtained from the rats by making a small incision in the caudal epididymis and minced on a petri-dish containing 1 ml normal saline for some few minutes to allow the sperm cells become motile and swim out of the caudal epididymis (Saalu*et al*., 2007).

3.6.2.4 Determination of Sperm Count

The sperm count was determined according to the method described in the WHO (1999) laboratory manual. The obtained sperm was diluted 1:200 in sperm immobilization solution (5% formol solution). Sperm count was determined using an improved Neubauer haemocytometer. 20µl of the sperm cell mixture was placed on sperm examination area and a cover slide was placed on the examination area and examined under X10 magnifications. The sperm cells within five boxes were counted and the number recorded and the sperm cell concentration was calculated.

$$sperm\ count = number\ of\ sperm\ counted \times dilution\ factor \times 1000$$

3.6.2.5 Determination of Sperm Motility

The sperm motility was determined according to the method described in the WHO (1999) laboratory manual. Sperm was diluted in 1ml normal saline and then a drop was placed on a glass slide, covered with a cover slip and examined under a microscope at 10x magnifications on the microscope. The motile sperm cells in forward progression were counted and percentage motility was calculated.

$$sperm\ motility = \frac{motile\ sperm}{motile\ sperm + non - motile\ sperm} \times 100$$

3.6.2.6 Determination of Sperm Viability

The sperm viability was determined according to the method described in the WHO (1999) laboratory manual. A drop of the sperm mixture was placed on a glass slide and a drop of eosin-nigrosin stain was added to it and a thin smear was made using a cover glass. It was then allowed to dry and examined under the microscope at X1000 (by using oil-immersion objective).The live sperm cells remain unchanged and the dead cells take the colour of the stain. The percentage live-dead ratio of the sperm cells was calculated.

$$live - dead\ ratio = \frac{live\ \ cells}{live\ cells + dead\ \ cell} \times 100$$

3.6.2.7 Determination of Sperm Morphology

The sperm morphology was determined according to the method described in the WHO (1999) laboratory manual. The sperm cells were examined under the microscope and the percentage number of sperm that appeared to have an abnormal morphology was calculated.

3.6.2.8 Statistical Analysis

Data were expressed as mean ± standard error of mean and analysed using the student's *t*-test and ANOVA. The Duncan post hoc test was used to analyse results where there was significant difference using statistical application package, Statistical Package for Social Scientists, version 16.0 and values with $p < 0.05$ was considered as significant (SPSS, 2007).

CHAPTER FOUR

4.0 RESULTS

4.1 Evaluation of Pharmacognostic Characters of the Leaves of *D. arborea*

4.1.1 Quantitative Leaf Microscopy

The stomatal number was found to be 10.40-12.20-14.00 for the upper epidermis and 46.30 – 54.50 – 62.70 for the lower epidermis. The stomatal index was 1.30-1.50-1.70 for the upper epidermis and 7.80- 9.20- 10.60 for the lower epidermis. The palisade ratio was found to be 5.60- 6.60- 7.60 (Table 4.1).

Table 4.1: **Quantitative Leaf Microscopy of *D. arborea***

	Results	
	Upper epidermis	**Lower epidermis**
Stomatal Number	10.40 – **12.20** – 14.00	46.30 – **54.50** – 62.70
Stomatal Index	1.30 – **1.50** – 1.70	7.80 – **9.20** – 10.60
Palisade Ratio	5.60 – **6.60** - 7.60	

Results are given as lower value – mean - upper value. Values are Mean value of 5 counts

4.1.2 Qualitative Leaf Microscopy

The surface preparations of the upper and lower epidermis showed single layered epidermal cells with no trichomes. The epidermal cells have straight walled and elongated polygonal cells (hexagonal).

The stomata are anomocytic in nature with a length of 36.0µm and width of 24.2 µm. It is distributed on both upper and lower surfaces of the leaf with a higher number (46.30-54.0-62.70) on the lower compared with (10.40 – 12.20 – 14.00) on the upper (Plate II and III).The transverse section showed an isobilateral tissue arrangement with a single layer of palisade cells beneath both lower and upper epidermis and calcium oxalate crystals were in some cells. The spongy mesophyll is made up of a loose tissue of parenchyma cells and collenchyma cells. The midrib portion showed the vascular tissues which were grouped in form of bundles and consist of scalariform of xylem vessels (Plate IV and V).

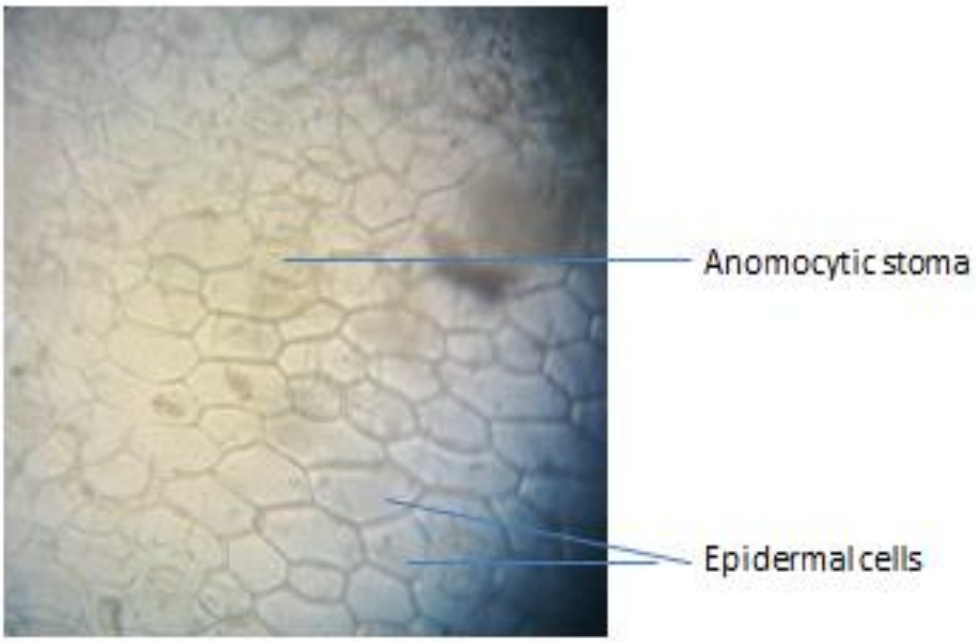


PLATE II: Photomicrograph of Upper Epidermis of Leaf of *D. arborea* showing Anomocytic Stomata (X 400)

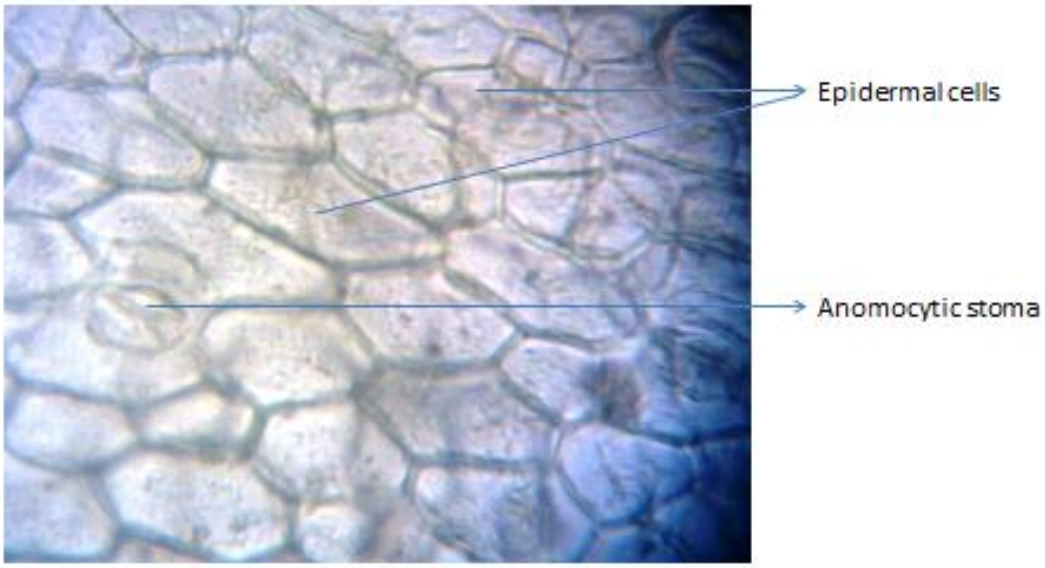


PLATE III: Photomicrograph of Lower Epidermis of the Leaf of *D. arborea* showing Anomocytic Stomata (X400)

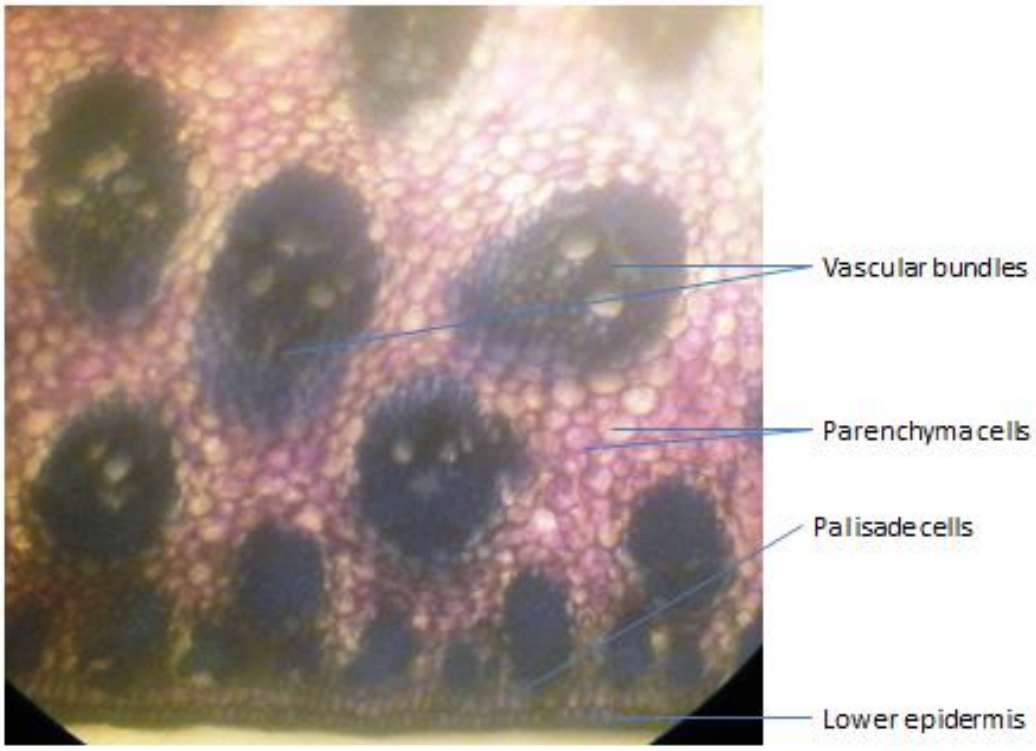


PLATE IV: Photomicrograph of Transverse Section of leaf of *D. arborea* stained with Toluidine Blue (X400)

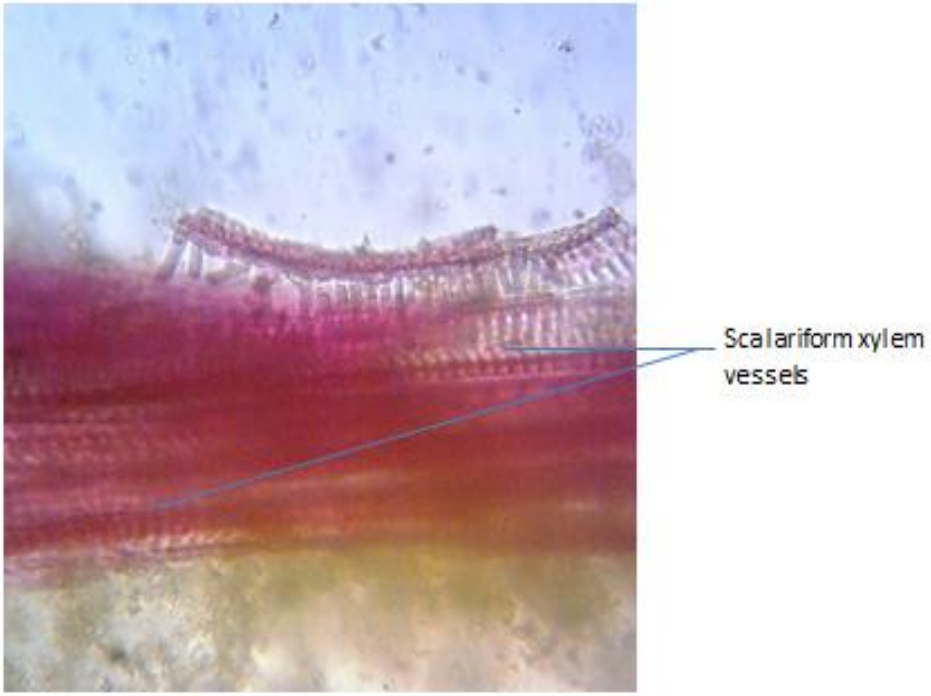


PLATE V: Photomicrograph Showing Xylem Vessels (Scalariform) of the Leaf of *D. arborea* (X100)

4.1.3 Chemo-Microscopy of the Leaf of *D. arborea*

The results of histochemical colour reactions indicated the presence of cell wall composed of cellulose, lignin, suberin, mucilage. Cell contents identified are starch, proteins, tannins, and calcium oxalate crystals (Table 4.2 (a) and (b).

Table 4.2(a): Chemomicroscopy of Cell Wall Materials of the Leaf of *D. arborea*

TEST	OBSERVATION	INFERENCE
Cellulose cell wall	Blue-violet colouration was observed on the outer cell walls on fibres and parenchyma cells. On addition of cuoxam the cell wall gradually dissolved.	Cellulose cell wall present
Lignified cell wall	Pink colouration was observed on the Xylem vessels (Scalariform) type.	Lignified cell wall present
Suberized cell wall	Orange red colouration was observed.	Suberized cell wall present
Mucilage	Pink colouration was observed.	Mucilage present

Table 4.2(b): Chemomicroscopy of Cell Contents of the Leaf of *D. arborea*

TEST	OBSERVATION	INFERENCE
Starch	Blue colouration was observed on starch grains/granules	Starch present
Hydroxyanthraquinones	No colouration	Hydroxyanthraquinone absent
Inulin	Brownish red colouration observed on crystals which later dissolved in sulphuric acid	Inulin present
Calcium oxalate	Prism-like crystals, small to medium size which dissolved in hydrochloric acid was observed	Calcium oxalate present
Calcium carbonate	No effervescence was observed on dissolution of crystals	Calcium carbonate absent
Proteins	Red colouration was observed	Proteins present

4.2 Evaluation of Physicochemical Parameters of the Leaf of *D. arborea*

Evaluation of the physicochemical parameters of the leaf showed that the total ash was 14.78 %, acid insoluble ash was 0.53 % and water soluble ash was 11.22 %.The ethanol soluble extractives were(1.13 %) and water soluble extractives were(1.29 %). The percentage moisture content was 3.89 %. The percentage quantity of tannins was found to be 4.42 %. The swelling index of the plant was 5.60 ml. The foaming index of the plant was found to be less than 100 as no foaming to a height of 1cm was observed (Table 4.3).

Table 4.3: Physicochemical parameters of *D. arborea*

PARAMETER	**VALUES(Mean ± SEM)**
	%
Moisture content	3.89 ± 0.61
Total ash	14.78 ± 0.61
Acid insoluble ash	0.53 ± 0.23
Water soluble ash	11.22 ± 0.48
Alcohol soluble extractives	1.13 ± 0.35
Water soluble extractives	1.29 ± 0.13
Swelling index	5.60 ± 0.13
Foaming index	<100
Tannins	4.42

* mean values of 3 counts.

4.3 Determination of Microbial Contaminants

The total viable aerobic count of bacteria was found to be 6,100 cfu/ml and fungi to be 14,800 cfu/ml. The presence of *E. Coli* was observed as red non-mucoid colonies but *Salmonella spp, P. aureginosa and S. Aureus* were absent (Tables 4.4a and b).

Table 4.4(a): Total viable aerobic count in powdered plant of *D. arborea*

MICRO-ORGANISM	NO OF COLONIES(CFU/ml)
Bacteria	6,100
Fungi	14,800

Mean values of 3 counts

Table 4.4 (b): Specific Micro-Organisms in Powdered Plant of *D. arborea*

MICRO-ORGANISM	OBSERVATION	INFERENCE
Eschericia coli	Red non-mucoid colonies were formed	Present
Salmonella species	No colonies formed	Absent
Pseudomonas aeruginosa	No colonies formed	Absent
Staphylococcus aureus	No colonies formed	Absent

4.4 Phytochemical Studies of Leaves of *D. arborea*

4.4.1 Extraction of Powdered Leaves of *D. arborea*

The 70% aqueous methanol extract yielded a brownish mass of 34.98g (6.99%).

4.4.2 Thin layer Chromatographic Studies of *D. arborea*

The chromatographic resolution of the aqueous methanol extract with solvent system chloroform: ethyl acetate (3:1) showed about 7 spots after spraying with 10% sulphuric acid (Plate VI and Table 4.5).Specific detecting spray reagents namely ferric chloride gave a greenish black colour reaction which indicated the presence of phenolic compounds and vanillin in sulphuric acid gave a violet colour reaction which indicated the presence of steroids while Dragendoff spray gave no colour reaction which showed the absence of alkaloids(Plates VII, VIII, and Table 4.6).

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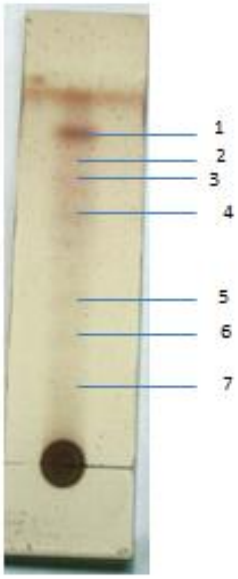

PLATE VI: Thin Layer Chromatogram of Crude Extract after Spraying With 10% Sulphuric Acid Spray.

Solvent system: Chloroform:Ethyl acetate (3:1)

Table 4.5: Spots and R_f Values of Thin Layer Chromatogram of the Aqueous Methanol Leaf Extract of *D. arborea* with 10% Sulphuric Acid

SPOT	R_f VALUE
1	0.81
2	0.75
3	0.64
4	0.58
6	0.45
7	0.29

Solvent system: Chloroform: Ethyl acetate (3: 1)

Plate VII: Thin Layer Chromatogram of Aqueous Methanol Leaf Extract of *D. arborea* after Spraying with Vanillin in Sulphuric acid Spray.

Solvent system: Chloroform: Ethyl acetate (3:1)

PLATE VIII: Thin Layer Chromatogram of Aqueous Methanol Leaf Extract of *D. arborea* after Spraying with Ferric Chloride Spray.

Solvent system: Chloroform: Ethyl acetate (3:1)

Table 4.6: Thin Layer Chromatography of Aqueous Methanol Leaf Extract of *D. arborea*

Spray reagent	Colour on spot	Inference
Ferric chloride	Greenish black	Phenolic compounds present
Vanillin in sulphuric acid	Violet	Steroids present
Dragendoff	No spot	Alkaloids absent

Solvent system: chloroform: ethyl acetate (3: 1)

4.5 Biological Studies of Aqueous Methanol Leaf Extract of *D. arborea*

4.5.1 Acute- Toxicity Studies

In the acute-toxicity study, no death was observed in the adult male wistar rats with oral administration of doses of up to 5000 mg/kg of the methanol extract of *D. arborea* after 48 hours of observation. The LD_{50} value was found to be above 5000 mg/kg body weight.

4.5.2 Sperm Function Analysis of Aqueous Methanol Leaf Extract of *D. arborea* in Male Wistar Rats

The aqueous methanol leaf extract of the plant had no significant ($p < 0.05$) pro-fertility effects in male wistar rats after 14 days of treatment at 250 and 500 mg/kg. However, there were significant ($p < 0.05$) pro-fertility effects in the male wistar rats after 28 days of treatment. The parameters evaluated were sperm count (76.00 ± 10.17 and113.33 ± 13.10 at doses 250 mg/kg and 500 mg/kg respectively), sperm motility (76.60 % ± 8.81and 80.00% ± 0.00 at doses 250 mg/kg and 500 mg/kg respectively) and sperm viability (79.66% ± 7.96 at 500 mg/kg).There was no significant difference in sperm abnormal morphology after 28 days of treatment (4.50 ±1.32 and 6.17 ± 0.33 at 250 mg/kg and 500 mg/kg respectively) as compared with the controls (Tables 4.8(a) and 4.8(b) and Plate IX).

Table 4.7(a): Sperm Function Analysis in Male Wistar Rats after 14 days of Treatment with Methanol Extracts

Group	Count* (10^6/ml)	Motility (%)*	Viability (%)	Abnormal morphorlogy (%)
Control	63.00 ±5.23	70.00 ±5.77	83.33 ±1.66	9.00 ±0.76
250mg/kg	8.30 ±2.96	46.66 ±3.33*	45.00 ±2.88	11.83 ±3.41
500mg/kg	22.67 ±8.73	53.33 ±8.81*	55.00 ±5.77	10.33 ±2.89

n =3, * p<0.05. Values expressed as mean ±SEM

Table 4.7 (b): Sperm Function Analysis in Male Wistar Rats after 28 days of Treatment with Methanol Extracts

Group	Count (10^6/ml)	Motility (%)	Viability (%)	Abnormal morphorlogy(%)
Control	41.33 ± 15.16	73.33 ± 6.66	80.00 ± 5.77	6.83 ±1.1.36
250mg/kg	76.00 ±10.17*	76.60 ± 8.81*	78.33 ± 4.40	4.50 ± 1.32
500mg/kg	113.33 ±13.1*	80.00 ± 0.00*	79.66 ± 7.96	6.17 ± 0.33

n=3, * p<0.05. Values expressed as mean ±SEM

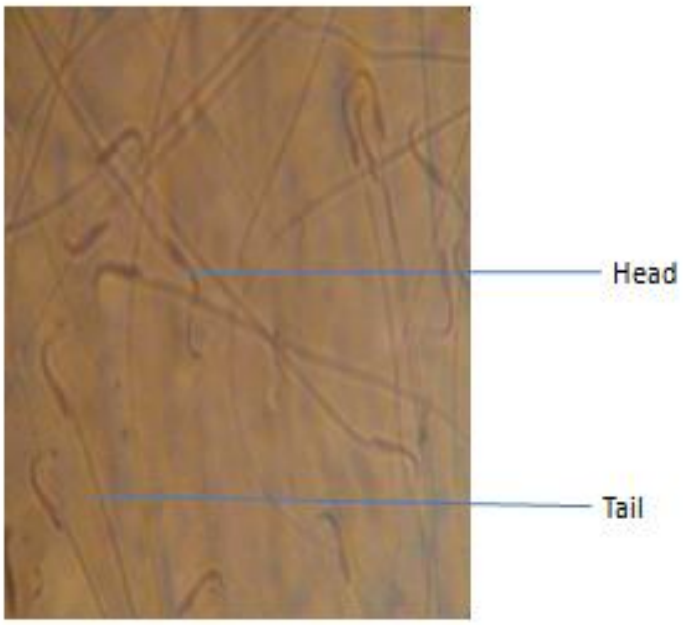

PLATE IX: Photomicrograph of Viable Rat Sperm Cells after Treatment with Methanolic Extract of *D. arborea* (X100)

CHAPTER FIVE

5.0 DISCUSSION

The anomocytic stomata measuring 36.00µm in length and 24.20 µm in width were more on the lower epidermis(46.30 – **54.50** – 62.70) than on the upper epidermis (1.30 – **1.50** – 1.70) is a characteristic of most of the species of *Dracaena* (Klimko and Szymanska, 2008), and this arrangement helps the plant to minimize the rate of transpiration through the leaves and it could be an adaptation to withstand harsh weather conditions during the dry season which helps the plant to remain evergreen all year round (Akpovughaye, 2009).

The absence of trichomes, the straight walled and elongated polygonal cells of the upper and lower epidermis, the anomocytic stomata with average length of 36.0µm and width of 24.2µm are all characteristics of most of the species of *Dracaena* and are similar to those reported by Klimko and Szymanska, (2008).

The histochemical colour reactions were carried out on the powdered leaf sample and sections of *D. arborea*. The results of the chemo-microscopy indicates the presence of cellulose cell wall, lignified cell wall, suberized cell wall, and also presence of mucilage, starch, proteins, tannins, and calcium oxalate crystals in the leaf of *D. arborea*. Histochemical localization of certain important compounds helps to get a preliminary idea of the types of compounds and their accumulation in the plant tissues (WHO, 1998).

The Total ash of 14.78 %, acid insoluble ash of 0.53 % and water soluble ash of 11.22 % are within acceptable ranges. These values obtained could be used as a reference for future studies. Ash values are important qualitative standards useful in determining authenticity and purity of drugs (Kokate, 1994). The residue remaining after

incineration of plant material represents inorganic salts naturally occurring in crude drugs or adhering to it or deliberately added to it as a form of adulteration (Evans, 2002).

The result of the extractive values showed little difference between ethanol soluble extractives (1.13 %) and water soluble extractives (1.29 %). The extractive values in different solvents are indicative of the quantity and nature of the chemical constituents present in the extract. These extractives are primarily useful for the detection of exhausted or adulterated drugs (WHO, 1998).

The percentage moisture content of 3.89 % is below 10 %, which is the maximum recommended value for crude drugs. An excess of water in medicinal plants will encourage microbial growth, colonization by fungi, insects, and deterioration following hydrolysis (WHO, 1998).

The percentage of tannins present in *D. arborea* was found to be 4.42 %which shows that the plant is astringent in nature and this could also be responsible for its therapeutic application (WHO, 1998). Tannins are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes (WHO, 1998).

The swelling index of 5.60 ml indicates the leaves of *D. arborea* contain an appreciable amount of mucilage, pectin or hemicelluloses (WHO, 1998). Swelling index is the volume, in millilitres, taken up by the swelling of one gram of plant material under specified conditions. This is important as many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling property especially those containing an appreciable amount of mucilage, pectin or hemicelluloses (WHO, 1998).

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming index of less than 100 (<100) indicates a small quantity of saponins in the plant.

Microbial contamination affects the quality of drugs as a result of the activities of viable aerobic bacteria and increased number of fungi. High microbial contaminants in crude drug materials could result in infections from bacteria and in the production of mycotoxins and toxic metabolites from fungi and these toxins possess powerful mutagenic and carcinogenic effects and therefore can be hazardous to humans who unknowingly consume these crude drugs.

The total viable aerobic count of bacteria (6,100 cfu/ml) and fungi (14,800 cfu/ml) indicates that the plant has a low level of microbial contamination as these values are below the maximum limits for fungi (10^5 per gram) and bacteria (10^4 per gram) in medicinal plant materials. Medicinal plant materials carry a great number of bacteria and moulds and the practise of harvesting, handling and storing may cause additional contamination and microbial growth. The microbial count of fungi in the plant even with the low level of moisture content could be that there was some growth of these organisms established before the complete drying of the plant since some fungi species are capable of growing at very low water content and also the ability of the fungi spores to withstand adverse environmental condition. (Tatjana *et al*., 2012).

The determination of bacteria and moulds indicates the quality of collection, processing and handling practices (WHO, 1998).The presence of *E. coli* was observed and may be as a result of the habitats proximity to settlements and animals that could contaminate the herbs with urine and faeces (Tatjana *et al*., 2012).

The absence of *salmonella, P. aeruginosa and S. aureus* in the plant is also indicative of its quality as the maximum acceptable limit for these micro-organisms is 10^3 per gram (WHO, 1998).

The use of 70% aqueous methanol as solvent of extraction was with the view to mimic the traditional method of use of the plant thus lending some explanation and support to the traditional preparation of leaves of *Dracaena arborea* in "palm wine", a traditional alcoholic solution for handling sexual impotency in males (Watcho *et al.*, 2007; Epidi and Njoku, 2009).

Watson and Dallwitz (1992) established that alkaloids are absent in *D. arborea* which is confirmed by the TLC of the extract which showed no coloured spot. Phenolic compounds were shown to be present on spraying with ferric chloride solution. Phenolic compounds are known to be the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts (Thabrew *et al.*, 1998). Presence of phenolic compounds can be further confirmed as a number of flavonoids have been isolated and characterized from *Dracaena* (Meksuriyen and Cordell, 1988; Liu *et al.*, 2009; Gupta *et al*.,2008).Preliminary phytochemical screening by Watcho *et al*., (2007) and Nnamani *et al*., (2008) showed the presence of flavonoids, condensed tannins and pseudotannins.

The presence of steroids in the TLC of the methanol extract is further confirmed as steroidal saponins are characteristic of the *Dracaenaceae* family (Watson and Dallwitz, 1992).Kougan *et al*., (2008) reported the isolation of steroidal saponins from *D. arborea*. Also Watcho *et al.*, (2007) and Nnamani *et al*., (2008) reported the presence of steroidal saponins, triterpenes, and sterols.

The aqueous methanol leaf extract of *D. arborea* can be said to be safe, as there was no death recorded after the first and second phases of the acute-toxicity study (LD_{50} > 5000 mg/kg) body weight per oral, in male wistar rats. This was supported by Lorke (1983) who stated that LD_{50} values greater than 5000 mg/kg body can be considered to be safe.

The sperm function analysis showed a significant ($p < 0.05$) increase in sperm count, sperm motility and sperm viability after 28 days of treatment with the aqueous methanol leaf extract on male rats in a duration dependent manner as compared with the controls while there was no significant ($p < 0.05$) difference in sperm abnormal morphology after 14 and 28 days of treatment as compared with the controls. Sperm functions analyses are often used as a measure of sperm production, testicular function and/or male fertility (Raji *et al.*, 2003). Low sperm count and motility and a high percentage of abnormal spermatozoa have been associated with reduced fertility (Adeeko and Dada, 1998). At 14 days of treatment the extract seems to reduce the sperm count, motility and viability and the ab. morphology was not affected, this could be as a result of some other underlying factors that affected the action of the drugs as there was an increased improvement in sperm functions at 500 mg/kg compared with 250 mg/kg. At 28 days there was significant difference in sperm count but motility, viability and abnormal morphology was not remarkably affected, but there was significant difference in sperm functions at 28 days compared to at 14 days which shows that length of administration of treatment influences the desired effects as it is known that the normal process of spermatogenesis is 52 - 53 days (Wankeu-Nya *et al.*, 2013).

The result of the present study even though not fully supported by statistical data suggests that *D. arborea* has some beneficial effect on increasing and enhancing male

reproductive functions. This data is confirmed by observations on some increased sperm parameters. The bioactive compounds (phenolic compounds and steroids) present in the extract may account for the pro-fertility effects observed and the observed increase in sperm functions due to a favourable increase in spermatogenic activities as a result of high testosterone levels (Watcho *et al.,* 2009). The result of this study can be compared to that done by Wankeu-Nya *et al*., (2013) in which the aqueous and ethanolic extract of *D. arborea* at a dose of 500 mg/kg showed a significant increase in sperm functions in rats with chemically induced diabetes, also Valentine *et al.,* (2013) showed that sub-chronic administration of the methanol extract at 500 mg/kg for 44-77 days enhances sperm functions.

One of the most important compounds concerned with fertility is cholesterol from which male steroidal hormones (testosterone in humans and adrostenedione in animals) are synthesized under the influence of luteinizing hormone (also called interstitial cell stimulating hormone). Testosterone is known to be an important hormone in the development of sperm cells, thus an increased testosterone level could be responsible for the increased sperm count noted in the treatment group as compared with the control. Because follicle stimulating hormone (FSH) binds to sertoli cells and promotes the synthesis of androgen binding protein (ABP), a high concentration of androgen (in this case, testosterone) is thus made locally available at the seminiferous tubules, which is the site of spermatogenesis (Morakinyo *et al*., 2008).

The increase in secretion of steroids and phenolic compounds increases the level of testosterone for spermatogenesis (Ukwenya *et al*., 2008).Also, it is known that a major function of the caudal epididymis is sperm maturation; therefore improvement in the activities of the epididymis through the influence of the methanol extract of *D. arborea* could have led to an increase in sperm functions in the experimental groups

(Morakinyo *et al*., 2008).The increase in sperm count and sperm motility has shown that treatment with *D. arborea* can possibly improve and enhance the fertilizing capacity of the semen.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The pharmacognostic study showed that the leaves of *Dracaena arborea* had an anomocytic type of stomata with no trichomes, a mean stomatal number of 12.20, stomatal index of 54.50, and a palisade ratio of 6.60, the total ash, acid insoluble ash and water soluble ash of 14.78 %, 0.53 % and 11.22 % respectively. The ethanol and water soluble extractives were 1.13 % and 1.29 % respectively. The moisture content was found to be 3.89 % and the quantity of tannins to be 4.42 %. The swelling index was5.60 ml and the foaming index was found to be less than 100 as no foaming to a height of 1cm were observed. The total viable aerobic count of bacteria and fungi was 6,100 cfu/ml and 14,800 cfu/ml respectively. TLC of the aqueous methanol extract showed the presence of phenolic and steroidal compounds while alkaloids are absent.

The aqueous methanol extract of the plant had a wide margin of safety after oral administration to male wistar rats, with an LD_{50} greater than 5000 mg/kg as no death occurred. The extract had a significant ($p < 0.05$) increase in sperm motility(76.60 % ± 8.81 and 80.00 % ± 0.00 at 250 mg/kg and 500 mg/kg respectively), sperm count (76.00 ± 10.17 and113.33 ± 13.10 at 250 mg/kg and 500 mg/kg respectively), and sperm viability(79.66% ±7.96 at 500 mg/kg) but no significant ($p> 0.05$) difference in abnormal sperm morphology(4.50 ± 1.32 and 6.17 ± 0.33 at 250 mg/kg and 500 mg/kg respectively) when compared with the negative controls after 28 days of treatment.

6.2 Conclusion

The study had determined the following properties of the leaves of *D. arborea*:

a. Some Pharmacognostic diagnostic features including :anomocytic stomata, polygonal epidermal cells, stomatal number, stomatal index, palisade ratio, moisture content, ash values, extractive values, swelling index, foaming index, tannins content ,microbial contaminants.
b. The aqueous methanolic leaf extract of the plant had a wide range of safety as its LD_{50} value was above 5000 mg/kg in male wistar rats.
c. The extract also has a significant (p <0.05) pro-fertility effects in male wistar rats after 28 days of oral administration at 250 and 500 mg/kg b/w when compared with control.

6.3 Recommendations

The following recommendations are made:-

1. More studies should be carried out to find out what active compound is responsible for the pro-fertility activity of the plant and possibly its mechanism of action.
2. More research should be carried out on the pro-fertility activity of the plant in terms of long-term toxicity studies, hormonal profile, reproduction, and human clinical trials.

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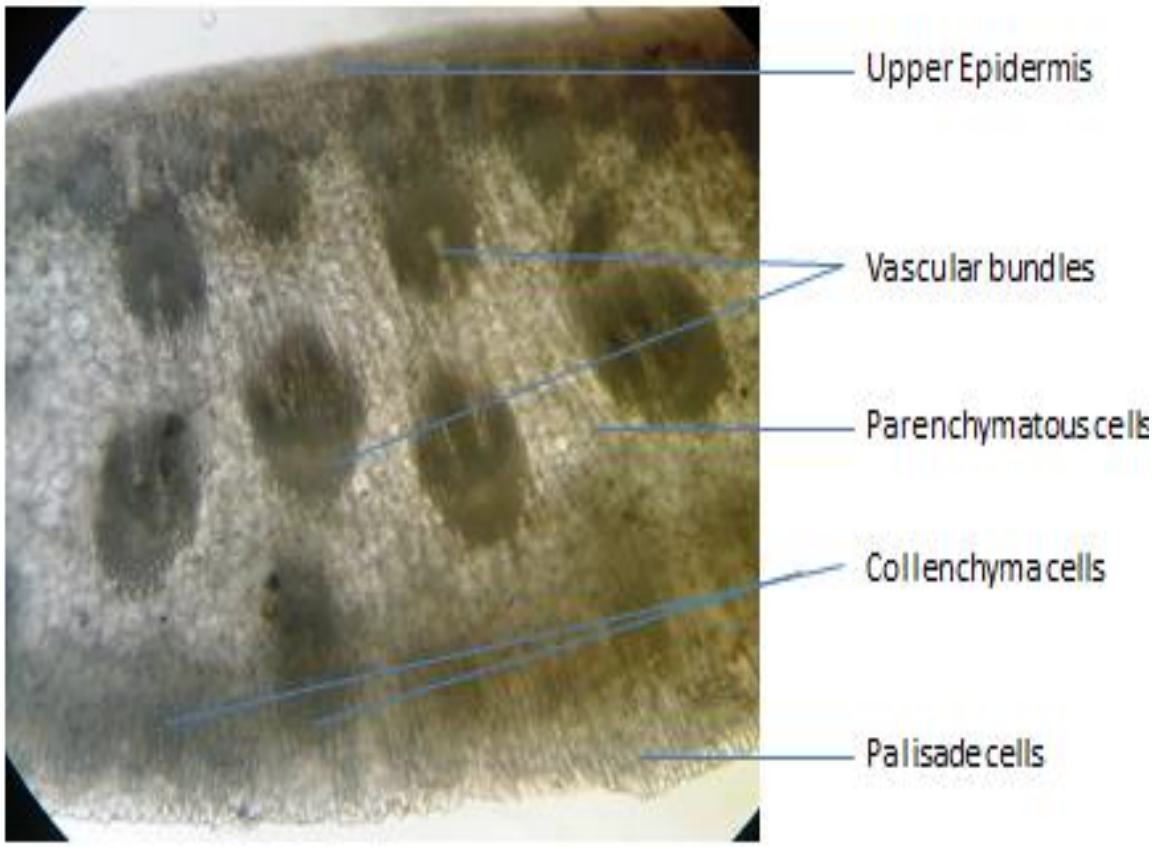
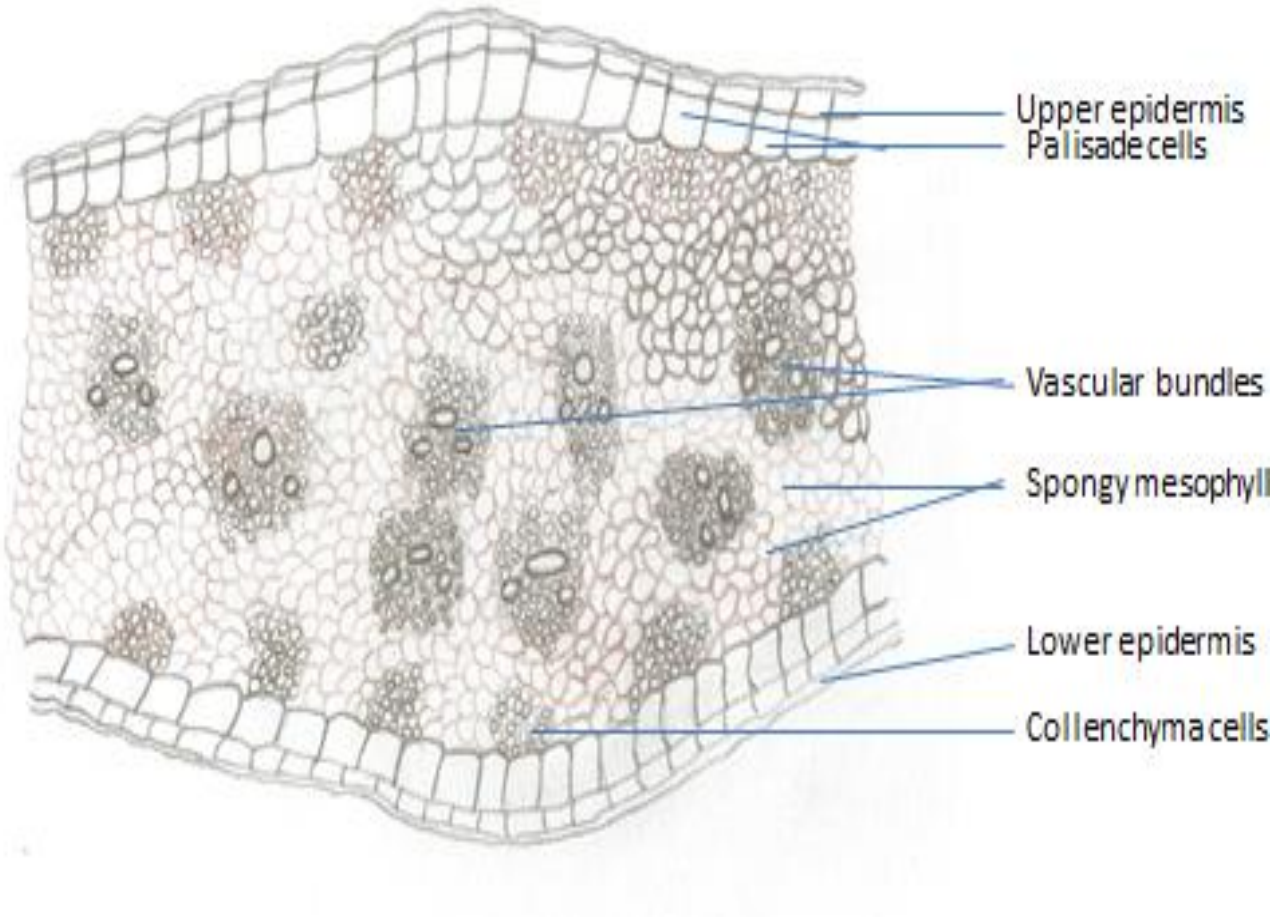
Appendices
Appendix I



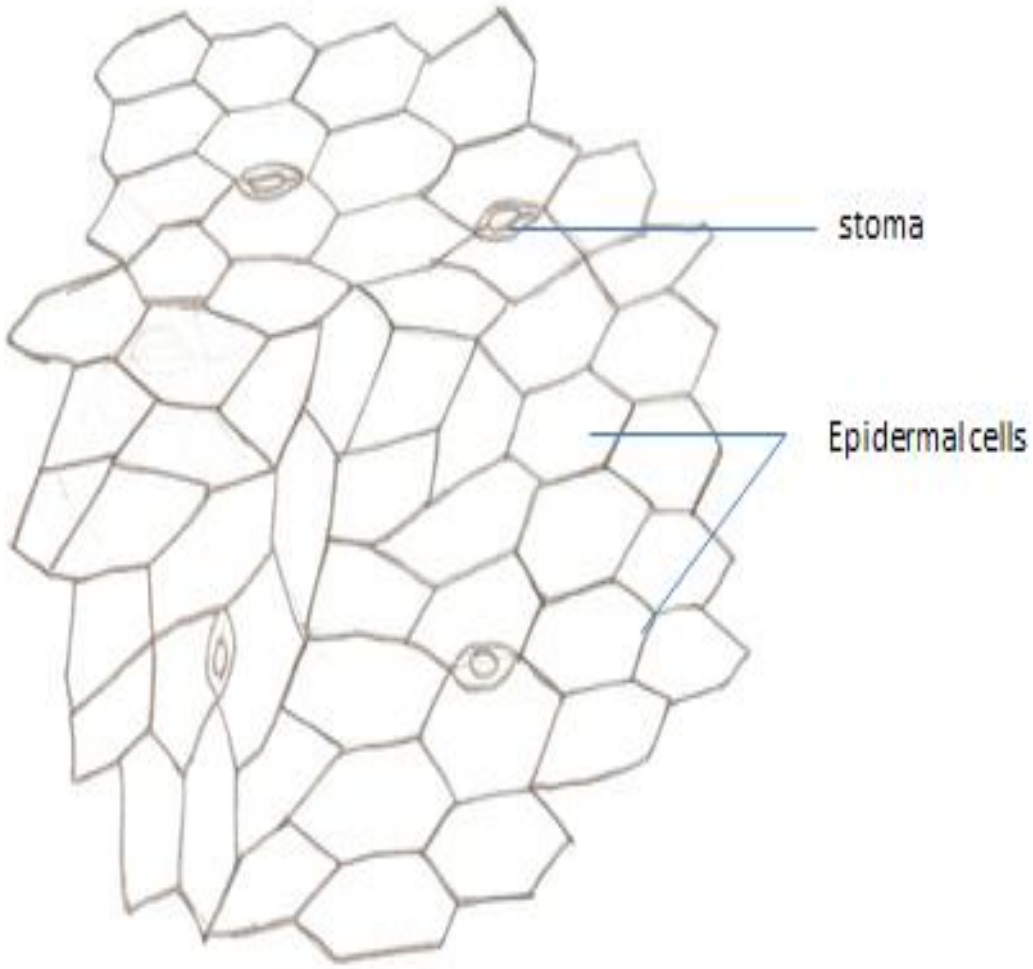
Photomicrograph of Transverse Section of Leaf of *Dracaena arborea*(X100)

Appendix II



Transverse Section of leaf of *D.arborea* through the midrib of the Leaf (X100)

Appendix III



Lower Epidermis of Leaf of *D.arborea* showing Anomocytic Stomata (X400)