APHRODISIAC PROPERTIES OF EXTRACT AND FRACTONS OF *VERNONIA AMYGDALINA* (ASTERACEAE) IN MALE WISTAR RATS

BY

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CERTIFICATION

This research project titled '**Aphrodisiac properties of flavonoid rich fraction of *Vernonia amygdalina* in male wister rats'** is an original research work carried out by Ezema Peace Udochukwuka under the supervision of Dr. (Mrs.) Josephine Omose Ofeimun, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City**,** Nigeria.

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DEDICATION

This project is dedicated to God Almighty for his infinite mercy, love, grace, guidance and protection throughout my stay in the University

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My greatest appreciation goes to Almighty God for his grace that has kept me thus far and for making this project a success.

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ABSTRACT

Phytochemicals constituents were screened for using different reagent to test for the presence of alkaloids, glycosides, tannins, saponins, anthraquinones, reducing sugars and flavonoids.

Diverse ethno medical applications exist for *Vernonia amygdalina*. However, the plant has only been the subject of a limited number of pharmacological research, and there hasn't been a thorough scientific investigation of its aphrodisiac properties. As a result, this study examined the aphrodisiac potential of extract and fractions of *Vernonia amygdalina* leaves utilizing physical and behavioral sexual parameters as well as in-vitro tests of the plant's effects on the corpus cavernosum muscles in male Wister rats. The powdered plant material was extracted with ethanol and the extract was subjected various solvent fractionation to obtain n-hexane, chloroform, ethyl acetate and residual aqueous fractions. After 2 weeks of acclimatization, oestrus was induced in the female rats by giving them 100mcg of ethinyl oestradiol orally and 1mg of progesterone subcutaneously, respectively, 24 hours and 3 hours before mating. The 35 male wister rats were randomly divided into 7 groups of 5 animals each, while 10 female rats were also obtained for the study. Animals in group 1 received 0.5mL of distilled water and each animal in this group received 20% Tween 80 (0.5mL) and this served as the negative group. Animals in groups 2, 3, 4, 5 and 6 received 50 mg/kg of the ethanolic extract, n-hexane, chloroform, ethyl acetate and aqueous fractions of *V. amygdalina* respectively in each group. Group 7 animals were given sildenafil 100mg/kg and served as the positive control. All administrations were done orally, and the physical parameters of aphrodisiac activity were measured. Also, the Corpus cavernosum smooth muscle was obtained from intact male rat and mounted in a 10 mL organ bath chamber containing Kreb's solution to evaluate the effects of the plant extract and fractions on the muscle. The direct effects of cumulative plant fractions concentrations were examined after tissue equilibration and response recording for 15 minutes without flushing. The plant fractions were utilized at doses of 0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/ml. In the presence of potassium, a pre-contractile agent, the identical process was done for the plant fractions. On a computer, the changes in isometric tension were monitored and noted. Following the administration of each concentration, a contact period of five minutes was permitted. The tissues were then cleansed three times and given 30 minutes to equilibrate before the next round of administration. The measurement of sexual behavior parameters, such as anogenital grooming, genital sniffing, mounting frequency, intromission frequency, ejaculatory frequency, mounting latency, intromission latency, and ejaculatory latency, showed a significant improvement in sexual activities. The plant fractions also generated a similar amount of relaxation of the corpus cavernosum smooth muscle to that brought on by the reference medication. In conclusion, the results of this study showed that the ethyl acetate and aqueous fraction of *V. amygdalina* leaves have strong aphrodisiac qualities and can relax the corpus cavernosum smooth muscle.

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CHAPTER ONE

1.0 INTRODUCTION

Humans have always depended on medical treatment for the prevention or cure of diseases. The first form of medical treatment was Herbal Medicine.

Herbal medicine (also called herbalism, phytomedicine or phytotherapy) is the study of pharmacognosy and the use of medicinal plants - roots, stems, leaves, flowers, or seeds of plants to improve health, prevent disease, and treat illness. It's a product made from a plant or plant part that is used to maintain or improve health. Plants have been used as medicine since ancient times, and we have relied on plants to formulate many of the drugs we use today (Bent 2008). Pharmacognosy, the study of crude drugs of plant and animal origin, incorporates the quality control and authentication of such herbal medicines (Sarker, 2012).

Herbal medicine is a product made from a plant or plant part that is used to maintain or improve health and Herbs are classified as dietary supplements under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (Bent 2008).

Consumers often perceive herbal medicines as safe because of their natural composition, although this may not invariably be the case, owing to the regulatory framework surrounding them. As per the Dietary Supplement Health and Education Act (DSHEA), herbal medicines do not necessitate substantiating their safety or efficacy prior to being marketed. Consequently, the dependability and safety of over-the-counter (OTC) herbal medicine products are often unreliable. Furthermore, the presence of contaminants and herb-drug interactions can give rise to unforeseen side effects.(Bent 2008)

In particular, herbal medicines can affect cytochrome P450 (CYP) enzymes, P-glycoprotein, and other metabolic enzymes/drug transporters, which can alter the efficacy and safety of prescription medications and other drugs (Asher *et al* ., 2017)

Furthermore, adequate research regarding the safety and efficacy of herbal medicine is lacking. Certain indications may suggest the efficacy of commonly used herbs, such as St. John's wort and gingko biloba, based on scientific evidence. However, it is worth noting that studies pertaining to herbal medicines frequently exhibit inadequate methodologies and yield conflicting results. Understanding proper dosing and administration is imperative for the safety and efficacy of all drugs, including herbal medicine, particularly since significant concerns regarding inconsistencies with product ingredients and herb-drug interactions have been noted in the medical literature (Glisson *et al.,* 2003).

This lack of knowledge and evidence may lead health care professionals to be uncertain of the role herbal medicine should play in modern health care approaches (Bent 2008).The use of herbal medicine has been increasing for a long time, despite disagreement among medical specialists. Since 2004, the sale of herbal supplements has risen in the US, with $8.085 billion projected to have been spent on them in 2017. This is an 8.5% increase over 2016. Some of the top-selling herbal supplements in supermarkets and pharmacies were echinacea, cranberry, turmeric, green tea, ginger, and saw palmetto. (Smith,2017).

Herbal Medicine is a sole part of Traditional medicine and Traditional medicine is often termed alternative or complementary medicine in many countries, though in some developing countries, Traditional medicine is the only attainable or affordable medicine.

88% of all countries are estimated to use traditional medicine, such as herbal medicines, acupuncture, yoga, indigenous therapies, and others.

About 80% of Africans rely on phytomedicine, which has demonstrated a wide range of applications in the treatment of diseases, particularly critical diseases of Africa like HIV/AIDS, malaria, sickle cell anemia, diabetes, and hypertension. Medicinal plants have played a significant role in global health care.

In Nigeria, using pure compounds obtained from plants, plant extracts, or both to cure illness has become a medicinal approach that has withstood the test of time. Today, a natural product prototype can be found in a number of pharmacological groups of medications. For the study of medicinal plants to be successful, a collaborative effort by ethnobotanists, ethnopharmacologists, doctors, and phytochemists is required.

However, there are certain issues that are impeding the growth of phytomedicine, including a lack of standards, insufficient efficacy and quality control of the plants utilized, the extinction of some plant species, a lack of funding, and others. When these issues are completely resolved, it will aid in the future development and synchronization of phytomedicines.

Hippocrates, who is widely regarded as the father of modern medicine and was born in 460 BC, made his appearance in the 18th century as the globe developed. Western Europe and the Americas both experienced a tremendous expansion of their economies at this time.

Acupuncture, homeopathy, herbal medicine, art therapy, traditional Chinese medicine, and many others are examples of alternative and complementary methods of medicine. Modern medication draws its source from plants, however it is synthesized.

1.2 SEXUAL DYSFUNCTION

Every stage of the sexual response cycle has the potential to experience sexual dysfunction. It keeps you from enjoying yourself during sexual engagement.

Excitation, plateau, orgasm, and resolution often make up the sexual response cycle. The excitement phase of the sexual response includes both arousal and desire. Remember that women don't always experience these phases in chronological order.

Despite the fact that evidence reveals sexual dysfunction is widespread, many people find it uncomfortable to discuss. However, you should discuss your worries with your spouse and your healthcare practitioner because there are therapy choices available.

Sexual dysfunction can affect people of any age, but it is more prevalent in people over 40 since it is frequently linked to a loss in health brought on by aging.

Sexual dysfunction can be any problems that prevent a person or couple from experiencing satisfaction from sexual activity. Some 43% of women and 31% of men report some degree of sexual dysfunction.

Aphrodisiacs might be something you want to try to help spice up your sex life, but in certain situations, people might want to take them to help address sexual difficulties. The majority of sexual dysfunction can be treated by treating the underlying physical or psychological disorders.

Not everyone who uses aphrodisiacs does so because of sexual issues. It makes sense that since amazing sex is enticing, some people would seek out products that claim to heighten sexual desire and participation. It's a common misconception that aphrodisiacs make sex more exciting,

delightful, and attractive. However, it is still unclear whether they have any actual ability to achieve these effects.

1.3 MALE SEXUAL DYSFUNCTION

Sex disorders of the male are classified into disorders of sexual function, sexual orientation, and sexual behavior. In general, several factors must work in harmony to maintain normal sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide system and androgens (Guay, *et al.,* 2003). Thus, malfunctioning of at least one of these could lead to sexual dysfunction of any kind.

Inability to engage in typical sexual activity is referred to as sexual dysfunction in men. It can also be seen as a group of illnesses that prevent a full cycle of sexual response.

The ability to enjoy or engage in sexual activity is compromised by certain illnesses. Sexual dysfunction rarely poses a risk to one's physical health, but it can have a devastating psychological impact, leading to feelings of hopelessness, anxiety, and melancholy. Unfortunately, the health care team has often neglected this problem.

Sexual dysfunction is more prevalent in males than in females and thus, it is conventional to focus more on male sexual difficulties (Guay, *et al.,* 2003).

Men's absolute Leydig cell numbers decline by roughly 40% as they age, and pulsatile luteinizing hormone release loses some of its power. Free testosterone levels also decrease by roughly 1.2% year in correlation with these occurrences. These have contributed in no small measure to prevalence of sexual dysfunction in the aged (Guay, *et al.,* 2003).

1.3.1 COMPONENTS OF MALE SEXUAL DYSFUNCTION (MSD)

MSD could be caused by various factors, which includes: psychological disorders (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure), androgen deficiencies (testosterone deficiency, hyperprolactinemia), chronic medical conditions (diabetes, hypertension, vascular insufficiency (atherosclerosis, venous leakage), penile disease (Peyronie's, priapism, phinosis, smooth muscle dysfunction), pelvic surgery (to correct arterial or inflow disorder), neurological disorders (Parkinson's disease, stroke, cerebral trauma, Alzheimer's spinal cord or nerve injury), drugs (side effects) (anti-hypertensive, central agents, psychiatric medications, antiulcer, antidepressants, and anti-androgens), life style (chronic alcohol abuse, cigarette smoking), aging (decrease in hormonal level with age) and systemic diseases (cardiac, hepatic, renal pulmonary, cancer, metabolic, post-organ transplant) (Guay, *et al.,* 2003 & Feldman *et al.,* 2001).

Sexual dysfunction takes different forms in men. A dysfunction can be life-long and always present, acquired, situational, or generalized, occurring despite the situation.

A man may have a sexual problem if he:

- Ejaculates before he or his partner desires.
- Does not ejaculate, or experiences delayed ejaculation.
- Is unable to have an erection sufficient for pleasurable intercourse.
- Feels pains during intercourse.
- Lacks or loses sexual desire.

Male sexual dysfunction can be divided into four categories: failure of detumescence, disorders of desire, disorders of orgasm, and disorders of ejaculation.

Disorders of desires: Deficient or compulsive sexual desire can be a symptom of desire disorders. The following dysfunctions can develop during the desire phase:

(i) **Hypoactive sexual desire (HSD):** It is challenging to establish a common definition of what constitutes an unacceptable want versus a lack of desire because sexual desires often involve feelings, ideas, and dreams that stimulate sexual hunger. It appears there is a great deal of variation in sexual desires between and within individuals and partners are rarely in sync when sexual desire is experienced. (Weeks & Gambescia 2002)

HSD can be said to be persistently or recurrently deficient (or absent) sexual fantasy and desire for sexual activity leading to marked distress or interpersonal difficulty. The complete or almost complete lack of desire to have any type of sexual relation is it's result.

(ii) **Compulsive sexual behaviors (CSBs)** encompass a broad spectrum of intricate sexual behaviors that exhibit remarkably repetitive, compelling, or driven characteristics. Typically, these behaviors manifest in the form of obsessive-compulsive sexuality, wherein individuals engage in excessive masturbation and promiscuity. Furthermore, they may display excessive sex-seeking tendencies in conjunction with affective disorders, such as major depression or mood disorders. Additionally, CSBs can also manifest as addictive sexuality, where individuals form attachments to another person, object, or sensation solely for the purpose of sexual gratification, disregarding all other aspects of life. Lastly, CSBs may also involve sexual impulsivity, which refers to the failure to resist impulses or temptations for sexual behavior that is harmful to oneself or others, such as exhibitionism, rape, or child molestation.

Disorders of Ejaculation: There exists a spectrum of

(i) **Premature Ejaculation:** also referred to as Early (EE) or Rapid Ejaculation (RE) is the commonest sexual compliant, affecting 30-40% of sexually active men in an age-dependant way (Frank *et al.,* 1970; Schein *et al.,* 1988) can be any of the following:

a) Persistent or recurrent ejaculation with minimum sexual stimulation that occurs before, upon, or shortly after penetration and before the person wishes it;

b) Marked distress or interpersonal difficulty; and

c) The condition does not arise as a direct effect of substance abuse. Premature ejaculation and disorders related to sexual desire were the commonly reported issues among young adult males experiencing unfavorable familial relationships.

(ii) **Painful Ejaculation**, arising from the adverse effects of tricyclic antidepressants, manifests as enduring and repetitive discomfort experienced in the reproductive organs during the act of ejaculation or immediately thereafter.

(iii) **Inhibited or Retarded Ejaculation:** This phenomenon denotes the absence of ejaculation.

(iv) **Retrograde Ejaculation:** This is the phenomenon in which the process of ejaculation is redirected towards the bladder instead of proceeding through the urethra and subsequently exiting the penis upon orgasm.

Disorders of Orgasm: Male orgasmic disorder is characterized as the continuing or recurring postponement in, or nonexistence of climax subsequent to a customary phase of sexual arousal during sexual engagement.

Failure of Detumescence: This is an extended state of tumescence typically persisting for a duration of four hours or more. It is marked by discomfort and consistently lacking in sexual inclination, notwithstanding the frequent occurrence of customary sexual stimuli preceding it.

Diagnostic options for male sexual dysfunction include: patient's history which embodies medical history (evaluating historical events like chronic disease, pharmacological agents, endocrine disorders, surgeries and trauma), psychological history (assessing individual's upbringing relationships, early sexual experiences, inadequate sexual information and general psychological health), sexual history (to ascertain the time and manner of onset, its course, current status, and associated medical or psychological problems), physical examination (entails general and systemic evaluation, assessment of gonadal function, vascular competence, neurological integrity, and genital organ normalcy), diagnosis testing (include blood tests, vascular assessment, sensory testing and nocturnal penile tumescence and rigidity testing) (Guay, *et al.,* 2003 & Feldman, 2001).

1.3.2 CAUSES OF SEXUAL DYSFUNCTION IN MALES.

Physical causes:

- Low testosterone levels.
- Prescription drugs (antidepressants, high blood pressure medicine).
- Blood vessel disorders such as atherosclerosis (hardening of the arteries) and high blood pressure.
- Stroke or nerve damage from diabetes or surgery.
- Smoking.
- Alcoholism and drug abuse.

Physiological causes:

- Concern about sexual performance.
- Marital or relationship problems.
- Depression, feelings of guilt.
- Effects of past sexual trauma.
- Work-related stress and anxiety.

1.4 ERECTILE DYSFUNCTION (ED)

This presents a concern pertaining to sexual stimulation. ED can be delineated as the challenge in attaining or sustaining an erection satisfactory for engaging in sexual activity or penetration, at a minimum of 50% of the instances, over the course of six months. It leads to substantial psychological, societal, and bodily morbidity and eradicates one's fundamental sense of masculinity.

1.4.1 CAUSES OF ERECTILE DYSFUNCTION

Achieving a regular erection is a multifaceted phenomenon that encompasses cognitive stimuli originating from the cerebral cortex; appropriate concentrations of the androgenic hormone, testosterone; a properly operating neural network; and sufficient and wholesome blood vessels in the male reproductive organ. A disorder of any of these systems may lead to ED. (Hirsch, 2013)

Although ED is more prevalent in older men, it is not directly caused by aging. Roughly 80% of ED cases can be attributed to physical factors. (Knott, 2013) Moreover, ED can arise from health issues, emotional difficulties, or a combination of both. It can serve as an early indicator of a

more severe medical condition. Identifying and addressing the underlying causes of ED may enhance overall health and quality of life.

1.4.1.1 PHYSICAL CAUSES

Reduced penile blood circulation: The diminished blood circulation to the male reproductive organ is unquestionably the predominant etiology behind erectile dysfunction in males aged 40 and above. Similar to other bodily regions, the arteries responsible for delivering blood to the penis can undergo constriction. Consequently, the blood flow might prove insufficient to induce an erection. The presence of risk factors may augment the likelihood of arterial stenosis. These include high blood pressure, high cholesterol and smoking. (Knott, 2013) Diseases that impact the nerves innervating the male genitalia may lead to the manifestation of Erectile Dysfunction (ED). Less common nerve disorders that cause ED include spinal cord injury, multiple sclerosis, Parkinson's disease, dementia and strokes.(Cunningham, 2014; Hirsch, 2013 & Knott, 2013) In addition, pelvic trauma, prostate surgery or priapism may cause ED.(Cunningham, 2014)

1.4.1.2 HORMONAL CAUSES

Testosterone plays an integral role in normal male sexual function.(Cunningham, 2014)

Hormonal disturbances, such as abnormally low levels of circulating testosterone, tend to decrease the sex drive, but can also result in ED. (Hirsch, 2013)

The restoration of sexual potency occurs with the normalization of testosterone levels. ED is often linked with other hormonal imbalances such as hyperprolactinemia, hyperthyroidism, and hypothyroidism. Restoration of the normal hormonal state usually results in the return of erectile function.(Cunningham, 2014)

Diabetes mellitus: Diabetes can affect the blood vessels and nerves.(Knott, 2013) The recognition that one third of men with type 2 diabetes mellitus have subnormal testosterone concentrations suggests that this hormone deficiency, and not just diabetic vasculopathy or neuropathy, may play a role in the ED seen in men with diabetes.(Cunningham, 2014)

1.4.1.3 PSYCHOLOGICAL CAUSES

Psychological problems, such as depression, performance anxiety or factors that decrease a man's energy levels, such as fatigue, illness or stress and relationship conflicts may cause or contribute to ED. Erectile dysfunction (ED) can arise in specific circumstances, encompassing a distinct location, moment or individual. Frequently, numerous elements contribute to the occurrence of ED. For example, a man with a slight decrease in erectile function caused by diabetes or peripheral. (Cunningham, 2014)

The harmony between the mind and body is a prerequisite for the realization of sexual activity. Psychological, emotional or relationship problems can cause or worsen ED and include the following: (Knott, 2013)

1.4.1.4 VASCULAR DISEASE

Vascular disease can develop severe ED after starting a new medicine or as a result of increased stress.(Hirsch, 2013)

The blood circulation to the male reproductive organ may experience obstruction or constriction due to vascular ailments like atherosclerosis, wherein the arteries undergo hardening.

1.4.1.5 NEUROLOGICAL DISORDERS

Impulses transmitted to the male genital organ can suffer from impairment due to stroke, diabetes, or other etiologies, encompassing multiple sclerosis. While incipient manifestations of carcinoma of the prostate do not instigate erectile dysfunction, its advanced presentations can. Invasive tumor excision of the prostate gland and radiotherapy for prostate malignancy possess the potential to elicit erectile dysfunction. Furthermore, certain pharmaceutical agents employed in the treatment of prostate cancer may also engender erectile dysfunction.

1.4.1.6 PSYCHOSOCIAL CAUSES

It is imperative to acknowledge that there exists a potential convergence between medical and psychosocial etiologies. To illustrate, in the case of an obese individual, alterations in blood circulation can impede the individual's capacity to sustain an erection, constituting a physiological cause. However, concurrently, the individual may experience diminished self-worth, thereby influencing erectile function, which can be classified as a psychosocial factor.

1.4.1.7 PSYCHOGENIC CAUSES

Psychogenic erectile dysfunction can potentially be ascribed to the presence of relationship stress, apprehension about sexual performance, or explicit psychological disorders, such as depression or schizophrenia, which can be further intensified by medications administered to address these conditions.

1.5 RISK FACTORS OF ERECTILE DYSFUNCTION

DIABETES MELLITUS

Diabetic men have a well-known increased risk of developing ED, with prevalence rates ranging from 35% to 90% (El-Sakk *et al.,* 2003; Malavige *et al.,* 2004; Giuliano *et al.,* 2004; Cho *et al.,* 2006; Siu *et al.,* 2001 & De Berardis *et al.,* 2002). Additionally, diabetic men tend to develop ED 10 to 15 years earlier than the average ED patient.

They appear to present with more severe ED and suffer a greater diminishment in health-related quality of life components than the general population (Penson *et al.,* 2003). ED secondary to DM is more resistant to medical management with phosphodiesterase 5 inhibitors

A systematic review by (Binmoammar *et al.,* 2016) of five cross-sectional studies found that poor glycemic control in patients with type II DM contributes significantly to the development and severity of ED. The various facets of erectile physiology are affected by the reactive oxygen species produced due to hyperglycemia. The likelihood of developing erectile dysfunction in diabetic individuals is heightened by the enduring consequences of macrovascular alterations, microvascular alterations, neuropathy, and endothelial dysfunction.

TOBACCO

Smoking causes oxidative stress and is a generally accepted risk factor for the development of ED (Peluffo *et al.,* 2009). A recent rat model documented several of the mechanisms that may be involved in ED and smoking. Rats exposed to 24 weeks of cigarette smoke had decreased penile neuronal NOS expression, decreased endothelial integrity, and diminished smooth muscle content compared to controls. Smoking has also been shown to impair endothelial Nitric oxide

synthase (NOS) -mediated vascular dilation in young men (Huang *et al.,* 2015). In conjunction with the vascular harm linked to tobacco consumption, there is some evidence indicating a potential decline in testosterone levels.

A review by (Dorey, 2001) that examined 18 studies showed that smokers were 1.5 times more likely to suffer from ED compared to nonsmokers. This increased risk has been confirmed by multiple cross-sectional studies, with the increased risk varying from 1.5 to 3.1 fold when compared to nonsmokers (Wu *et al.,* 2012, Chew *et al.,* 2009; Austoni *et al.,* 2005 & Ghalayini *et al.,* 2010). The dose-dependent and cumulative nature of smoking has been observed to contribute to a negative impact on erectile function. Studies have demonstrated that the increased risk of ED associated with smoking only becomes statistically significant after 20 pack-years or more (Natalie *et al.,* 2005 & Gades *et al.,* 2005)

HYPERTENSION

Hypertension is a well-established risk factor for ED, with 30% to 50% of hypertensive men being affected (Nunes *et al.,* 2012).

There are downstream effects with subsequent decreased relaxation of the erectile tissues and collagen remodeling and fibrosis within the corpora cavernosa (Behr-Roussel *et al.,* 2005).

There exist several alternative pathways that establish a connection between hypertension and ED, comprising of endothelial dysfunction, arteriosclerosis, and adverse effects resulting from the medical management of hypertension. Although there is variation within each category of antihypertensive medications, it is widely acknowledged that aldosterone receptor antagonists, beta-blockers, and thiazide diuretics have an adverse impact on erectile function. Non-selective

beta-blockers have a higher occurrence of causing ED compared to selective agents like metoprolol, which specifically targets the beta-1 receptor.

DEPRESSION

There exists a robust correlation between depression and erectile dysfunction (ED), and the antecedent condition is not always unequivocal. Moreover, numerous therapeutic interventions for major depressive disorders can instigate ED. The Morisky Medication Adherence Scale (MMAS) has yielded cross-sectional data that reveals depressed males are twice as prone to experiencing ED as the general populace. ED is a common side effect of selective serotonin reuptake inhibitors (SSRIs), with the incidence ranging from 20.8% to 80.3% depending on the medication utilized (Serretti *et al.,* 2009). Recent data raises the concern that after SSRIs ED and genital anesthesia may persist upon discontinuation (Csoka *et al.,* 2008) .Bupropion, nefazodone, and mirtazapine are associated with a less than 10% incidence of ED, which is lower than that documented with standard SSRIs (Gregorian *et al.,* 2002)

ATHEROSCLEROSIS

Although ED has several causes, including low testosterone and some medications, atherosclerosis is the most common reason. Cholesterol build up in the blood vessel walls causes the blood flow to slow down and extra blood flow to the penis is essential to obtain and maintain an erection.

In the public's mind, atherosclerosis is usually associated with heart attacks, but the condition is not confined to the arteries that supply the heart with blood. It affects the blood vessels throughout your body, including those in the penis, where the condition often shows up years before heart trouble does.

PROSTATECTOMY

A radical prostatectomy is a surgical intervention targeting the prostate gland, aiming to combat prostate cancer. This particular procedure frequently leads to a diverse range of adverse effects. Among these, a particularly daunting challenge is erectile dysfunction (ED), rendering the maintenance of an erection a formidable task.

1.6 SYMPTOMS OF ERECTILE DYSFUNCTION

Erectile dysfunction encompasses more than just the total incapacity to attain an erect phallus. Additionally, indications may involve difficulties in sustaining an erection for a sufficient duration to consummate sexual intercourse or an incapability to ejaculate. Emotional manifestations frequently emerge, including feelings of embarrassment, shame, anxiety, and a diminished inclination towards engaging in sexual intercourse.

1.7 DIAGNOSIS OF ERECTILE DYSFUNCTION

The diagnosis of ED involves a clinical evaluation including medical/physical examination as well as documentation of sexual and psychosocial history.(Cavallini, 2005 & Chen, 2001). Erectile dysfunction is among the various indications of sexual disorders, encompassing premature ejaculation, extended latency time connected to age, psychosexual relationship difficulties, and diminished sexual desire. When diagnosing erectile dysfunction, it is crucial to acknowledge and consider other sexual dysfunctions, such as diminished sexual desire. (A few validated instruments are used in diagnosing ED, grading its severity, (Choi, 2003 & Chopping, 2001) and assessing treatment satisfaction.

Recommendations based on biochemical investigation may consist of hormonal screening to detect hypogonadism or other underlying common diseases such as hyperprolactinemia, diabetes, and dyslipidemia (Dunzendorferet *et al.,* 2002)

Other methods that may be used are urine analysis, blood count, lipid levels, or prostate-specific antigen (PSA) concentration (Abdel-Naser *et al.,* 2004)

There are also specialized evaluation techniques such as duplex ultrasonography, penile tumescence studies, RigiScan, test injections, audio-visual stimulation, and penile brachial index measurement.

1.8 MANAGEMENT OF ERECTILE DYSFUNCTION.

There are various treatment /management options for ED depending on the cause and severity and on whether there is any underlying health condition. They are;

1.8.1 DRUG MANAGEMENT OF ERECTILE DYSFUNCTION (ORTHODOX THERAPY

Orthodox Drug therapy for the management of ED includes;

- **Oral medications:** Oral medications have been found to be successful in treatment of erectile dysfunction for many men. These medications include;

i. Tadalafil(Cialis, Adcirca)
ii. Sildenafil (Vega)
iii. Avanafil (Stendra)
iv. Vardenafil (Levitra, Staxyn)

These medications belong to the Phosphodiesterase-5-inhibitors (PDE5) class of drug. They reversibly inhibit the cyclase Guanosine Monophosphate (cGMP)-specific phosphodiesterase type 5 enzyme, thereby enhancing erectile functions.

All of these medications enhance the impact of nitric oxide, a naturally occurring vasodilator within the body that aids in the relaxation of penile muscles, consequently boosting blood circulation and facilitating penile erection in response to sexual stimulation. Nevertheless, the ingestion of one of these tablets does not automatically lead to an erection; sexual stimulation is still necessary in order to trigger the release of nitric oxide from the penile nerves. These medications amplify this signal, enabling certain individuals to carry out their sexual function in a normal manner. Oral medications for erectile dysfunction do not possess aphrodisiac properties, therefore they do not induce sexual excitement and are not required by men who are able to achieve an erection without assistance. These medications differ in terms of dosage, duration of effectiveness, and potential side effects. Possible adverse effects may include headaches, flushing, nasal congestion, alterations in vision, and gastrointestinal discomfort.

Other medication for Erectile Dysfunction Includes;

- **Alprostadil Self-Injection:** This method entails the utilization of a delicate needle to administer alprostadil (Caverject impulse, Edex) into the foundation or flank of the male reproductive organ. In certain circumstances, injections employed for alternative ailments are employed solely or in combination for penile injections. Phentolamine and Papavarine are examples of such medications. When these medications are combined, they are referred to as bimix (if two medications are used) or trimix (if three are used). The needle employed for this procedure is exceedingly delicate, resulting in minimal

discomfort at the site of injection. Each medication is administered in a manner that ensures an erection lasting no longer than sixty minutes. The therapy is associated with adverse effects such as priapism, and in rare cases, the development of fibrous tissue at the site of injection.

- **Alprostadil Urethral Suppository:** This therapeutic intervention entails the insertion of a minuscule alprostadil suppository into the penile urethra, facilitated by a distinctive applicator. Following the placement of said suppository, the onset of an erection commonly transpires after a period of approximately 10 minutes. The duration of this effect, when the therapy proves efficacious, typically spans between 30 and 60 minutes. Potential adverse effects associated with this treatment encompass discomfort, minor hemorrhaging within the urethra, and the development of fibrous tissues within the phallus.
- **Testosterone Replacement Therapy:** Insufficient quantities of the hormone testosterone have the potential to instigate or exacerbate erectile dysfunction. For males encountering erectile dysfunction, testosterone replacement therapy may be suggested as an initial measure or administered concurrently with other treatment approaches.

1.8.2 NON-DRUG THERAPY FOR ERECTILE DYSFUNCTION

Non-pharmacological intervention for erectile dysfunction (ED) encompasses the application of vacuum erection devices, insertion of penile prosthesis, penile vascular surgery, and sexual therapy.

1.8.2.1 VACUUM ERECTION DEVICE THERAPY

Vacuum erection devices have been commercially accessible since the early 1980s. This apparatus comprises three constituents: a vacuum cylinder, a pump that generates a regulated negative pressure, and one or multiple constrictor rings. The pump can be either manually or battery driven, contingent on the model. The constrictor ring(s) are situated at the exposed extremity of the lubricant-covered cylinder. The cylinder is then positioned atop the previously lubricated penis, and subsequently, the pump is activated to initiate a controlled vacuum. Once the swelling of the penile tissues is achieved, one or more rings are shifted onto the penile base.

1.8.2.2 PENILE PROSTHESIS IMPLANTATION

Penile prosthesis is available in two forms: the non-inflatable and the inflatable. This device has a major advantage of being able to cause the penis to alternate between the flaccid and erect states. Furthermore, it causes penile flaccidity and erection that is very much like that produced by natural mechanism.

1.8.2.3 PENILE ARTERIAL REVASCULARIZATION

The prevalence of vascular disease rises with advancing age among males, and it is likely the primary etiology of organic erectile dysfunction in men over the age of 50. The utilization of coronary arterial bypass grafts in managing coronary artery disease has indicated that a comparable procedure may be efficacious in restoring typical erectile function in males afflicted with vasculogenic erectile dysfunction.

1.8.2.4 SEX THERAPY

Sexual therapy is deemed appropriate for psychogenic erectile dysfunction (ED), and in some cases, it serves as a valuable supplement to other treatments for men with both psychogenic and organic ED. The potential causes encompass childhood sexual trauma, issues related to sexual identity, unresolved attachment to a partner or parent, as well as cultural or religious taboos.

Sexual therapy on its own has the potential to resolve psychogenic ED, owing to the fact that psychological factors often play a significant role in all forms of organic ED. Additionally, it can be employed in conjunction with pharmacological interventions for ED.

1.9 APHRODISIAC

An aphrodisiac refers to any substance, whether edible or medicinal, that stimulates the innate sexual drive, incites carnal longing, and enhances both gratification and sexual prowess. This word is derived from ëAphroditaeí the Greek Goddess of love and these substances are derived from plants, animals or minerals and since time immemorial they have been the passion of man. (Yakubu *et al.,* 2005)

There exist several rationales why individuals might desire to employ an aphrodisiac for the purpose of enhancing their sexual experiences, with sexual dysfunction serving as a prevalent motive.

1.9.1 MECHANISM OF ACTION OF APHRODISIACS

Sexual desire is regulated by the central nervous system which integrates three main stimuli; tactile, mental and olfactory stimuli (Patel *et al.,* 2011). On stimulation of sexual function , the axons of the parasympathetic nerves release the gas nitric oxide (NO) which diffuses into the

smooth muscle cells that line the arteries of the erectile tissues (Corpus cavernosum) and activates the enzymes guanylatecyclase (GC). This enzyme then converts the nucleotide guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), which in turn causes relaxation of the smooth muscle cells around the penis leading to dilation of the arteries and increased blood influx into the penile tissues. The blood is mainly trapped in the penis and results in an erection (Palmer, 2009). The erection ceases after a while, as a result of hydrolysis of cGMP by the phosphodiesterase type -5 enzyme (PDE-5) to form inactive GMP. Aphrodisiac plants inhibit the hydrolyzing action of PDE-5 thereby leading to retention of active cyclic GMP.

1.9.2 HISTORY OF APHRODISIAC

The introductions of the drug sildenafil (Viagra) have elicited heightened interest in the utilization of aphrodisiacs, however their usage can actually be traced back to antiquity. Across the annals of human history, individuals have sought solace in comestibles and other natural elements to augment desire and even fecundity. Comestibles such as oysters, chocolate, cloves, cinnamon, ginger, and thyme have all been postulated at distinct junctures in human history to contribute to heightened arousal and performance. The reason why aphrodisiacs have persevered in popularity for millennia is their pledge to engender positive affect and facilitate enhanced gratification. While further research is essential to scrutinize the influence of comestibles and substances on sexual functioning, it is plausible that aphrodisiacs will persist in captivating those desiring to ameliorate their intimate lives.

1.9.3 CLASSIFICATION OF APHRODISIAC

The categorization of aphrodisiacs centers on the functional activities that take place during the sexual cycle by introducing an initial stage of desire or libido. Consequently, the customary male

sexual response cycle can be functionally separated into five interconnected occurrences that transpire in a specific order: libido, erection, ejaculation, orgasm, and detumescence.

Libido or Sexual Desire: Libido refers to the innate biological urge for engaging in sexual activity, commonly known as the sex drive, and is often manifested through the pursuit of sexual encounters. The degree of its strength varies among individuals, as well as within an individual across different periods.

Erection: Erection denotes the augmented and inflexible condition of the sexually stimulated phallus that meets the necessary criteria for vaginal penetration. This phenomenon emerges as a consequence of numerous psychogenic and sensory stimuli originating from imaginative, visual, auditory, olfactory, gustatory, tactile, and genital reflexogenic sources.

Ejaculation: Ejaculation refers to the physiological process of expelling semen. This action is triggered through a reflex response to sexual stimulation. It comprises two successive stages. The initial stage, known as emission, involves the deposition of seminal fluid into the posterior urethra. Subsequently, the true ejaculation transpires, wherein the expulsion of seminal fluid occurs through the penile meatus.

Orgasm: This represents the pinnacle of arousal experienced during sexual stimulation. The entire period of emission and ejaculation is known as the male orgasm (Guyton *et al.,* 2000).

Detumescence: This is the process of subsiding from a state of tension, swelling, or (especially) sexual arousal.

1.9.4 PHYTOMEDICINE IN MANAGEMENT OF ERECTILE DYSFUNCTION

CHLOROPHYTUM BORIVILIANUM

Chlorophytum borivilianum San. and Fern. (Liliaceae) commonly known as Safed Musli is an important plant used against various disorders in indigenous systems of medicine, such as antioxidant, analgesic, and as a remedy for male impotence. (Ryan and Gajraj 2011). This particular plant should be regarded as a threatened species owing to its significant utilization in numerous indigenous regions and its extensive application within the domain of Traditional Medicine.

Safed musli, scientifically known as *Chlorophytum borivilianum*, is a highly esteemed botanical species that is often regarded as the "white gold". Historically, *Chlorophytum borivilianum* has gained recognition for its efficacious properties in addressing male sexual dysfunction. The multi therapeutic and nutritional importance of *Chlorophytum borivilianum* is attributed to the rich source of phytochemicals particularly saponins. (Barve *et al.,* 2010). Recently, *Chlorophytum borivilianum* has garnered a firmly established domestic and international market for serving as a herbal alternative to "Viagra" in a manner devoid of any adverse effects.

Within the tubers, one can find saponins which possess aphrodisiac, adaptogenic, antiaging, health-rejuvenating, and health-enhancing characteristics. The root of this plant is replete with steroidal and triterpenoidal saponins, sapogenins, and fructans, all of which function as therapeutic agents and assume a pivotal role in numerous therapeutic applications. It is a rich source of over 25 alkaloids, vitamins, proteins, carbohydrates, steroids, saponins, potassium, calcium, magnesium, phenol, resins, mucilage, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose.

(Barve *et al.,*2010). Safed musli is currently being endorsed as a botanical agent for augmenting sexual prowess and has traditionally served as a natural aphrodisiac.

In a research investigation performed on rodents, it was ascertained that safed musli elicited a conspicuous augmentation in the sexual drive of rats, accompanied by an escalation in sexual vitality and stimulation. In simpler terms, it can be concluded that safed musli is efficacious as an aphrodisiac for rats. Subsequent to a continuous intake of the pulverized root of safed musli for a duration of 60 days, the rats exhibited a significant elevation in their sperm count. (Thakur, *et al.,* 2009).

They also came to the conclusion that "roots of *chlorophytum borivillianum* (safed musli) can be useful in the treatment of certain forms of sexual inadequacies such as premature ejaculation and oligosperma" (Thakur *et al.,* 2009).

MYRISTICA FRAGANS

Myristica fragrans belongs to the family Myristicaceae and kingdom (Plantae). *Myristica fragrans* Houtt is commonly named nutmeg or mace. It is an aromatic evergreen tree with spreading branches and a yellow fleshy fruit similar in appearance to an apricot or peach. (Tajuddin *et al.,* 2005).

Oral administration of the extract of *Myristica Fragrans* produces significant augmentation of sexual activity in male rats (Ahmad, *et al.,* 2005).

It significantly increases the Mounting Frequency, Intromission Frequency, Intromission Latency and causes significant reduction in the Mounting Latency and Post Ejaculatory Interval. (Tajuddin, *et al.,* 2005).

It also significantly increases Mounting Frequency with penile anaesthetization as well as Erections, Quick Flips, Long Flips, and the aggregate of penile reflexes with penile stimulation. (Tajuddin, *et al.,* 2005).

TRIBULUS TERRESTRIS

Tribulus terrestris, a member of the Zygophyllaceae family, is a blossoming plant. It is colloquially referred to as devil's thorn, puncture vine, caltrop, yellow vine, and goat head. Within Nigeria, it is a commonly occurring herb. The augmentation of sexual function through the utilization of *T. terrestris* extracts is ascribed to the increase in testosterone, dihydrotestosterone, and dehydroepiandrosterone. *T. terrestris* extract increases the body's natural testosterone levels and thereby improves male sexual performance and helps build muscle. (Eardley *et al.,* 2010).

Tribulus terrestris has the capacity to enhance sexual libido, augment sexual contentment, and alleviate manifestations of erectile dysfunction (ED) in males. The mechanism by which *Tribulus terrestris* ameliorates erectile function potentially encompasses an augmentation in the relaxation of penile smooth muscle tissue, heightened intracavernous pressure, and escalated penile blood flow. These effects seem to be dependent on a greater release of nitric oxide (NO) from the endothelium of blood vessels.(Adaikan and Gauthaman 2002).

MONDIA WHITEI

Mondia whitei (Apocynaceae) has been used by African people to treat various ailments. The roots were used for the treatment of anorexia, stress, bilharzias and sexual dysfunction as well as for general aches and pains.(Aremu, *et al* ., 2011).

The acrid-flavored roots possess a sugary post-taste and an aroma reminiscent of vanilla. Individuals typically masticate the root or steep it in hot water as a beverage. It is perceived as a distinctive African scent or condiment. The foliage may be utilized as a replacement for spinach.

Parts Used;

The roots and leaves.

The utilization of the roots as a traditional remedy for issues regarding fertility has been well-documented. This remedy possesses properties that stimulate sexual desire and foster sexual stimulation, augment sexual performance, heighten sensitivity, and ameliorate erectile dysfunction in males. The efficacy of *Mondia whitei* as an aphrodisiac is not solely substantiated by its capacity to boost testosterone production or unwind corpus cavernosum tissue, but also by its ability to enhance human sperm functionality. Furthermore, it has been demonstrated that it exerts a comparable effect on erectile dysfunction as Sildenafil (commonly recognized as Viagra), which initiates the release of nitric oxide, thus inducing muscle relaxation, promoting blood circulation, and resulting in erections.

As an aphrodisiac for the treatment of impotence, erectile dysfunction and infertility, men eat the barks of the root or drink an extract of the roots. (Martey and He, 2010).

PHOENIX DACTILIFERA

The pollen grain of *Phoenix dactylifera* is a minute and ellipsoid gametocyte covered with a delicate exocarp. It belongs to Arecaceae or palm family. (Al-Alawi, *et al.,* 2017).

The *Phoenix dactylifera*, commonly known as date palm pollen (DPP), has been utilized for centuries as a means to enhance fertility and act as an aphrodisiac in individuals suffering from sexual dysfunction or infertility.

Experimental studies have revealed that DPP increases both sperm count and quality (Hassan *et al.,* 2012; Abedi *et al.,* 2012) .

It harbors facilitative impacts that heighten sexual stimulation, the condition of sexual exhilaration or yearning in the course of sexual engagement. It also enhances penile erection and other sexual behaviors. (Abedi *et al.,* 2012).

Effects of DPP is due to the presence of steroids, alkaloids and flavonoids through a multitude of central and peripheral means therefore, it may be useful to solve the sexual problems such as pre-ejaculation and impotency.(Abedi *et al.,* 2012).

It can influence the sexual arousal and performance. The aphrodisiac effect of the DPP extract is due to the presence of alkaloids, saponins and flavonoids through a central and peripheral pathway.(Abedi *et al.,* 2012).

DPP improves fertility rate in adult male with sexual dysfunction through a significant increase in serum level of testosterone, LH, and estradiol, number and motility of sperm, and diameter of seminiferous tubules. (Marbeen *et al* ., 2016).

The DPP extract exhibits antitoxic characteristics in addition to its antioxidant and anti-inflammatory properties. As an illustration, cadmium (Cd) is a hazardous metallic element capable of causing harm to multiple bodily organs, notably the reproductive system. Some

animal studies demonstrated that treatment with DPP lowers the level of Cd in testis and consequently attenuates reproductive damage. (Mohamed and collaborators 2012).

1.10 JUSTIFICATION

Plants like Dehydroepiandrosterone (DHEA), Horny goat weed (epimedium), Ginkgo, L-arginine, Red ginseng and others have been used over the ages to treat erectile dysfunction but, some of these herb aren't at an immediate reach to those who need it. Therefore, this research is to find out the potency of *Vernonia Amygdalina* in treating erectile dysfunction.

1.11 *VERNONIA AMYGDALINA*

Vernonia amygdalina, belonging to the daisy family, is a diminutive shrub that thrives in the tropical regions of Africa. *Vernonia amygdalina* generally attains an altitude ranging from 2 to 5 m (6.6 to 16.4 ft). The leaves possess an elliptical shape and have a maximum length of 20 cm (7.9 in). The outer covering of this plant is characterized by a coarse texture.

1.11.1 TAXONOMIC CLASSIFICATION OF *VERNONIA AMYGDALINA*

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Asterales

Family: Asteraceae

Genus: *Vernonia*

Species: *Vernoniaamygdalina*

1.11.2 BOTANICAL DESCRIPTION

Vernonia amygdalina, which is widely recognized as bitter leaf, is a shrub that attains a height of up to 3 meters in the African tropics as well as various regions of Africa, specifically Nigeria, Cameroon, and Zimbabawe. It is renowned for its numerous advantageous attributes. The plant's organic portion extractions have been demonstrated to exhibit cytotoxic properties against malignant cells of the nasopharynx. It has antimicrobial and antiparasitic activities.

The biologically active compounds of *Vernonia amygdalina* are saponins and alkaloids (Muraina, *et al.,* 2004) terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone, edotides and sesquiterpenes.

Vernonia amygdalina, commonly known as Bitter leaf, is used as a vegetable, sole or mixed with other vegetables. Bitter leaf has been used as food and medicine in centuries in Africa and has even been turned into capsule in the United States of America.

The African Traditional medicine utilizes the plant for a wide range of purposes, encompassing the management and treatment of various health conditions. African Traditional practitioners throughout the African continent employ the plant in the healing and control of numerous ailments, including fever, malaria, stomach discomfort, skin infections such as ringworm and acne, diabetes, cancer, insomnia, hepatitis, jaundice, toothache, diarrhea, bilharzia, pneumonia, tuberculosis, stroke, arthritis, wounds, fatigue, and cough. Additionally, it functions as an

antiparasitic, antibacterial, anti-inflammatory, and anthelmintic agent; as a laxative, an appetizer, a tool for weight loss, and an aphrodisiac.

Bitter leaf is rich in vitamins A, C, E, B1, and B2, as well as minerals like zinc, manganese, iron, potassium, and calcium.

Figure 1: Picture of *Vernonia Amygdalina* twig and leaf

Ecology

Vernonia amygdalina is naturally present in riparian and lacustrine habitats, as well as in the periphery of forests, woodland areas, and grasslands, thriving at elevations of up to 2000 m. It frequently thrives in disturbed localities, such as deserted agricultural land, and exhibits spontaneous growth in secondary forests. Optimal growth necessitates an abundance of sunlight. The flowering process is initiated by shorter periods of daylight. While it exhibits a reasonable

tolerance to arid conditions, it generally favors a moist environment. It can be found on all soil types, but performs best in humus-rich soils. (Fomum and Bernard 2004).

Propagation and planting

Propagation can be achieved through the use of seeds, although the majority of farmers opt to utilize stem cuttings. The choice of cuttings for propagation from mature stems is made based on various attributes, including the level of bitterness, the size of the leaves, and the growth characteristics. In domestic gardens, it is common to cultivate more than one type of plant, as younger individuals tend to prefer the sweeter and less bitter varieties, while older individuals favor the more bitter ones. When planting cuttings, they can be positioned upright or at a 45-degree angle to encourage the growth of additional side-shoots. Cuttings tend to exhibit faster growth compared to seedlings. To obtain seeds, it is necessary to collect them from dry flower heads. These seeds are then scattered over nursery beds that have been prepared using soil rich in humus and are protected from excessive heat and sunlight. The germination process for the seeds typically takes around 2-3 weeks. It is crucial to frequently water the nursery beds during dry periods. Approximately 4-6 weeks after the emergence of the seedlings, they can be transplanted. In home gardens people plant bitter leaf amongst other crops or as a hedge or live fence; in commercial fields it is planted in rows. (Fomum and Bernard 2004)

Yield

The period of peak productivity occurs during the rainy season, with the highest levels of yield observed between the months of May to August.

1.11.3 ETHNOMEDICINAL USE OF *VERNONIA AMYGDALINA*

Vernonia amygdalina has several medical, industrial, food, and traditional uses. The plant is used as a tonic in the treatment of fever, constipation, and many illnesses in traditional and herbal Nigerian medicine (Howard *et al.,* 2016).

Tonics derived from this botanical specimen are employed in the management of sexually transmitted infections. Broadly speaking, the cultivation of this plant serves as a substantial means of supplying consumable produce.

The plant is also used in the brewing industry as an alternative to hops in the production of beer. The Congolese maximizes *V. amygdalina* 's medicinal potential by using it to treat cough and hemorrhoids (Ngatu *et al.,*2012).

The leaves are frequently utilized in the treatment of malaria in Ethiopia. Several scientific studies have found that the herb has antioxidative, anti-inflammatory, and anticancer properties (Bihonegn *et al.,* 2019; IfedibaluChukwu *et al.,* 2020).

1.11.4. PHARMACOLOGICAL PROPERTIES OF VERNONIA AMYGDALINA

Vernonia amygdalina had been reported to possess several pharmacological effects like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, anthelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Iwalokun *et al.,* 2006; Erasto *et al.,,* 2007; Gresham *et al.,,* 2008; Khalafalla *et al.,,* 2009; Ilondu, 2010; Farombi and Owoeye, 2011; Anibijuwon *et al.,,* 2012; Ngatu *et al.,,* 2012; Adetunji *et al.,,* 2013; Atangwho *et al.,,* 2013; Akinyele *et al.,,* 2014; Ezeadila *et al.,,* 2015; Udochukwu *et al.,* 2015; Alara *et al.,* 2017).

Antioxidant Effect

The crude extracts from *V. amygdalina* had been studied to possess an antioxidant property by scavenging the free radicals cells. The aqueous extracts from the leaf showed a significant reduction in the malondialdehyde levels of oxidative stressed streptozotocin-induced diabetic rats (Nwanjo, 2005).

The leaves extracts had been examined to scavenge 75-99.3% DPPH radicals and 96.2-100% of the ABTS radicals (Erasto *et al.,* 2007). The presence of flavonoids in the *V. amygdalina* extracts had been attributed to their antioxidant property (Ayoola *et al.,* 2008; Farombi and Owoeye, 2011).

In vivo biochemical analysis of *V. amygdalina* leaf extracts on the rats showed an appreciable increase in the level of the antioxidants, superoxide dismutase, catalase, glutathione, and malondialdehyde. In addition, daily administration of the extracts to rats resulted in the reduction of their lipid profile when compared to the control (Imaga and Bamigbetan, 2013).

The chemo preventive effects of *V. amygdalina* extracts had been attributed to their ability to scavenge free radical cells, interfere with DNA binding of some transcription factors, and induced detoxification (Amodu *et al.,* 2013).

Moreover, the extracts from this plant had been found to inhibit bleaching B-carotene, lipid peroxidation induced by iron ion ascorbate in a rat liver microsomal preparation, and linoleic acid (Khalafalla *et al.,* 2009; Yeap *et al.,* 2010).

Anti-diabetic Effect

Diabetes mellitus has been associated with a fasting venous plasma glucose concentration higher than 7.8 Mmol/l (140 mg/dl) 2 h after an oral ingestion of 75 g glucose equivalent or carbohydrate meal (Nwanjo, 2005; Letchuman *et al.,,* 2010; Jan Mohamed *et al.,,* 2015).

Studies had shown that aqueous extracts from *V. amygdalina* leaves reduced the blood glucose, increased the serum triglyceride levels and increased the LDL-cholesterol, and normalized cholesterol concentrations in streptozocin-induced diabetic rats (Nwanjo, 2005).

In another study on the effect of *V. amygdalina* leaf extracts on blood glucose of diabetic rats, the results showed that decrease in blood glucose after administration of the extracts may be associated with the presence phytochemicals, vitamins and other nutrients in the extracts (Osinubi, 2008; Nwaoguikpe, 2010; Ejike *et al.,* 2013).

The aqueous extracts had been administered to alloxan-diabetic rats, the blood glucose and serum triglyceride levels were significantly reduced (Akah *et al.,,* 2004).

Justin *et al.,*2012 had reported that decoction of *V. amygdalina* and *Azadirachta indica* leaves promptly lowered blood glucose and Journal of Chemical Engineering and Industrial Biotechnology V2(2017)80-96 89 maintained a relatively steady level over the study period. Likewise, the study on the activities of *V. amygdalina* leaves aqueous extracts on the haemostatic, hematological and biochemical profile of induced male diabetic albino rats showed a significant reduction in the glucose level (Oguwike *et al.,* 2013).

Anti-allergic Effect

The extracts from *V. amygdalina* leaves had been reported to inhibit and prevent atopic or eczema dermatitis syndrome in mice (Ngatu *et al.,* 2012).

Anti-inflammatory Effect

Vernonia Amygdalina leaves extracts had been reported to possess anti-inflammatory activity when applied to the ear of rat suffering from inflammation. It produced a significant reduction when compared with the application of acetylsalicylic acid (Georgewill and Georgewill, 2010). The percentage of inhibition of leaves extracts was higher than roots extracts (Egharevba *et al.,* 2014).

Anti-cancer Effect

Breast cancer has been the second leading cause of deaths of women in the world (American Cancer Society, 2016). *V. amygdalina* leaves extracts had been reported to inhibit the proliferation of MCF-7 and MDA-MB-231 which involved the stimulation of cell-type specific G1/S phase cell cycle.

Owoeye *et al.,* 2010 had also reported the presence of epivernodalol in the methanolic extract of *V. amygdalina* leaf which was active against HT-144 (skin melanoma) cell line. On the other hand, the aqueous extracts were administered to mice for 4 weeks at dose 10 to 100 µg/ml per day, there was a significant reduction in CYP1A2 expression. Methanol and chloroform extracts were as well inhibited human leukemia monocyte THP-1 cell line with IC50 values of 19.1 and 243.4 µg/ml, respectively (Yeap *et al.,* 2010).

Anti-microbial Effect

The aqueous and ethanol extracts of *V. amygdalina* leaves had shown antimicrobial effects against *Staphylococcus aureus*, Escherichia coli, *Pseudomonas aeruginosa, Klebsiella spp*., and *Candida albicans* with the Microbial inhibitory concentration (MIC) values ranged between 12.5 and 50 mg/ml (Ghamba *et al.,,* 2014). In another study, ethanolic and aqueous extracts of *V. amygdalina* leaves had shown a higher value of MIC inhibitions on Streptococcus mutans at 25 and 55 mg/ml, respectively (Akinpelu, 1999; Anibijuwon *et al.,* 2012).

Anti-malaria Effect

Leaves and roots of *V. amygdalina* extract possessed antimalarial effect against drug sensitive *Plasmodium berghei* in mice which resulted in 67% and 53.5% suppression of parasitaemia after the four days of administration, respectively (Audu *et al.,* 2012).

The leaves extracts had exhibited a significant antiplasmodial effect in mice against *Plasmodium berghei* with 73% inhibition (Njan *et al.,* 2008). In the same vein, isolated sesquiterpene lactones from *V. amygdalina* had been reported to show antiplasmodial property with IC50 < 4 µg/ml against Plasmodium falciparum (Egharevba *et al.,* 2014).

V. amygdalina leaf extracts dose had dependently restored the efficacy of chloroquine against *Plasmodium berghei* in mice which had developed resistance (Iwalokun, 2008).

Journal of Chemical Engineering and Industrial Biotechnology V2(2017)80-96 90 Significant results were obtained antiplasmodial effects of ethanolic and aqueous extracts at IC50 values 44.03 and 41.690 µg/ml, respectively (Egharevba *et al.,* 2014).

Anti-Infertility Effect

The anti-fertility impact of *V. amygdalina* leaf's 95% ethanolic extracts on the mouse uterus, when compared to the control agonist acetylcholine (1 g/kg), was documented at doses of 0.385, 0.5, and 1.0 g/kg body weight of mice. Significant reduction in a mean number of implantation sites, the number of live fetuses and survival percentage were recorded (Egharevba *et al.,* 2014).

Anti-fungal Effect

Sesquiterpene lactones had been known to be highly antifungal (Barrero *et al.,,* 2000; Wedge *et al.,,* 2000). The presence of vernodalol and vernolide which belongs to sesquiterpene lactones in the *V. amygdalina* leaves extracts made them exhibit higher antifungal effect against *Candida albicans* and *Aspergillus flavus* with LC50 values of 0.4 mg/ml each (Erasto *et al.,* 2006).

Anti-bacterial Effect

Sesquiterpene lactones from *V. amygdalina* leaves had exhibited antibacterial against five gram-positive bacteria with the MIC value of 0.25 mg/ml, but lack efficacy against gram-negative bacteria (Erasto *et al.,,* 2006).

Likewise, the ethanolic stem extracts inhibited antibacterial effect against *Staphylococcus aureus* with 50 mg/ml concentration (Akinyele *et al.,* 2014). The aqueous, ethanolic and methanolic extracts of the leaves had been reported to exhibit strong potency against clinical bacteria: *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* (Alo *et al.,,* 2012; Adetunji *et al.,* 2013).

Anti-leukemia Effect

The root culture of cold water, hot water, and ethanolic *V. amygdalina* extracts had been tested on the patients suffering from acute leukemia and myeloid leukemia. The extracts showed a higher level of anti-leukemia activity on the patients (Khalafalla *et al.,* 2009).

1.11.5 PHYTOCHEMICAL COMPOUNDS IN MANAGEMENT OF ERECTILE DYSFUNCTION

Alabi and Adeyemi (2021) uncovered several flavonoids (luteolin7-O-b-glucuronide, luteolin 7-O-b-glucoside) in *V. amygdalina* ethanolic preparations. All three flavones have strong antioxidant properties, particularly luteolin (3′,4′,5,7 tetrahydroxyflavone). Other phytochemicals present include alkaloids, anthraquinone, steroid, phenol, phytate, oxalate, cyanogenic glycoside, tannins and saponins.

(Hasibuan *et al.,* 2020) used LC-MS/MS analysis to investigate the phytochemicals found in *V. amygdalina*. The findings revealed the presence of the following flavonoids: apigetrin, apigenin, luteolin, diosmetin, baicalin, rhoifolin, and scutellarin.

Toyang and Werpoorte (2013) examined the isolated phytochemicals obtained from *V. amygdalina* extracts and showed that vernonioside A3, vernodalol, vernolepin, vernodalin,11,13-dihydrovernodalin, and hydroxyvernolide are among the isolated bioactive chemicals and flavonoids.

The reports of Adaramoye *et al., (*2008) showed that an increased content of flavonoids such as luteolin-7-O-glucoside in mice treated against liver toxicity might be connected to a reduction in lipid peroxidation (LPO) levels in irradiated animals pretreated with *V. amygdalina* extracts.

Using LC-MS analysis, Erukainure *et al., (*2018) identified the phytochemicals found in *V.*

amygdalina. The study revealed the presence of nicotinic acid, cumidine, and 3-methyl-isoquinoline. *V. amygdalina* alkaloids were discovered and described by(Omojokun *et al.,.*(2019).

The extract of alkaloids was quantified using GC-MS. 1-Hexanamine, dimethylamine, 1-fluorononane, 1,3-cyclooctadiene, and hexadecanamide are examples of isolated alkaloid compounds. Iwalokun,(2008) identified phytoconstituents with anti-plasmodial action from the extract and quinoline alkaloids such as cephantharin, cryptolepine, isocryptolepine, and neocryptolepine, as well as courmarins and terpenoids, are among these compounds.
(Hasibuan *et al.,* 2020) used LC-MS/MS analysis to investigate the phytochemicals contained in *V. amygdalina*.

The results unveiled the existence of diterpene (ingenol-3-angelate) and phenolics (chlorogenic acid and 4-methoxycinnamic acid), in addition to coumarines (7-hydroxycoumarine, 4-methylumbelliferone, and 4-methylumbelliferylglucuronide). Alara *et al.* employed the Soxhlet method and MAE to determine the bioactive constituents in ethanolic extracts of *V. amygdalina*. The gas chromatography-mass spectroscopy (GC-MS) examination was employed for further identification, and a confirmatory test was conducted using Fourier transform infrared spectroscopy analysis. 2-pentanol, pentanoic acid, 2-methyl-3-hexanol, and ethyl ester linoleic acid were among the bioactive components that were isolated and characterized.

Structures of some compound identified and isolated in *V. amygdalina*

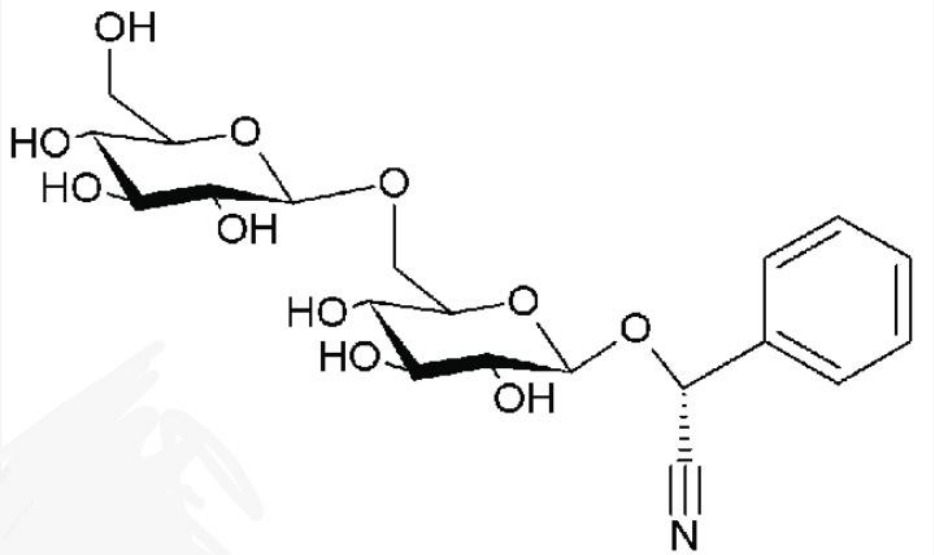

Figure 2: Cynanogenic glucoside

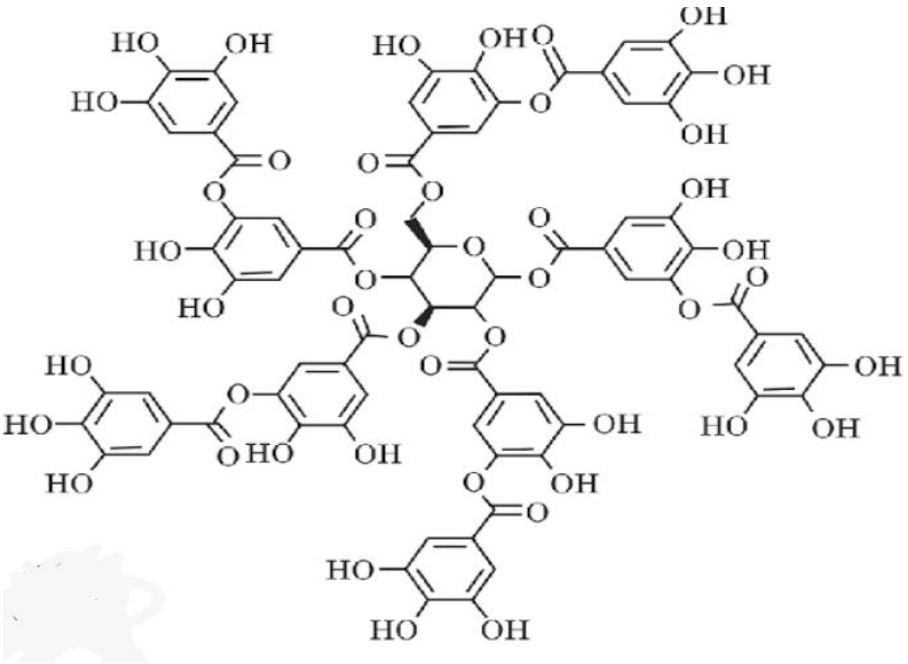

Figure 3: Tannins

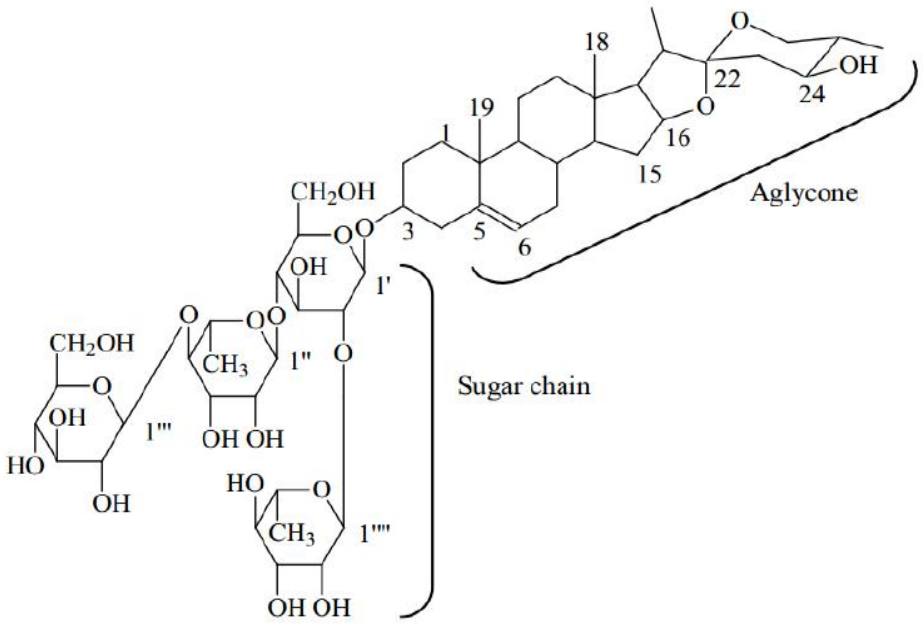

Figure 4: Saponins

1.12 STATEMENT OF PURPOSE (JUSTIFICATION).

Easy and common consumptions among claims of the medicinal and nutritional effects of bitter leaf as it's classification as an Aphrodisiac has hesitated studies to be conducted on the plant.

Moreover, the increasing incidence of male sexual dysfunction both in developing and developed countries of the world has led to renewed interest in quest for natural products with sexual enhancing properties and herbal remedies in a view to discovering new therapies for these diseases.

The literature available on *Vernonia Amygdalina* is quiet insufficient, hence this research seeks to add to pre-existing information.

1.13 AIMS AND OBJECTIVES

The aim of the study is to investigate the aphrodisiac properties of the extract and fractions leaves of *vernonia amygdalina*.

The objectives are:

1. To evaluate the aphrodisiac activities of extract and fractions of leaf of *vernonia amygdalina* by physical means.
2. To investigate the effect of the active fractions of the leaf of *vernonia amygdalina* from above on the smooth muscle of the corpus cavernosum in rats.
3. To determine the secondary metabolites, present in the active fractions using spectroscopic means.

CHAPTER TWO

MATERIALS AND METHOD.

2.1 EQUIPMENT.

The equipment used include: Separating funnel, Soxhlet apparatus, digital weighing balance, electric milling machine, rotary evaporator, 10 ml organ bath, centrifuge, Thermo-regulated digital water bath and retord stand.

2.2 MATERIALS.

Materials used include: porcelain dishes, glass jars, mortar and pestles, orogastric tubes, measuring cylinders, beakers, ink markers, distilled water, ethinyl oestradiol and progesterone were obtained from reliable sources, Aphrodisiac screening box, Kreb's solution, thread, sildenafil tablets, mortar and pestles, syringes, sample bottles, dissecting kit, and micro pipettes. This was purchased from Pharmatrend Nigeria limited

The analytical-grade solvents listed below, including ethanol, n-hexane, chloroform, ethyl acetate, and tween 80, were purchased from reputable suppliers (Pharmatrend Nig. Lmtd.).

2.3 PLANT MATERIAL COLLECTION AND AUTHENTICATION

The plant, *Vernonia amygdalina* leaves were collected in March 2023 from a farmland around the University of Benin Edo State Nigeria, identified and authenticated by Dr. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Edo state, Nigeria. A voucher specimen was deposited at the departmental herbarium and the herbarium number UBH-V342 was issued.

2.3.1 Preparation of Plant Material.

The *Vernonia amygdalina* leaves that had been collected had any foreign materials removed, and they were then spread out on a spotless surface in the shade to air dry until totally dry. A contemporary laboratory electric milling machine was used to grind the air-dried plant material into a coarse powder. It was weighed and kept in an airtight container for later use.

2.3.2 Extraction of Plant Material.

The powdered plant material (1.5 kg) was extracted with 5 litres of 99.9% ethanol. A "soxhlet" apparatus was used to perform the extraction in batches. A rotary evaporator was also used to concentrate the acquired plant extract. The semi-solid plant extract obtained was weighed and noted. The extract (70 g) was fractionated with n-hexane, chloroform, and ethyl acetate and to obtain the distinct fractions of the various solvents respectively as well as a residual aqueous fraction. The obtained plant fractions were concentrated using a rotary evaporator. Thereafter, the semi-solid plant fractions obtained was weighed and stored in the refrigerator at 4^{o}C for further use.

2.4.0 Phytochemical analysis of aqueous extract of *Vernonia amygdalina* leaves

The presence of secondary metabolites such as alkaloids, saponins, flavonoids, phenols, and tannins in the aqueous extract of *Vernonia amygdalina* leaves was investigated using standard tests as described by (Yadav *et al*.,2013). The specific tests for these phytochemicals were conducted as follows:

2.4.1 Test for Alkaloids

The powdered plant material of *Vernonia amygdalina* leave (5 g) was extracted in 20 ml of water on a water bath for 30 minutes. It was filtered and the filtrate was tested with alkaloidal reagents. Another 5 g of the powdered plant material was extracted using dilute acid (10% H_2SO_4). The purpose of extracting the powdered plant material in dilute acid was to convert the alkaloidal base in the plant to salts.

1. Dragendoff's Test: Three drops of Dragendoff reagent was added to 2 ml of aqueous extract of *Vernonia amygdalina.*

Hager's Test: Three drops of Hager's reagent was added to 2 ml of aqueous extract of *Vernonia amygdalina*.

Mayer's Test: Three drops of Mayer's reagent was added to 2 ml of aqueous extract of *Vernonia amygdalina.*

Wagner's Test: Three drops of Wagner's reagent was added to 2 ml of aqueous extract of *Vernonia amygdalina.*

The same procedure was repeated using acidic solution of the extract. Observation for the colour change was taken. Blank test was carried out for each of the reagents.

2.4.2 Test for Carbohydrates

The powdered plant of *Vernonia amygdalina* (1 g) was extracted in 5 ml of water and two drops of 10% alcoholic solution of alpha- naphthol was added followed by the addition of of 2 ml of concentrated H_2SO_4 with which the tube inclined at angle 45°.

2.4.3 Test for Anthracene Derivatives

The powdered plant material (5 g) was extracted in 20 ml of water. The resultant solution was used for the various test for anthracene derivatives.

(a) To 7 ml of water to, 3 ml of extract was added to make up to 10mL. The resultant solution was shaken gently with 5mL of chloroform from which 3mL of the chloroform layer was pipetted off into a clean dried test tube and was shaken gently with 1.5mL of dilute Ammonia solution.

(b) The aqueous solution of the powdered plant (1 ml) was taken into a test tube. 2.5mL of 10% H_2SO_4 was added and heated on a water bath for 5 minutes and then allowed to cool. 5mL of chloroform was added. The resultant solution was made up to 10 ml. About 3mL of chloroform layer was shaken gently with 1.5 ml of dilute Ammonia.

(c) Exactly 2.5 ml of 10% H2SO4 and 2.5 ml of H_2O_2 was added to 3 ml of the aqueous extract. The resultant solution was heated on a water for about 5 minutes and then allowed to cool. The solution was made to 10 ml with water. The solution was shaken gently with 5 ml of chloroform. The chloroform layer was pipetted off into a test tube after which it was shaken gently with 1.5 ml of dilute Ammonia solution.

2.4.4 Test for saponins

(a) The powdered plant (0.5 g) was extracted in 2.5 ml of water and was shaken vigorously. The solution was added 0.1g of Sodium Bicarbonate followed by the addition of equal volume of Fehling's solution A and B and boiled on a water bath.

(b) To another test tube, 5mL of the extract was added followed by the addition of equal volume of 90% alcohol and was shaken.

2.4.5 Test for Phenolic compounds (Tannins)

(a) A drop of aqueous solution of Ferric chloride was added to 2 ml of the extract

(b) Iron complex test: About 2 drops of 0.25% of Ferric ammonium citrate solution followed by the addition of 1g of Sodium acetate after which the mixture was boiled on a water bath and allowed to cool

(c) Modified Iron complex Test : A drop of 33% acetic acid and 1g of Sodium Potassium tartarate was added to 5mL of the extract and then warmed. The mixture was then filtered and washed. The washings was added to the filtrate after the resultant solution of 0.25% Ferric ammonium citrate was added and boiled for 5 minutes (Wadood *et al.,* 2103).

2.4.6 Test for flavonoids (alkaline reagent or NaOH test)

The powdered plant (0.5g) was extracted in 2 ml of water. To this, three drops of 20% sodium hydroxide solution were added. An intense yellow colour was formed which turned colourless with the addition of three drops of 20% hydrochloric acid, indicated the presence of flavonoids in the extract. Besides, a lead acetate test was performed. To the same solution used above, three drops of 10% lead acetate were added and the formation of yellow precipitate was observed for the presence of flavonoids (Ogbu *et al*., 2020).

2.4.7 Test for steroids

The powdered plant (0.5 g) was extracted in 5 ml of water. The extract was then dissolved in 0.5 ml dichloromethane to produce a dilute solution. To this solution, 0.5 mL of acetic anhydride

was added, followed by the addition of three drops of concentrated sulphuric acid (Yadav *et al*., 2014). The colour change was observed.

2.4.8 Test for cyanogenic glycosides

The powdered plant (0.5 g) was extracted in 2 ml of water in a test tube. Sodium picrate test paper was inserted into the test tube and was covered with cotton wool. The test was observed for 5 minutes.

2.5 PHARMACOLOGICAL EXPERIMENTS

2.5.1 Source of Laboratory Animals

For this investigation, adult male and female albino rats were used. Ten albino female rats with an average weight of 165.50 g and thirty five albino male rats with an average weight of 275.00 g were obtained from the Faculty of Pharmacy Department of Pharmaceutical Chemistry and kept in the department of Pharmacology and Toxicology Animal house at the University of Benin. The animals were kept in separate, clean plastic cages with bedding made of wood shavings, and they were kept in an environment that was clean and well ventilated. They were given clean water and regular palletized animal food (Premier Feed, Ibadan). They were kept in an environment with an average room temperature of 27 +/-3°C and a 12-hour cycle of light and darkness. The animals were given two weeks to acclimatize.

2.5.2 Ethical Clearance

The guidelines as specified by the Ethics Committee of the University of Benin's Faculty of Pharmacy's were followed in all experiments.

2.5.3 Evaluation of the aphrodisiac activities of extract and fractions of *Vernonia amygdalina* by physical means.

2.5.3.1 Test for aphrodisiac activities of extract and fractions.

This was carried out according to the method of Yakubu *et al.,* (2007). The female animals were sequentially be put into artificial heat (oestrous) in 24 hours and 3 hours before mating by oral dose of ethinyl oestradiol (100 micrograms) and subcutaneous treatment of progesterone (1 mg) respectively.

The female animals was trial tested with male animals other than those used for the experiment. Only female animals that showed receptivity towards the male animals was selected for the experiments. The male animals were grouped into seven groups of five animals each. Animals in group 1 received 0.5mL of distilled water and each animal in this group received 20% Tween 80 (0.5mL) and this served as the negative group. Animals in groups 2, 3, 4, 5 and 6 received 50 mg/kg of the ethanolic extract, n-hexane, chloroform, ethyl acetate and aqueous fractions of *V. amygdalina* respectively in each group. Group 7 animals was given sildenafil 100mg/kg and served as the positive control. All administrations were done orally with the aid of an orogastric tube.

2.5.3.2 Measurement of Physical sexual parameters.

Thirty minutes after the administration of extract and fractions or control agent, animals (one at time) was placed in transparent plastic cages (30 cm x18cm x 30 cm) and allowed 10 minutes to acclimatize to the environment of the cages. Thereafter, the receptive female animals was introduced into the box. The observation of the mating behavior started right away. An independent observer and a recording camera were used to observe the animals for 30 minutes

for each group. For the objective of accuracy and eliminating bias, video camera recordings were transcribed and compared with that of the independent observer.

The following sexual behavior parameters were observed, noted, and assessed: Anogenital sniffing (AS), Genital grooming (GG), Mounting frequency (MF), Mounting latency (ML), Intromission frequency (IF), Intromission latency (IL) and Ejaculatory latency (EL) (Fouche *et al.,* 2015).

Anogenital Sniffing

Sniffing is an inherent respiratory action that plays a vital role in the attainment of scents. Potentially interconnected with this, sniffing is frequently exhibited during motivated and social actions. The rats sniffed each other to reinforce their social hierarchy and prevent aggressive behavior.

Genital grooming

This is a behavior that is exhibited by rodents in its cleaning function. Aside from its cleaning function, this can be led by arousing experience. This occurred when prepubescent males and female rats were placed alone in a novel cage, males more than females were found to clean their genitals, but not other body regions.

Mounting Frequency

In the context of this experiment, "mount" refers to a behavioral act where a male rat, climbs onto the back of another animal, typically a female rat. The mounting frequency is the number of mounts from the time of introduction of the female until ejaculation.

Mounting Latency

This is the time between the addition of a receptive female into the arena and the first mount.

Intromission Frequency

An intromission is when the male achieves vaginal penetration during a mount. Intromission frequency is defined as the number of intromissions to ejaculation.

Intromission Latency

The time between addition of a receptive female into the arena and the first intromission.

Ejaculatory Latency

This is the time taken for rat to ejaculate, from first intromission, following addition of a receptive female into the arena.

2.5.4 Evaluation of effect of aqueous fraction and ethyl acetate fraction of *Vernonia amygdalina* on smooth muscle of corpus cavernosum.

2.5.4.1 Experimental Set-up.

For this investigation, the approach used by Tang *et al.,* 2017 was adopted. Prior to the actual experiment, 1 liter of Kreb's solution was prepared using Sodium chloride (NaCl : 8 g/L), Potassium chloride (KCl : 0.2 g/L), Calcium chloride (CaCl2 : 0.2 g/L), Magnesium sulfate (MgSO4 : 0.1 g/L), Sodium Bicarbonate (NaHCO3 : 1.2 g/), D-glucose (0.1 g/L). The stock solution of each ingredients was prepared and the amount of each chemical was dissolved in distilled water. Appropriate volume of each solution was combined to make the final Krebs' solution. The solutions were mixed thoroughly to ensure complete dissolution.

The chosen male rat was weighed and mercifully killed by cervical dislocation, and the penis was surgically extracted en bloc. Care was taken to preserve the tunica albuginea.

After carefully separating the corpus cavernosum tissue from the tunica albuginea and surrounding fat, it was inserted into a container filled with ice-cold Krebs solution for preservation. Strips of the corpus cavernosum tissue were then prepared for mounting and testing. The tissue strips were placed in a 10 mL organ bath chamber filled with Krebs solution. One end of each strip was fastened to a metallic support to hold it in place. The other end was attached to a free displacement transducer with cotton thread to measure tension changes. The organ bath chamber was maintained at 37°C and continuously aerated with 95% oxygen and 5% carbon dioxide to mimic physiological conditions. Before testing, the tissue strips were subjected to a tension of 1.0 g using the transducer and allowed to equilibrate for 1 hour in the bath solution. This allowed the tissue to normalize and achieve a stable baseline. A computer was connected to the transducer to measure and record the variations in isometric tension generated by the tissue during testing.

2.5.4.2 Evaluation of effect of aqueous fraction and ethyl acetate fraction of *Vernonia amygdalina* on basal contraction of Corpus cavernosum muscle.

Using multiple fractions provides comparative data to see if different components have different biological activities or potencies. This gives more complete information about the plant's effects.

V. amygdalina is known to contain various bioactive components like alkaloids, tannins, saponins, and flavonoids. The study is to evaluate if more nonpolar compounds extracted in ethyl acetate versus more polar compounds from the aqueous fraction affect corpus cavernosum

contractility differently. Using both ethyl acetate and aqueous fractions allows for a more comprehensive screening of *V. amygdalina's* effects on corpus cavernosum muscle contractility.

The direct impact of cumulative concentrations of aqueous fraction and ethyl acetate fraction of *V. amygdalina* on the basal contractility of corpus cavernosum smooth muscle was examined following tissue equilibration and response recording for 15 minutes without flushing. To find concentration-response associations, 6 different concentrations (0.78, 1.56, 3.125, 6.25, 12.5 and 25 mg/ml) were used. Following each concentration of the fractions administered, a 5-minute contact period was permitted.

2.5.4.4 Evaluation of the effect of ethyl acetate fraction of *V. amygdalina* on Potassium Chloride-Induced contraction of Corpus Cavernosum muscles.

After tissue equilibration for 1 hour, the physiological stock solution were washed out thereafter, 10mL of 0.0596g of Potassium chloride was added to the organ bath and was allowed enough contact time for maximum tissue contraction.

The effects of cumulative concentrations of the ethyl acetate fraction of *V. amygdalina* on the corpus cavernosum muscle were examined after maximum tissue contraction had been reached, without washing. To determine the concentration-response relationship, 6 different concentrations of the fraction (0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/ml) were utilized. Following administration of each fraction concentration, a 5-minute contact period was permitted.

2.6.0 Statistical Analysis.

Data were expressed as the mean +/- standard error of mean (S.E.M). Comparison between the treatment groups and negative control was carried out using one-way analysis of variance (ANOVA) followed by Dunnett post hoc test. Analysis and data presentation was done using GraphPad Instat version 2.05 software. Results were considered significant when $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 Yield of extraction

The weight of the concentrated extract of *Vernonia amygdalina* was taken after the extraction and the yield of the extract is;

(a) Weight of concentrated extract of *Vernonia amygdalina* = 87.1 g

(b) Percentage yield of *V.amygdalina* extract = 5.81 %

3.1.0 Phytochemical analysis

The phytochemical characteristics *Vernonia amygdalina* was tested. Secondary metabolites were screened for. The aqueous leaf extract of *Vernonia amygdalina* was found to contain alkaloids, cardiac glycosides, reducing sugars, flavonoids, phenols and saponins. Anthraquinone and cyanogenic glycosides were observed to be absent in the extract as presented in table below.

Table 1: The result of phytochemical analysis of aqueous extract of *V. amygdalina* leave.

TEST	OBSERVATION	INFERENCE
Alkaloids		
Dragendoff	Black colouration observed, no precipitate	Alkaloidal salt absent
Hager	Yellow precipitate observed	Alkaloidal salt present
Mayer	Cream precipitate observed	Alkaloidal salt present
Wagner's	Reddish brown precipitate observed	Alkaloidal salt present
Anthraquinone	Brown colouration observed	Anthraquinone absent
Tannins	Bluish colour observed	Presence of tannins
Saponins	Formation of frothing	Saponins present
Reducing sugars	Brick red precipitate observed	Presence of reducing sugars
Cardiac glycosides	A brick red interface observed	Cardiac glycosides present
Cyanogenic glycosides	There was no change in test paper colour	Cyanogenic glycosides absent
Phenols	Deep blue colouration observed	Presence of phenols
Flavonoids	Yellow colour observed	Flavonoids present

3.2Results of *Invivo* aphrodisiac activity evaluation

Table 2a: Effects of *Vernonia amygdalina* leaf extract and fractions on mounting, intromission, and ejaculatory frequencies and genital grooming and sniffing

Groups	Doses (mg/kg)	Mean±SEM MF	Mean±SEM IF	Mean±SEM EF	Mean±SEM GS	Mean±SEM GG
Control	DW	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.67±0.33^{a}	1.67±0.33^{a}
VALEE	50	2.00±0.10^{a}	0.00±0.00^{a}	0.00±0.00^{a}	9.00±2.51^{b}	10.33±2.54^{b}
VALNHF	50	5.33±0.43^{b}	5.00±0.86^{b}	1.67±0.13^{b}	4.67±0.67^{a}	12.00±3.09^{b}
VALCF	50	9.00±2.02^{b}	8.00±1.23^{b}	1.67±0.10^{b}	3.67±0.63^{a}	11.00±2.82^{b}
VALAF	50	20.33±5.03^{c}	15.00±4.86^{c}	1.67±0.18^{b}	5.33±1.03^{b}	24.67±6.45^{c}
VALEAF	50	25.33±4.20^{c}	20.33±3.68^{c}	4.00±1.82^{b}	3.00±0.55^{a}	26.33±5.82^{c}
Sildenafil	100	28.67±3.33^{c}	21.67±3.83^{c}	4.00±0.08^{b}	7.00±1.58^{b}	28.00±5.69^{c}

$p < 0.05$. superscript a showed no significant difference KEYS:VALEE (*Vernonia amygdalina* leaf ethanol extract); VALNHF(*Vernonia amygdalina* leaf n-hexane fraction); VALCF(*Vernonia amygdalina* leaf chloroform fraction); VALEAF(*Vernonia amygdalina* leaf ethy l acetate fraction); VALAF(*Vernonia amygdalina* leaf aqueous fraction).

Table 2b: Effects of *Vernonia amygdalina* leaf ethanol extract and fractions on Mounting , intromission and ejaculatory latencies

Groups	**Doses (mg/kg)**	**Mean±SEM ML (sec)**	**Mean±SEM IL (sec)**	**Mean±SEM EL (sec)**
Control	DW	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
VALEE	50	140.00±15.95[c]	0.00±0.00[a]	0.00±0.00[a]
VALNHF	50	183.30±21.30[c]	350.70±34.76[c]	360.00±32.60[c]
VALCF	50	245.00±38.30[c]	143.33±5.23[b]	172.00±19.42[b]
VALAF	50	110.00±29.93[c]	215.70±26.79[c]	297.70±41.19[c]
VALEAF	50	99.67±10.54[b]	110.70±12.67[c]	189.70±24.22[b]
Sildenafil	100	87.00±26.65[b]	74.33±9.55[b]	4.00±21.26[b]

$p < 0.05$. superscript a showed no significant difference KEYS:VALEE (*Vernonia amygdalina* leaf ethanol extract); VALNHF(*Vernonia amygdalina* leaf n-hexane fraction); VALCF(*Vernonia amygdalina* leaf chloroform fraction); VALEAF(*Vernonia amygdalina* leaf ethy l acetate fraction); VALAF(*Vernonia amygdalina* leaf aqueous fraction).

CUMMULATIVE EFFECTS OF ETHYL ACETATE FRACTION OF *Vernonia amygdalina* LEAVES ON POTASSIUM PRE-CONTRACTED CORPUS CAVERNOSUM SMOOTH MUSCLE

The outcome demonstrates that potassium chloride produced effect on the mean response and cumulative dosages of ethyl acetate fraction of *V. amygdalina* led to a dose dependent reduction in the mean response after a plateau has been reached. This shows that the fraction cumulative dose induced the smooth muscle of potassium-precontracted corpus cavernosum to relax.

The cumulative dose of ethyl acetate fraction only also caused the relaxing effect on the corpus cavernosum muscles.

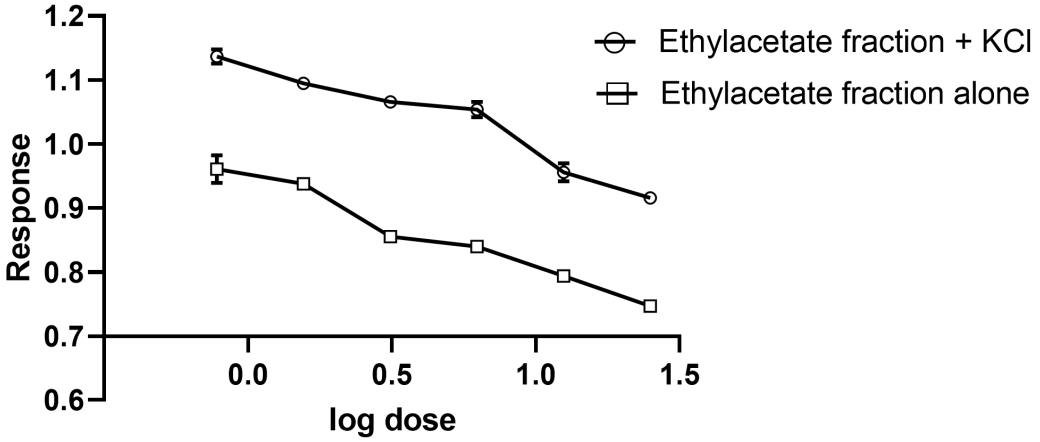

Figure 1: Dose response curve of ethylacetate fraction of *Vernonia amygdalina* with and without KCl

CUMMULATIVE EFFECTS OF AQUEOUS FRACTION OF *Vernonia amygdalina* LEAVES ON POTASSIUM PRE-CONTRACTED CORPUS CAVERNOSUM SMOOTH MUSCLE

The outcome demonstrates that potassium chloride produced effect on the mean response and cumulative dosages of aqueous fraction of *V. amygdalina* led to a dose dependent reduction in the mean response after a plateau has been reached. This shows that the fraction cumulative dose induced the smooth muscle of potassium-precontracted corpus cavernosum to relax.

The cumulative dose of aqueous fraction only also caused the relaxing effect on the corpus cavernosum muscles.

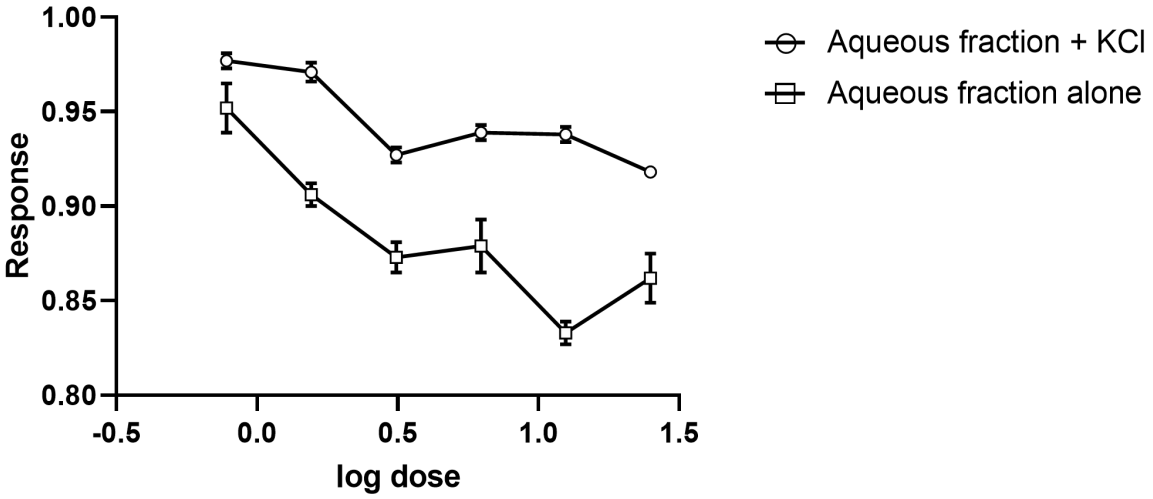

Figure 2: Dose response curve of the aqueous fraction of *Vernonia amygdalina* with and without KCl

CHAPTER FOUR

DISCUSSION

Phytochemical analysis

Phytochemicals are intrinsic organic compounds found within the plant kingdom, playing an indispensable role in the enhancement of well-being and vigor.

The outcomes of the phytochemical examinations unveiled that the extracts encompassed phytochemicals with advantageous consequences. Phytochemicals, besides their physiological activity in plants, also function as reservoirs of micronutrients and macronutrients for the well-being of humans and animals (Hasler and Blumberg, 1999). Flavonoids are polyphenolic compounds synthesized by plants that possess a broad spectrum of biological and pharmacological impacts. They manifest spermatogenic, aphrodisiac, antioxidant attributes as well as antimicrobial, cytotoxic, anti-inflammatory, anticancerous, oestrogenic, anti-allergic, and hematopoietic characteristics (Itodo *et al*., 2022). These compounds safeguard biological systems against detrimental oxidative processes on the macromolecules of the organism (Atmani *et al*., 2009).

Saponins are widely recognized for their anti-inflammatory properties and hemolytic activity; they also possess the ability to bind cholesterol and exhibit bitter characteristics. Tannins, derived from plants, are antinutrients that precipitate proteins, thereby impeding the function of digestive enzymes and diminishing the absorption of vitamins and minerals within the body (Ryszard 2007). Alkaloids provide a diverse range of medicinal effects, including anti-hypertensive properties as well as the ability to combat malaria or carcinogens (Saxena *et al*., 2013). *Vernonia amygdalina* leaf has been demonstrated to contain substantial amounts of

phenols and flavonoids while showcasing remarkable steroidogenic capabilities and antioxidant properties.

The various phytochemical test conduct was able to elucidate the phytochemical constituents of Vernonia amygdalina. The test was positive for alkaloids, tannins saponins, flavoniods, reducing sugars and glycosides. This study was able to demonstrate that *Vernonia amygdalina* contains a considerable amount of phytochemicals which includes; alkaloids, tannins, saponins, cardiac glycosides, flavonoids and reducing sugar. Anthraquinones and cyanogenic glycosides are observed to be absent.

Aphrodisiac activity

The field of traditional medicine encompasses a wide array of attributes and components, thereby justifying its designation as specified by the World Health Organization (WHO) (Oreagba *et al.,* 2011).

Sexual relationships are some of the most important social and biological relationship in human life. Male impotence also called erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide (Montorsi *et al.,* 2003). Medicinal plants that possess aphrodisiac properties have been observed to manifest their effects through diverse mechanisms, encompassing peripheral, hormonal, and neuronal components, potentially integrating all of these mechanisms (Abedi *et al.,* 2013; Anderson and Wagner, 2005). The peripheral component entails the relaxation of the smooth muscles within the penile region as a result of heightened generation and release of nitric oxide, activation of cyclic adenosine monophosphate (cAMP), and/or activation of the efflux of potassium ions, thereby leading to an

augmented blood flow towards the penis, subsequently culminating in erection (Anderson, 2007; Burnett, 2007).

Penile erection is a vascular phenomenon that occurs in three steps. These steps involve increased arterial inflow, relaxation of sinusoidal smooth muscle, and decreased venous drainage. The control of this process is exerted by both upper centers and peripheral regulatory mechanisms. At the peripheral level, the mechanisms involved are similar to those found in other vascular beds. They comprise a complex interplay between sympathetic (adrenergic) and parasympathetic (cholinergic) neurotransmitters, as well as endothelial and smooth muscle autacoids such as endothelins. These specific mechanisms have been extensively discussed in a previous study by (Maggi *et al.,* 2000). The mechanism of aphrodisiac activity is equally engaged by the neural system, and it relies on the stimulation or suppression of dopamine receptors in the central nervous system to augment or hinder sexual behavior (Lee *et al.,* 2006).

This research was conducted with the purpose of assessing the aphrodisiac properties exhibited by ethanolic extract and fractions derived from the leaves of *Vernonia amygdalina* in mature male rats through physical methods. The investigation primarily focused on examining the impact of ethanolic extract and fractions obtained from *V. amygdalina* leaves on the smooth muscle of the isolated corpus cavernosum. The outcome of this investigation unveiled that *V. amygdalina* effectively induced an amplified stimulatory response in male sexual behavior.

Mounting frequency (MF) and Intromission frequency (IF) are considered to be indicators of sexual desire and potency. These parameters are typically quantified in the assessment of sexual motivation, penile erection, efficacy, and ejaculatory reflex action (Dabhadkar and Zade, 2013). An augmentation in MF and IF may suggest the potential of a medication to regulate erectile

dysfunction. According to the findings of the investigation, the ethyl acetate and aqueous fraction derived from the leaves of *V. amygdalina* were observed to elicit an increase in MF, IF, and EF. These effects were found to be significantly heightened in comparison to those induced by the reference drug, implying that the ethyl acetate and aqueous fractions of *V. amygdalina* leaves possess the capacity to positively influence sexual desire and potency. An identical outcome was seen with the aqueous fraction of the methanol extract of the root of *R. beninensis Planch ex Benth.* (Ofeimun and Ayinde 2020).

Anogenital sniffing refers to a specialized respiratory behavior that is essential for the acquisition of odors. This is commonly displayed during motivated and social behaviors. The rats sniffed each other to reinforce their social hierarchy and prevent aggressive behavior. Genital sniffing, denoted as GS, pertains to the frequency with which the male animal engages in pursuit of the female animal wherein it engages in olfactory examination of the anal region without mounting. This particular parameter serves as a metric for evaluating pre-copulatory sexual conduct. The objective of genital sniffing is to elicit sexual arousal and an elevation in this variable is a sign of pre-copulatory sexual arousal in males (Padashetty and Mishra, 2007). In this investigation, it was observed that the ethyl acetate and aqueous fraction of *V. amygdalina* leaves elicited a statistically significant augmentation in the act of sniffing the genital region in comparison to the control. This particular effect was similar to the effect produced by the reference drug, thereby implying a heightened state of sexual stimulation and excitement mediated by the fractions of *V. amygdalina* leaves. The result observed with ethanol extract, n-hexane and chloroform fractions of *V. amygdalina* gave a very little response in producing sexual stimulation and excitement. Similarly, animal studies also reported increases in libido and erectile functions in rats fed *yohimbe* root (Ojatula, 2017).

Similarly, Increase in the act of anogenital grooming can be seen as an indication of heightened sexual arousal in the male rat population. According to (Giuliano *et al.*2003), anogenital grooming (AG) is a key indicator of the erectile health of the penis and plays a significant influence in the adult male rat's readiness for reproduction. Anogenital grooming is a behavior that is exhibited by the rats in its cleaning function. Aside from its cleaning function, this can be led by arousing experience. This occurred when prepubescent males and female rats were placed alone in a novel cage, males more than females were found to clean their genitals, but not other body regions.

In this investigation, it was observed that the ethyl acetate and aqueous fraction of *V. amygdalina* leaves elicited a statistically significant augmentation in the act of grooming the genital region in comparison to the control. This particular effect was similar to the effect produced by the reference drug, thereby implying a heightened state of sexual stimulation and excitement mediated by the fractions of *V. amygdalina* leaves. The result observed with ethanol extract, n-hexane and chloroform fractions of *V. amygdalina* gave a very little response in producing sexual stimulation and excitement.

Similarly, animal studies also reported increases in libido and erectile functions in rats fed *yohimbe* root (Ojatula, 2017).

The characteristics known as mounting latency (ML) and intromission latency (IL) are typically acknowledged as indicators of sexual motivation. These variables are inversely correlated with sexual arousal; an increase in them denotes a lack of sexual drive (Yakubu *et al.,* 2005); a decrease in them denotes sexual arousal, motivation, and potency (Rampin *et al.,* 2003).

It has been noted that the administration of ethyl acetate and aqueous fractions from the leaves of*V. amygdalina* resulted in a decrease in ML and IL in rats compared to other fractions. This decrease suggests an enhancement in sexual arousal and motivation in the rats, indicating a positive impact of the plant fractions on sexual stimulation, potency, and vigor. Furthermore, the significant increase in Ejaculation Latency (EL) observed in the treated rats implies that the plant fractions has the potential to prolong the duration of copulation, thereby suggesting an improvement in sexual vigor, potency, motivation, and performance in the treated rats. The EL was notably elevated with the administration of ethyl acetate, exhibiting a comparable effect to that of the reference drug. A similar outcome has also been observed with the aqueous fraction of the methanol extract derived from the root of *R. beninensis* (Ofeimun and Ayinde, 2020).

An Invitro study was carried out using ethyl acetate and aqueous fractions to compare each of the fractions that have a more relaxing effect.

In the initial stages of the natural process of relaxation of penile arteries and corpus cavernosum smooth muscle, nitric oxide released from vascular endothelial and neural sources is crucial. According to (Burnett *et al.* 2002) and (Moreland *et a.l,*2001), the Cyclic Guanosine Monophosphate (cGMP) pathway mediates its effects. According to (Robertson *et al.,* 2003) and (Lincoln *et al.,* 2001), cGMP is believed to relax smooth muscles by decreasing the intracellular calcium ion (Ca2+) concentration. This is accomplished by either increasing Ca2+-ATPase activity or by opening potassium ion channels, which causes hyper polarization and reduces Ca2+ influx through voltage-gated calcium ion channels.

According to the research done on isolated corpus cavernosum smooth muscle, cumulative dosages of the ethyl acetate and aqueous fractions of *V. amygdalina* leaves resulted in a dose-

dependent reduction in the mean baseline response. This shows that the fraction may have a relaxing effect on the smooth muscles of the corpus cavernosum. It was found that the ethyl acetate fraction's effect was comparable to that brought about by the aqueous fraction. Comparatively speaking, the ethyl acetate fraction seems to have a more relaxing impact than the aqueous fraction. An in vivo and in vitro investigation of the aphrodisiac effects of *Allium tuberosum* seed extract on corpus cavernosum smooth muscle revealed comparable effects on relaxation. (Tang *et al.,* 2017).

Following the administration of a pre-contractile agent, potassium chloride, the effect of the plant fractions on corpus cavernosum smooth muscle was studied to ascertain the potential mechanism of action of the *Vernonia amygdalina* fractions. It was found that potassium chloride caused the smooth muscles in the corpus cavernosum to contract; normally, this effect is not known to cause the smooth muscles in the corpus cavernosum to contract directly. In fact, potassium's principal function in muscle physiology is to keep the membrane potential at rest and help control muscular contraction and relaxation. The findings of the study showed that cumulative doses of *V. amygdalina* fractions respectively (ethyl acetate and aqueous) were effective in causing the potassium chloride-precontracted corpus cavernosum smooth muscle to relax, indicating that *V. amygdalina*-induced relaxation of the corpus cavernosum smooth muscle is closely linked to the reduction of intracellular Ca2+ concentration by promoting Ca2+-ATPase activity. It was observed that the ethyl acetate fraction has more relaxing effect compared to the aqueous fraction. And this could be because of ethyl acetate fraction containing a higher concentration of bioactive compounds that have relaxant effects on smooth muscles, including those of the corpus cavernosum.

4.1. CONCLUSION

The aqueous extract contains phytochemicals with beneficial effects, such as steroids flavonoids, saponins, alkaloids, cardiac glycosides, reducing sugars and tannins. These phytochemicals have a wide range of biological and pharmacological effects, including antioxidant capabilities and anti-inflammatory properties.

The results of this study show that the ethyl acetate and aqueous fractions of *V. amygdalina* leaves considerably improve sexual behavior parameters, which supports the use of *V. amygdalina* leaves as an aphrodisiac in traditional medicine. The aphrodisiac activity of *V. amygdalina* in this study was fraction dependent, with ethanol extract, n-hexane, and chloroform fractions having lower aphrodisiac activity than the ethyl acetate and aqueous fractions.

The ethyl acetate and aqueous fractions of *V. amygdalina* leaves have a relaxing effect on the corpus cavernosum smooth muscle, and they can also cause the relaxation of potassium-precontracted corpus cavernosum smooth muscle, indicating that *V. amygdalina*-induced relaxation of corpus cavernosum smooth muscle is closely linked to the reduction of intracellular Ca2+ concentration by promoting Ca2+-ATPase activity.

Further research is required to determine the pharmacologically active principle in the plant that is responsible for the activity seen and to identify the secondary plant metabolites that may have contributed to the effects seen with the ethyl acetate and aqueous fractions of *V. amygdalina leaves.*

REFERENCES

Abdel-Naser MB, Imam A, Wollina U. (2004).'Sildenafil citrate significantly improves nocturnal penile erections in sildenafil non-responding patients with psychogenic erectile dysfunction'. International Journal of Impotence Research; 16(6), pp.552-556.

Abedi A, Parviz M, Karimian SM, SadeghipourRodsari HR. (2012).'The Effect of Aqueous Extract of *Phoenix Dactylifera* Pollen Grain on Sexual Behavior of Male Rats'. Journal Physiology and Pharmacology Advance ;6(1), pp.235-242.

Abedi A., Parviz M., Karimian SM, and Rodsari HZ. (2013). 'Aphrodisiac activity of aqueous extract of *phoenix dactylifera* pollen in male rats'. *Advance Sex Medicine*. 3(1), pp.28-34

Adaramoye O., Ogungbenro B., Anyaegbu O., FafunsoM., (2008). 'Protective effects of extracts of *Vernonia amygdalina, Hibiscus sabdariffa* and vitamin C against radiation induced liver damage in rats'. Journal of Radiation Research 49 (2), pp. 123–131. doi:10.1269/jrr.07062.

Adetunji CO, Olaniyi O.O., Ogunkunle AT, Oluwola J. (2013). 'Bacterial Activity of Crude Extracts of *Vernonia amygdalina* on Clinical Isolates,. Journal of Microbiology and Antimicrobials. 5(6), pp. 60–64.

Aizenberg D., Zemishlany Z., Hermesh H., Karp L., Weizman A. (1991).'Painful ejaculation associated with antidepressants in four patients'. Journal of Clinical Psychiatry, 52 (1), pp. 461–463.

Akah, P., Njoku, O., Nwanguma A., and Akunyili D. (2004). 'Effects of Aqueous Leaf Extract of *Vernonia amygdalina* on Blood Glucose and Triglyceride Levels of Alloxan–Induced Diabetic Rats (*Rattus rattus*). Animal Research International. 1(1), pp. 90–94.

Akinpelu, D.A. (1999). 'Antimicrobial Activity of *Vernonia amygdalina* leaves'. *Fitoterapia*. 70(4), pp. 432–434

Akinyele BJ, Oladejo BO, Akinyemi AI. and Ezem LO. (2014). 'Comparative Study of the Antibacterial Effect of Mouth Washes and *Vernonia amygdalina* (Del.) on Some Tooth Decay Causing Bacteria'. British Microbiology Research Journal. 4(7), pp. 749–758.

Alabi QK, Adeyemi WJ.(2021). '*Vernonia amygdalina* (Del) as an antioxidant aspirin toxicity, and oxidative stress'. Toxicology. pp. 491–504. doi: 10.1016/B978-0-12-819092-0.00048-0

Alo MN, Anyim C., Igwe JC, Elom M., Uchenna, DS. (2012). 'Antibacterial Activity of Water, Ethanol and Methanol Extracts of *Ocimum gratissimum, Vernonia amygdalina* and *Aframomummelegueat'*. Pelagia Research Library. 3(2), pp. 844–848.

American Cancer Society. (2016). Cancer Facts & Figures. pp. 1–72.

American Psychiatric Association (APA) (1994)., 'Diagnostic and Statistical Manual of Mental Disorders, ed 4'. American Psychiatric Association, Washington DC.

Amodu A., Itodo SE., Musa DE., Mamu A. (2013). 'Nigerian Foodstuffs with Tumour Chemosuppressive Polyphenols'. International Journal of Pharmaceutical Science Invention. 2(1), pp. 12–17.

Anderson KE. (2007). 'Pharmacology of penile erection'. *Pharmacology Review*. 53(1), pp. 417-450.

Anderson KE., and Wagner G. (2005). 'Physiology of penile erection'. *Physiology Review.* 75(1) pp. 191-236.

Anibijuwon I.I, Oladejo BO, AdetitunDO, Kolawole OM. (2012). 'Antimicrobial Activities of *Vernonia amygdalina* against Oral Microbes'. Global Journal of Pharmacology. 6(3), pp. 178–185.

Asher GN, Corbett AH, Hawke RL. (2017) 'Common Herbal Dietary Supplement-Drug Interactions'. American Family Physician. 96(2), pp. 101–107.

Atmani D., Nassima C., Dina A., Meriem B., Nadjet D., Hania B. (2009). 'Flavonoids in Human Health': From Structure to Biological Activity.Current Nutrition & Food Science; 5(1), pp. 225-237.

Audu, SA, Alemika ET, Abdulraheem RO, Abdulkareem SS, Abdulraheem RB, Ilyas M. (2012). 'A Study Review of Documented Phytochemistry of *Vernonia amygdalina* (Family Asteraceae) as the Basis for Pharmacologic Activity of Plant Extract'. Journal of Natural Sciences Research. 2(7), pp. 1–9.

Austoni E., Mirone V., Parazzini F., Fasolo CB., Turchi P., Pescatori ES. (2005)'Smoking as a risk factor for erectile dysfunction: data from the Andrology Prevention'. A study of the Italian Society of Andrology (s.I.a.) European Urology. 48(1), pp. 810–817.

Ayoola GA, Sofidiya T., Odukoya O., Coker HA. (2006).'Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants'. Journal of Pharmaceutical Science & Pharmaceutical Practice. 8(1), pp. 133-136.

Bacon CG, Mittleman MA, Kawachi I, Giovannucci E, Glasser DB, Rimm EB. (2006).'A prospective study of risk factors for erectile dysfunction'. Journal of Urology.176 (1), pp. 217–221.

Barve KH, Rert WR, Foth GG, Verth B. (2010). 'Extraction of saponins from safed musli' Pharmacognosy Journal.

Behr-Roussel D., Gorny D., Mevel K., Compagnie S., Kern P., Sivan V.(2005). 'Erectile dysfunction: an early marker for hypertension? A longitudinal study in spontaneously hypertensive rats'. American Journal of Physiology Regulatory Integrative and Comparative Physiology. 288 (1), pp. 276–283.

Bent S. (2008). 'Herbal medicine in the United States: review of efficacy, safety, and regulation: grand rounds at University of California, San Francisco Medical Center'. Journal of General International Medicine. 23(6), pp. 854–9. doi: 10.1007/s11606-008-0632-y. doi. Epub.

Bihonegn T., Giday M., Yimer G., Animut A., Sisay M., (2019). 'Antimalarial activity of hydromethanolic extract and its solvent fractions of *Vernonia amygdalina* leaves in mice infected with *Plasmodium berghei'*. SAGE Open Medicine doi:10.1177/2050312119849766.

Binmoammar TA, Hassounah S., Alsaad S., Rawaf S., Majeed A.(2016). 'The impact of poor glycaemic control on the prevalence of erectile dysfunction in men with type 2 diabetes mellitus': a systematic review. Journal of the Royal Society of Medicine Open;7:2054270415622602.

Bosch RJ, Bernard F., Aboseif SR., Steif CG, Lue TF, Tanagho EA. (1991). 'Penile detumescence': characterization of three phases. Journal of Urology, 146, pp. 867.

Burnett AL. (2007). 'Nitric oxide in the penis'. Physiology and pathology. *Journal of Urology*. 157(1), pp. 320- 324.

Carson CC, Mulcahy JJ, Govier FE, Terma DE. (2000).'Efficacy, safety and patient satisfaction outcomes of the AMS 700CX inflatable penile prosthesis: results of a long-term multicenter study'. AMS 700CX Study Group. Journal of Urology, 164(1), pp. 376-8.

Cavallini G., Modenini F., Vitali G., Peter D. (2005).'Acetyl-L-carnitine plus propionyl-L-carnitine improve efficacy of sildenafil in treatment of erectile dysfunction after bilateral nerve-sparing radical retropubic prostatectomy'. Urology; 66(5), pp. 1080-1085

Chen KK, Hsieh JT, Huang ST.(2001). 'ASSESS-3: a randomised, double-blind, flexible-dose clinical trial of the efficacy and safety of oral sildenafil in the treatment of men with erectile dysfunction in Taiwan'. International Journal of impotence Research; 13(4), pp. 221-229.

Chew KK, Bremner A., Stuckey B., Earle C., Jamrozik K. (2009). 'Is the relationship between cigarette smoking and male erectile dysfunction independent of cardiovascular disease? Findings from a population-based cross-sectional study'. Journal of Sex Medicine.6(1), pp. 222–231.

Cho NH, Ahn CW, Park JY, Ahn TY, Lee HW, Park TS. (2006).'Prevalence of erectile dysfunction in Korean men with Type 2 diabetes mellitus'. Diabetes Medicine; 23(1), pp. 198–203.

Choi HK, Ahn TY, Kim JJ, Tim KW. (2003) .'A double-blind, randomised- placebo, controlled, parallel group, multicentre, flexible-dose escalation study to assess the efficacy and safety of sildenafil administered as required to male outpatients with erectile dysfunction in Korea'. International Journal of Impotence Research; 15(2), pp. 80-86.

Choppin A., Blue DR, and Hegde SS, Dert F., (2001). 'Evaluation of oral ro70-0004/003, an alpha1A-adrenoceptor antagonist, in the treatment of male erectile dysfunction'. international Journal of impotence Research; 13(3), pp. 157-161.

Cortés-González JR, Arratia-Maqueo JA, Gómez-Guerra LS, Holmberg AR. (2010). 'The use of *Butea superba* (Roxb.) compared to sildenafil for treating erectile dysfunction'. British Journal of Urology International. 105 (1), pp.225-8.

Csoka AB, Bahrick A., Mehtonen OP, Killen YT. (2008). 'Persistent sexual dysfunction after discontinuation of selective serotonin reuptake inhibitors'. Journal of Sex Medicine. 5(1), pp. 227–233.

Cunningham GR, and Rosen RC. (2014)'Overview of male sexual dysfunction'. UpToDate [homepage on the Internet].. Access from: http://www.uptodate.com/contents/overview-of-male-sexual-dysfunction.

Dabhadkar D.,& Zade, V., 2013. 'Evaluation of the potential aphrodisiac activity of *Psoralea corylifolia* in male albino rats'. Asian Journal of Biomedical and Pharmaceutical Sciences, 6(3), pp.18.

De Berardis G., Franciosi M., Belfiglio M., Di Nardo B., Greenfield S., Kaplan SH. (2002).'Erectile dysfunction and quality of life in type 2 diabetic patients: a serious problem too often overlooked'. Diabetes Care.25 (3), pp. 284–291.

Defeudis G., Gianfrilli D., Di Emidio C., Pofi R., Tuccinardi D., Palermo A. (2015).'Erectile dysfunction and its management in patients with diabetes mellitus'. Review Endocrine of Metabolism Disorder. doi: 10.1007/s11154-015-9321-4.

Dorey G. (2001).'Is smoking a cause of erectile dysfunction? A literature review'. British Journal of Nursing.10 (2), pp.455–465.

Dunzendorfer U., Behm A., Dunzendorfer E. (2002).'Drug combinations in the therapy of low response to phosphodiesterase 5 inhibitors in patients with erectile dysfunction'. In Vivo; 16(5), pp. 345-348.

ED: non-surgical management (erectile dysfunction). Urology Care Foundation [homepage on the Internet].c2014. Available from: http://www.urologyhealth.org/urology/index.cfm?article=60

Egharevba, C., Osayemwenre, E., Imieje, V., Ahomafor, J., Akunyuli, C., Udu-Cosi, A. A., Theophilus, O., James, O., Ali, I. and Falodun, A. (2014). 'Significance of Bitter Leaf (*Vernonia amgdalina*) In Tropical Diseases and Beyond'. A Review. Malaria Chemotherapy Control and Elimination. 3(1), pp. 1–10.

Ejike CE, Awazie SO, Nwangozi PA, Godwin CD. (2013). 'Synergistic postprandial Blood Glucose Modulatory Properties of *Vernonia amygdalina* (Del.), *Gongronemal atifolium* (Benth.) and *Occimum gratissimum* (Linn.) Aqueous Decoctions'. Journal of Ethnopharmacology.149(1), pp. 111–116.

El-Sakka AI, Tayeb KA. (2003).'Erectile dysfunction risk factors in noninsulin dependent diabetic Saudi patients'. Journal of Urology.169 (7) pp.1043–1047.

Erasto G., Grierson DS, Afolayan AJ. (2006).'Bioactive sesquiterpene from leaves of *Vernonia amygdalina'*. Journal of Ethnopharmacology.106 (1), pp. 117–129.

Erukainure, OL, Chukwuma, CI, Sanni, O., Matsabisa MG, Islam MS, (2018). 'Histochemistry, phenolic content, antioxidant, and anti-diabetic activities of *Vernonia amygdalina* leaf extract'. Journal of Food Biochemistry doi:10.1111/jfbc.12737.

Farombi EO, Owoeye O., Onwuka SK, Eruwa RT. (2011).'Gross morphometric reduction of rats cerebellum by gamma irradiation was mitigated by pretreatment with *Vernonia amygdalina* leaf extract'. Romanian Journal of Morphology and Embryology.

Farombi, EO, and Owoeye, O. (2011). 'Antioxidative and Chemopreventive Properties of Vernonia amygdalina and Garcinia biflavonoid'. International Journal of Environmental Research and Public Health. 8(1), pp. 2533–2555.

Feldman HA, Koussa VT, and Swerdloff RS. (2001). 'Male sexual function and its disorders' Physiology, Pathophysiology, Clinical Investigation and, Treatment Endocrinology. Review, 22(3), pp. 342-388.

Feldman HA, Goldstein I., Hatzichristou DG., Krane RJ, and McKinlay JB, (1994). 'Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study'. Journal of Urology., 151(1), pp. 54–61.

Frank E., Anderson C., Rubinstein D., (1978).'frequency of sexual dysfunction in 'normal' couples'. New England Journals of Medicine 299, pp. 111-115.

Fullick A., (1994). Biology. Heinemann Advanced Science. Oxford. pp. 332.

Gades NM, Nehra A., Jacobson DJ, McGree ME, Girman CJ, Rhodes T.(2005). 'Association between smoking and erectile dysfunction: a population-based study'. American Journal of Epidemiology. 161(3), pp. 346–351.

Gauthaman K., Adaikan PG, Prasad RN, Jict YH. (2002). 'Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats'. Life Science;71, pp. 1385–96.

Georgewill, OA, Georgewill UO. (2010). 'Evaluation of the Anti-inflammatory Activity of Extract of *Vernonia amygdalina*. Asian Pacific Journal of Tropical Medicine'. pp. 150–151.

Ghalayini IF, Al-Ghazo MA, Al-Azab R, Bani-Hani I, Matani YS, Barham AE. (2010). 'Erectile dysfunction in a Mediterranean country: results of an epidemiological survey of a representative sample of men'. International Journal of Impotence Research. 22(1), pp. 196–203.

Ghamba, P., Balla, H., Goje, L., Halidu, A. and Dauda, M. (2014). 'In Vitro Antimicrobial Activities of *Vernonia amygdalina* on Selected Clinical Isolates'. International Journal of Current Microbiology and Applied Sciences. 3(4), pp. 1103–1113.

Giuliano F., Rampin O., Schirar A., Jardin A., and Rousseau JP. (2003). 'Autonomic control of penile erection: modulation by testosterone in the rat'. *Journal of Neuroendocrinology*. 5(6), pp. 677-683.

Giuliano FA, Leriche A., Jaudinot EO, de Gendre AS. (2004). 'Prevalence of erectile dysfunction among 7689 patients with diabetes or hypertension, or both. Urology. 64 (2), pp. 1196–1201.

Glisson JK, Rogers HE, Abourashed EA, Ogletree R, Hufford CD, Khan I. (2003). 'Clinic at the Health Food Store? Employee Recommendations and Product Analysis. Pharmacotherapy'. 23(1), pp. 64–72.

Gregorian RS, Golden KA, Bahce A., Goodman C., Kwong WJ, Khan ZM. (2002) 'Antidepressant-induced sexual dysfunction and Pharmacotherapy'. 36(2), pp. 1577–1589.

Grenier G., and Byers ES. (1995). 'Rapid ejaculation: a review of conceptual, etiological and treatment issues'. Archives of Sexual Behavior . 22(1), pp. 447-472.

GuayAT, Spark RF, Bansal S., Cunningham GR, Goodman NF, Nankin HR, Petak SM, Perez JB. (2003).'American Association of Clinical Endocrinologist: Medical Guidelines for Clinical Practice for the evaluation and treatment of male sexual dysfunction'. Endocrinology. Practice., 9(1), pp. 78-95

Gupta BP, Murad MH, Clifton MM, Prokop L, Nehra A, Kopecky SL. (2011). 'The effect of lifestyle modification and cardiovascular risk factor reduction on erectile dysfunction: a systematic review and meta-analysis'. Archive International Medicine , 171 (1), pp. 803-1797.

Guyton AC, and Hall JE. (2000). 'Textbook of Medical Physiology (Tenth Edition).' (Harcourt International Edition) W. B. Saunder Company, Philadelphia. pp. 279-281.

Hasibuan F., Harahap P., Sitorus U., Satria P. (2020). 'The anticancer activities of *Vernonia amygdalina* Delile. leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway'. Heliyon 6 (7). doi:10.1016/j.heliyon.2020.e04449.

Hasler CM, Blumberg JB. (1999). 'Symposium on Phytochemicals: Biochemistry and Physiology'. Journal of Nutrition ; 129: 756S-757S.

Hirsch IH. (2014). Erectile dysfunction. The Merck Manual Home Edition [homepage on the Internet]. 2013. Available from: http://www.merckmanuals.com/home/mens_health_issues/sexual_dysfunction_in_men/erectile_dysfunction_ed.html?qt=erectile%20dysfunction&alt=sh

Howard CB, McDowell R., Feleke R., Deer E., Stamps S., Thames E., Singh V., Pervin S. (2016). 'Chemotherapeutic Vulnerability of Triple-negative breast cancer cell-derived tumors to pretreatment with *Vernonia Amygdalina* Aqueous extract'. Anticancer Research 36(8), pp. 3933-3943.

Huang YC, Chin CC, Chen CS, Shindel AW, Ho DR, Lin CS. (2015). 'Chronic cigarette smoking impairs erectile function through increased oxidative stress and apoptosis, decreased nNOS, endothelial and smooth muscle contents in a rat model'. Public Library of Science One.10:e0140728.

Ifedibaluchukwu EI, Aparoop D., Kamaruz Z. (2020). 'Antidiabetic, Anthelmintic, and anti-oxidation properties of novel and new phytocompounds isolated from the metanolicstem-bark of *Vernonia Amygdalina* Delile (Asteraceae)'. Scientific Africa 10. Doi: 10.1016/j.scaif.2020.e00578.

Imaga, NO, and Bamigbetan, DO. (2013). 'In vivo Biochemical Assessment of Aqueous Extracts of *Vernonia amygdalina* (Bitter leaf)'. International Journal of Nutrition and Metabolism. 5(1), pp. 22–27.

Iwalokun BA, Efedede BU, Alabi-Sofunde JA, Oduala T, Magbagbeola OA, Akinwande AI. (2006). 'Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice'. Journal Medicine of Food. 9(1), pp. 524–530.

Jang DJ, Lee MS, Shin BC, Lee YC, Ernst E. (2008). 'Red ginseng for treating erectile dysfunction': a systematic review. Journal of Clinical Pharmacology; 66(1), pp. 444-500.

JanMohamed HJ, Yap RW, Loy SL, Norris SA, Biesma RA. (2015). 'Prevalence and Determinants of Overweight, Obesity, and Type 2 Diabetes mellitus in Adults in Malaysia'. Asia Pacific Journal Public Health. 27(2), pp. 123–135.

Joe FM. (1990). 'Erectile Dysfunction: Sexual response in men'. Archive Sexual Behavior, 14, pp.33-39.

Justin, I., Ekong, P., Ubana, E., Zaini, M. and Ahmad, M. (2012). 'Synergistic Antidiabetic Activity of *Vernonia amygdalina* and *Azadirachta indica*: Biochemical Effects and Possible Mechanism'. Journal of Ethnopharmacology. 141(3), pp. 878–887

Kaplan HS. (1996). 'Erotic obsession: relationship to hypoactive sexual desire disorder and paraphilia'. American Journal of Psychiatry, 153(7), pp. 30–41.

Khalafalla, M. M., Abdellatef, E., Daffalla, H. M., Nassrallah, A. A., Lightfoot, D. A., Cocchetto, A. and El-shemy, H. A. (2009). Antileukemia Activity from Root Cultures of *Vernonia amygdalina.* Journal of Medicinal Plants Research. 3(8), pp. 556–562.

Kinzl JF, Mangweth B., Traweger C., and Bieble W. (1996). 'Sexual dysfunction in males: significance of adverse childhood experiences'. Child Abuse Neglect., 20 (2), pp. 759–766.

Knott L. (2013). 'Erectile dysfunction (impotence)'. Available from: http://www.patient.co.uk/health/erectile-dysfunction-impotence

Kupchan, S.M.; Hemmnigway, R.J.; Karim, A.; Werner, D. (1969). 'Tumor inhibitors. XLVII Vernodalin and Vernomygdin'. Two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. Journal of Organic Chemistry. , 34(5), pp. 3908-3911.

Laumann EO, Paik A., Rosen RC. (1999).'Sexual dysfunction in the United States: Prevalence and predictors'. Journal of American Medicine Association, 281(2), pp. 537-544.

Lee SW, Wang HZ, and Christ GJ. (2006). 'Characterization of ATP-sensitive potassium channels in human corporal smooth muscle cells'. *International Journal of Impotence Research.* 11(1), pp. 179-188.

Letchuman GR, Nazaimoon WM, Mohamad WBW, Chandran, LR, Tee, GH, Jamaiyah H., Faudzi YA. (2010). 'Prevalence of Diabetes in the Malaysian National Health Morbidity Survey III 2006'. Medical Journal of Malaysia. 65(3), pp. 173–179.

Maggi M., Filippi S., Ledda F., MaginiA., & Forti G. (2000).'Erectile dysfunction: from biochemical pharmacology to advances in medical therapy'. European Journal of Endocrinology 7 (1), pp. 143, 143–154.

Malavige LS, Jayaratne SD, Kathriarachchi ST, Sivayogan S, Fernando DJ, Levy JC. (2008) 'Erectile dysfunction among men with diabetes is strongly associated with premature ejaculation and reduced libido'. Journal of Sex Medicine. 5 (1), pp. 2125–2134.

McVary K. (2006). 'Lower urinary tract symptoms and sexual dysfunction: epidemiology and pathophysiology'. British Journal Urology International. 97(2), pp.23–28.

Metz ME, Pryor JL, Nesvacil LJ, Abuzzahab F., Koznar J. (1997). 'Premature ejaculation: a psychophysiological review'. Journal of Sex Marital Therapy. 23(5), pp. 3–23.

Michael A., and Owen A. (1997).'Venlafaxine-induced increased libido and spontaneous erections'. British Journal of Psychiatry, pp. 170:193.

Monga M. (1999). 'The aging penis: erectile dysfunction'. Geriatric Nephrology. Urol. 9(1), pp. 27-37

Montorsi F., Salonia A., Dehaf O., Cestari A., Guazzoni G., Rigatti P., Steef C. (2003) 'Pharmacological management of erectile dysfunction'. British Journal of Urology, 8(1), pp. 211-216.

Muraina IA, Adaudi AO, Mamman M, Kazeem HM, Picard J, McGaw LJ, Eloff JN. (2010). 'Antimycoplasmal activity of some plant species from northern Nigeria compared to the currently used therapeutic agent'. Pharmaceutical Biology. 48 (7), pp. 1103–1107

Muraina, IA, Adaudi AO, Mamman M., Kazeem HM, Picard J., McGaw LJ, Eloff JN, Cimanga RK, Tona L., Mesia K., Musuamba CT, De Bruyne, T., Apers S., Hernan N., Miert VS, Pieters L., Totte J., Vlietink AJ. (2004). 'In vitro antiplasmodiaacivity of extravts and fractions of seven medicinal plants used in the democratic republic of Congo'. Journal of Ethnopharmacology., 93(1), pp. 27-32.

Natali A., Mondaini N., Lombardi G., Del Popolo G., Rizzo M. (2005). 'Heavy smoking is an important risk factor for erectile dysfunction in young men'. International Journal Impotence Research. 17, pp. 227–230.

Navabi S., Gorrepati VS, Yadav S., Chintanaboina J., Maher S., Demuth P., Stern B., Stuart A., Tinsley A., Clarke K., Williams ED, Coates MD. (2018) 'Influences and Impact of Anxiety and Depression in the Setting of Inflammatory Bowel Disease'. Inflammatory Bowel Disease. Oct 12; 24(11), pp. 2303-2308. doi: 10.1093/ibd/izy143. PMID: 29788469.

Ngatu N R, Okajima M K, Yokogawa M, Hirota R, Takaishi M, Eitoku M, Muzembo B A, Sabah A B, Saruta T, Miyamura M, Kaneko T, Sano S, Suganuma N, (2012). 'Anti-allergic effects of Vernonia Amygdalina leafs extract in hapten-induced atopic dermatisis-like disease in mice'. Allergology International 61(4), pp. 597-607. doi: 10.2332/allergolint. 11-OA-0393.

Njan, AA, Adzu B., Agaba AG, Byarugaba D., Díaz-Llera S., Bangsberg, DR. (2008). 'The Analgesic and Antiplasmodial Activities and Toxicology of *Vernonia amygdalina'*. Journal of Medicinal Food. 11(3), pp. 574–81.

Nunes KP, Labazi H., Webb RC. (2012). 'New insights into hypertension-associated erectile dysfunction'. Current Opinion in Nephrology Hypertension. 21(9), pp. 163–170.

Nwanjo, H. U. (2005). 'Efficacy of Aqueous Leaf Extract of *Vernonia amygdalina* on Plasma Lipoprotein and Oxidative Status in Diabetic Rat Models'. Nigerian Journal of Physiological Sciences: Official Publication of the Physiological Society of Nigeria. 20(1–2): pp. 39–42.

Nwaoguikpe, RN. (2010). 'The Effect of Extract of Bitter Leaf (*Vernonia amygdalina*) on Blood Glucose Levels Of Diabetic Rats'. International Journal of Biology and Chemical Sciences. 4(1), pp. 721–729.

Ofeimun JO, and Ayinde BA. (2017). 'Investigation of aphrodisiac potential of methanol extract and fractions of *Rhaphiostylisbeninensis* Planch ex Benth'. *Journal of Science and Practice of Pharmacy* 4(1), pp. 182-188.

Oguwike, FN, Offor CC, and Onubeze DP, and Nwadioha AN. (2013). 'Evaluation of Activities of Bitterleaf (Vernonia amygdalina) Extract on Haemostatic and Biochemical Profile of Induced Male Diabetic Albino Rats'. Journal of Dental and Medical Sciences. 11(2): pp. 60–64.

Ojatula AO. (2017). 'Bioactivity and safety assessment of *Pausinystalia yohimbe* (K. Schum.) *Pierre ex Beille* root extract on male sexual and spermatic parameters in wistarrats'. Unpublished PhD Dessertation, PhytoMedicine. University of Benin, Faculty of Life Sciences, Benin City, Nigeria, pp.266.

Omojokun, OS, Famurewa, AJ, Jaiyeoba, OA, Oboh, G, Agbebi, OJ., (2019). 'Alkaloid extracts from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (Solanum nigrum) inhibit phosphodiesterase-5, arginase activities and oxidative stress in rats penile tissue'. J Food Biochem. doi:10.1111/jfbc.12889.

Oreagba AI, Oshikoya AK, Amachre M, Awefo A. (2011).'Herbal medicine use among urban residents in Lagos, Nigeria, BMC Complementary and Alternative Medicine', www.biomedcentral.com/1472-6882/11/117

Padashetty SA, and Mishra SH. (2007). Aphrodisiac studies of *Tricholepisglaberrima* with supportive action from anti-oxidant enzymes. *Pharmaceutical. Bioogyl*. 45: pp. 580 -586.

Peluffo G., Calcerrada P., Piacenza L., Pizzano N., Radi R. (2009). 'Superoxide-mediated inactivation of nitric oxide and peroxynitrite formation by tobacco smoke in vascular endothelium: studies in cultured cells and smokers'. American Journal of Physiology Heart Circulatory Physiology. 296(1), pp. H1781–H1792.

Penson DF, Latini DM, Lubeck DP, Wallace KL, Henning JM, Lue TF. (2003). 'Do impotent men with diabetes have more severe erectile dysfunction and worse quality of life than the general population of impotent patients?' Results from the Exploratory Comprehensive Evaluation of Erectile Dysfunction (ExCEED) database. Diabetes Care. 26, pp. 1093–1099.

Pourmand G., Alidaee MR, Rasuli S., Maleki A., Mehrsai A. (2004). 'Do cigarette smokers with erectile dysfunction benefit from stopping?'. a prospective study. British Journal of Urology International . 94(1), pp. 1310–1313.

Rampin O., Jerome N., and Suaudeau C (2003). 'Proerectile effect of apomorphine in mice'. *Life Science*. 79(21), pp. 2329-2336.

Rendell MS, Rajfer J., Wicker PA. (1999). 'Sildenafil for treatment of erectile dysfunction in men with diabetes'. Journal of the American Medical Association, 282(1), pp. 421-426.

Salmon PH. (1983). 'Psychosexual dysfunction in chronic renal failure: An overview'. Journal of European Dialysis and Transplantation Nursing Association, 12(3), pp. 209-212.

Sarker SD. (2012). 'Pharmacognosy in Modern Pharmacy Curricula'. Pharmacognosy Magazine. Apr-Jun; 8(30), pp. 91–92.

Scarano A., Gerardi C., D'Amico L., Accogli R., Santino A. (2018). 'Phytochemical analysis and antioxidant properties in colored Tiggiano carrots'. Agriculture 8(1), pp. 102.

Schein M., Zyzanski. SJ., Levine S., Medali JH., Dickman RL., & Alemagno S,A. (1988). 'The Frequency of sexual problem among family practice patients'. Family practitioner Research journal 7, pp. 122-134.

Serretti A., Chiesa A. (2009). 'Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis'. Journal of Clinical Psychopharmacology. 29(2), pp. 259–266.

Siu SC, Lo SK, Wong KW, Ip KM, Wong YS. (2001). 'Prevalence of and risk factors for erectile dysfunction in Hong Kong diabetic patients'. Diabetes Medicine. 18(1), pp. 732–738.

Smith T., Kawa K., Eckl V., Morton C., Stredney R. (2018). 'Herbal Supplement Sales in US Increased 8.5% in 2017'. Topping $8Billion. Herbal Gram. 119 (4) pp. 62–71.

Tajuddin, Ahmad S., Latif A., Qasmi IA. (2003). 'Aphrodisiac activity of 50% ethanolic extracts of Myristica fragrans Houtt. (nutmeg) and Syzygiumaromaticum (L.) Merr. & Perry. (clove) in

male mice: a comparative study'. BMC Complementary Alternative Medicine.;3:6. doi: 10.1186/1472-6882-3-6.

Tang X., Olatunji OJ, Zhou Y., and Hou X. (2017). 'In vitro and in vivo aphrodisiac properties of the seed extract from *Allium tuberosum* on corpus cavernosum smooth muscle relaxation and sexual behavior parameters in male Wistar rats'. *BMC Complementary Alternative Medicine,*17(2), pp.510.

Thakur M., Thompson D., Connellan P., Deseo MA, Morris C., Dixit VK. (2011). 'Improvement of penile erection, sperm count and seminal fructoselevels in vivo and nitric oxide release in vitro by ayurvedic herbs'.Andrology, 43(4), pp. 273–277.

TooneBK, Wheeler M., Nanjee M., Fenwick P., Grant R. (1983).'Sex hormones, sexual activity and plasma anticonvulsant levels in male epileptics'. Journal of Neurology and Neurosurgery Psychiatry, 46 (1), pp. 824–826.

Toyang, NJ, Verpoorte, R., (2013). 'A review of the medicinal potentials of plants of the genus *Vernonia* (Asteraceae)'. Journal of Ethnopharmacology, 146 (3), pp. 681–723. doi:10.1016/j.jep.2013.01.040.

Treating erectile dysfunction. Urology Care Foundation [homepage on the Internet]. c2014. Available from: http://www.urologyhealth.org.

Wagner G., and Green R. (1981). 'Erection: physiology and endocrinology. Impotence: Physiological, Psychological, Surgical Diagnosis and Treatment'. Plenum Press, pp. 25–36.

Weeks GR, Gambescia N. (2002). 'Hypoactive sexual desires: integrating sex and couple therapy'. New York: WW. Norton co. Inc.

Wessells H, Penson DF, Cleary P, Rutledge BN, Lachin JM, McVary KT. (2011). 'Effect of intensive glycemic therapy on erectile function in men with type 1 diabetes'. J Urol. 185(1), pp. 1828–1834.

Wu C., Zhang H., Gao Y., Tan A., Yang X., Lu Z. (2012). 'The association of smoking and erectile dysfunction: results from the Fangchenggang Area Male Health and Examination Survey (FAMHES)'. Journal of Andrology. 33 (2), pp. 59–65.

Yakubu MT, Akanji MA, and Oladiji AT. (2005). 'Aphrodisiac potentials of aqueous extract of *Fadogiaagrestis* (Schweinf. Ex Hiern) stem in male albino rats'. *Asian Journal of Andrology,* 7(4), pp. 399-404.

Yakubu MT, Akanji MA, Oladiji AT, Terdo EW. (2005). 'Aphrodisiac potentials of the aqueous extract of *Fadogiaagrestis* (Schweinf. Ex Hiern) stem in male albino rats'. Asian Journal of Andrology.7(1), pp. 399–404.

Yeap SK, Ho WY, Beh BK, Liang WS, Ky H., Hadi A., Alitheen, NB. (2010). '*Vernonia amygdalina*, an Ethnomedical used Green Vegetable with Multiple Bio-activities'. Journal of Medicinal Plants Research. 25(4), pp. 2787–2812.