CORPUS CAVERNOSUM SMOOTH MUSCLE MODULATORY EFFECTS OF ETHANOL EXTRACT AND FRACTIONS OF *MORORDICA CHARANTIA* Linn (CUCURBITACEAE)

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BENIN CITY.

FEBRUARY, 2025

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHARMACY (PHARM.D) DEGREE,

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CERTIFICATION

This investigative study, entitled ‘corpus cavernosum smooth muscle modulatory effects of ethanol extract and fractions of *Morordica charantia* Linn (cucurbitaceae) was conducted independently by ESOGBUE, Michael Chukwuemeka under the guidance of Dr. (Mrs.) Josephine Ofeimun. The research was undertaken within the Pharmacognosy Department at the Faculty of Pharmacy, University of Benin, located in Benin City, Nigeria.

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…………………………. ……………………………

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DEDICATION

I dedicate this project to God Almighty, my parents, Mr and Mrs Esogbue, for their unwavering support during my time at this institution, and to my siblings for their ongoing encouragement and backing.

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ABSTRACT

This study investigated the modulatory effects of ethanol extract and solvent fractions of *Momordica charantia* on corpus cavernosum smooth muscle, aiming to identify potential therapeutic agents for erectile dysfunction.

Powdered *M. charantia* was macerated in 1.5 L ethanol, yielding a concentrated extract (15.57 % yield, 77.85 g). The crude extract was dissolved and fractionated sequentially with n-hexane, chloroform, and ethyl acetate to obtain distinct fractions alongside a residual aqueous fraction. Solutions of 1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL were prepared for the extract, its fractions, and a sildenafil citrate extract (used as a standard)., The corpus cavernosum smooth muscle was isolated from male rats and mounted in an organ bath containing Kreb's solution. Incremental volumes (25 µL, 62.5 µL, 125 µL, and 250 µL) of stock solution of extract and fraction were administered with a four-minute contact time per dose. Each extract/fraction was tested in triplicate, and the responses were analyzed using one-way ANOVA. An F-statistic of 10.9523, which exceeded the F-critical value of 2.53, indicated statistically significant differences ($p < 0.05$) among the treatments. Phytochemical tests were carried out using standard procedures to determine secondary metabolites present in the plant material

Phytochemical analysis of the extract revealed the presence of alkaloids, tannins, saponins, reducing sugars, and cardiac glycosides. These bioactive compounds may contribute to smooth muscle relaxation via multiple mechanisms including nitric oxide (NO) release, cyclic guanosine monophosphate (cGMP) pathway activation, phosphodiesterase type 5 (PDE5) inhibition, calcium channel blockade, and antioxidant effects. Notably, the n-hexane fraction exhibited the

lowest EC50 (0.257 mg), suggesting it possesses the highest potency in modulating smooth muscle tone compared to other fractions and even the sildenafil citrate extract.

In conclusion, the ethanol extract and its fractions from *M. charantia* significantly modulate corpus cavernosum smooth muscle activity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 ERECTILE DYSFUNCTION

Erectile dysfunction (ED), previously referred to as impotence, is characterized by the inability to achieve or maintain a firm penile erection adequate for satisfactory sexual activity, (Muneer *et al.* 2014). While no specific time frame is required for diagnosis, some experts suggest that ED should persist for at least six months. This condition is common among men aged 40 and older, with prevalence increasing with age and the presence of other health issues, (Shamloul and Ghanem, 2013).

1.2 PATHOPHYSIOLOGY OF ERECTILE DYSFUNCTION

ED can result from a variety of underlying health problems and serves as an important but often overlooked cardiovascular risk factor, (Orimoloye, *et al*. 2019). Any condition that impacts the penile arteries, nerves, hormone levels, smooth muscle tissue, corporal endothelium, or tunica albuginea can lead to ED. It is closely associated with cardiovascular diseases, diabetes, hypertension, and hyperlipidemia, among other conditions. Endothelial dysfunction is another key pathway frequently observed in patients with ED, (Matsui, *et al*. 2015).

While most cases of ED are due to physical causes, some younger men may experience it primarily due to psychological issues. Even when the root cause is organic, ED often has psychological effects, including marital and relationship challenges, cultural pressures, loss of self-esteem, anxiety, depression, and feelings of shame. These emotional impacts can significantly affect the quality of life for both the patient and their partner. The good news is that ED is nearly always treatable.

The causes of ED are typically multifactorial, and it's important to determine whether the condition has a psychological or organic origin. Factors like depression, performance anxiety, and other sexual dysfunctions can contribute, even when physical causes are present. Aging plays a crucial role in the development of ED, as cardiovascular diseases, hypertension, and other co-morbidities become more prominent with age. Conditions like diabetes and metabolic syndrome can affect multiple organ systems, accelerating the decline of erectile function and disrupting the biological processes that support erections on a molecular level, (Kouidrat, *et al*. 2017). Other factors that can contribute to erectile dysfunction (ED) include neurological conditions (such as multiple sclerosis), hormonal imbalances (e.g. hypogonadism, thyroid disorders), traumatic injuries (such as pelvic fractures or spinal cord injuries), hyperlipidemia, stroke, sleep apnea, chronic obstructive pulmonary disease (COPD), glaucoma, the aftermath of priapism, depression, benign prostatic hyperplasia with lower urinary tract symptoms (BPH with LUTS), iatrogenic causes (such as post-transurethral resection of the prostate), and a range of medications, including antidepressants, anti-hypertensives, antipsychotics, opioids, and recreational drugs.

1.3. RISK FACTORS FOR ERECTILE DYSFUNCTION

1.3.1 CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is a major risk factor for ED. Nearly 50% of men with coronary artery disease, confirmed through cardiac catheterization, also experience significant ED, (Montorsi, *et al*. 2003). This is partly due to the similarity in size between the coronary arteries and penile cavernosal arteries, both of which can develop atherosclerotic issues in a similar manner. The smaller size of the cavernosal arteries makes them more prone to blockages from atherosclerotic plaques, leading to vasculogenic ED that can appear years before coronary artery disease becomes clinically evident. Both CVD and ED share endothelial dysfunction as a common factor in their pathophysiology, (Guay, 2005).

Patients may show signs of subclinical atherosclerosis up to 10 years before experiencing noticeable ED. Due to the smaller diameter of the cavernosal arteries, vasculogenic ED often occurs before coronary artery disease, heart attacks, and strokes, sometimes by as much as five years, (Imprialos *et al*. 2021). Younger men with unexplained ED are at a significantly higher risk for cardiovascular problems later in life, with a risk increase as much as 50-fold compared to their age-matched peers, (Inman, 2009).

Inform patients that erectile dysfunction (ED) is a significant marker for underlying heart disease, and refer them for further cardiovascular risk assessment and management, (Inman, 2009). The Prostate Cancer Prevention Trial database revealed that ED increases a patient's cardiovascular risk to a level comparable to the risks associated with smoking or having a family history of heart attacks, (Thompson *et al*. 2005). A meta-analysis of 14 studies, involving over 90,000 men with ED, showed that these individuals experienced 44% more cardiovascular events, 62% more heart attacks, 39% more strokes, and a 25% higher risk of death compared to those without ED, (Vlachopoulos *et al*. 2013).

ED serves as an independent predictor of future cardiovascular events, so it is crucial to screen all patients with ED for cardiovascular risk factors. Patients with intermediate risk, consider non-invasive testing for subclinical atherosclerosis and an exercise stress test, (Jackson, 2013)

1.3.2 CIGARETTE SMOKING

Cigarette smoking is indeed a significant risk factor for erectile dysfunction (ED). Research has consistently shown that smoking can damage blood vessels and reduce blood flow to the penis, making it harder to achieve and maintain an erection. The link between smoking and ED is thought to be related to the development of atherosclerosis, a condition in which plaque builds up in the arteries, restricting blood flow. This can lead to a range of cardiovascular problems, including ED. Studies have also shown that quitting smoking can help reduce the risk of ED. However, the extent to which quitting can improve ED symptoms is still debated, and more research is needed to fully understand the relationship between smoking cessation and ED, (Rosen *et al*, 1997*)*.

1.3.3 HYPERTENSION

Hypertension, or high blood pressure, is a well-established risk factor for erectile dysfunction (ED). Research has consistently shown that hypertension can damage blood vessels and reduce blood flow to the penis, making it harder to achieve and maintain an erection.

The link between hypertension and ED is thought to be related to the development of atherosclerosis, a condition in which plaque builds up in the arteries, restricting blood flow. This can lead to a range of cardiovascular problems, including ED, (Doumas, *et al*. 2006).

1.3.4 HYPERLIPIDEMIA

Hyperlipidemia, or high levels of lipids (cholesterol and triglycerides) in the blood, is a significant risk factor for hypertension, (Kannel, 1996), Research has consistently shown that hyperlipidemia can contribute to the development of hypertension through several mechanism such as;

a.) Endothelial dysfunction

High levels of low-density lipoprotein (LDL) cholesterol can damage the endothelium, the inner lining of blood vessels, leading to impaired vasodilation and increased blood pressure.

b.) Inflammation

Hyperlipidemia can trigger inflammation in the blood vessels, which can lead to the development of atherosclerosis (plaque buildup in the arteries) and increased blood pressure.

c.) Renal dysfunction

High levels of lipids in the blood can damage the kidneys, leading to impaired sodium excretion and increased blood pressure.

d.) Vascular remodeling

Hyperlipidemia can lead to changes in the structure and function of blood vessels, making them more susceptible to vasoconstriction and increased blood pressure.

1.3.5 DIABETES

Diabetes is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that diabetes can damage blood vessels and nerves, leading to impaired blood flow and erectile function, (Feldman, *et al*. 1994).

The link between diabetes and ED is thought to be related to several mechanisms:

a.) Neuropathy

Diabetes can damage the nerves that control erectile function, leading to impaired transmission of signals.

b.) **Vasculopathy**

Diabetes can damage blood vessels, reducing blood flow to the penis and making it harder to achieve and maintain an erection.

c.) **Endothelial dysfunction**

Diabetes can impair the function of the endothelium, the inner lining of blood vessels, leading to reduced blood flow and erectile function.

d.) Hormonal imbalance

Diabetes can disrupt hormonal balances, including testosterone levels, which can contribute to ED.

e.) **Inflammation and oxidative stress**

Diabetes can lead to chronic inflammation and oxidative stress, which can damage blood vessels and nerves, contributing to ED.

1.3.6 HYPOGONADISM

Hypogonadism, a condition characterized by low levels of testosterone, is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that hypogonadism can impair erectile function by affecting the physiological processes involved in erection, (Snyder, *et al*. 2016). The link between hypogonadism and ED is thought to be related to several mechanisms, (Cunningham *et al*. 2016).

Mechanisms linking hypogonadism to ED include:

a.) Reduced nitric oxide production:

Testosterone is essential for the production of nitric oxide, a molecule that helps to relax smooth muscle cells in the penis, allowing for increased blood flow and erection.

b.) Decreased erectile tissue responsiveness:

Low testosterone levels can reduce the responsiveness of erectile tissue to sexual stimulation, making it harder to achieve and maintain an erection.

c.) Neurological effects:

Testosterone has neuroprotective effects, and low levels of testosterone can lead to neurological damage, including damage to the nerves involved in erectile function.

1.3.7 OBESITY

Obesity is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that obesity can impair erectile function by affecting the physiological processes involved in erection, (Feldman, *et al*. 1994). Mechanisms linking obesity to ED include:

a.) Inflammation and oxidative stress

Obesity can lead to chronic inflammation and oxidative stress, which can damage blood vessels and nerves, contributing to ED.

b.) Insulin resistance and metabolic syndrome:

Obesity is often associated with insulin resistance and metabolic syndrome, which can lead to decreased blood flow and erectile function.

c.) Hormonal imbalance:

Obesity can disrupt hormonal balances, including testosterone levels, which can contribute to ED.

d.) Vascular dysfunction:

Obesity can damage blood vessels, reducing blood flow to the penis and making it harder to achieve and maintain an erection.

e.) Neurological effects:

Obesity can lead to neurological damage, including damage to the nerves involved in erectile function.

1.3.8 ALCOHOL USE

Alcohol use is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that excessive alcohol consumption can impair erectile function by affecting the physiological processes involved in erection. According to Laumann *et al*. (1999), mechanisms linking alcohol use to ED include:

a.) Neurological effects:

Alcohol can damage the nerves involved in erectile function, leading to impaired transmission of signals.

b.) Vasodilation and blood flow:

Alcohol can cause blood vessels to dilate, leading to decreased blood flow to the penis and making it harder to achieve and maintain an erection.

c.) Hormonal imbalance:

Excessive alcohol consumption can disrupt hormonal balances, including testosterone levels, which can contribute to ED.

d.) Sleep disturbances:

Alcohol can disrupt sleep patterns, leading to fatigue, decreased libido, and ED.

e.) **Psychological effects:**

Excessive alcohol consumption can lead to anxiety, depression, and relationship problems, all of which can contribute to ED.

1.3.9 BENIGN PROSTATIC HYPERPLASIA (BPH)

Benign prostatic hyperplasia (BPH) is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that BPH can impair erectile function by affecting the physiological processes involved in erection. According to Thompson, *et al*. (2003), mechanisms linking BPH to ED include:

a.) Urinary symptoms:

BPH can cause urinary symptoms such as frequency, urgency, and nocturia, which can disrupt sleep patterns and contribute to ED.

b.) Pelvic floor dysfunction:

BPH can lead to pelvic floor dysfunction, which can impair erectile function by affecting the muscles involved in erection.

c.) Bladder outlet obstruction:

BPH can cause bladder outlet obstruction, which can lead to increased pressure on the bladder and urethra, contributing to ED.

d.) Hormonal imbalance:

BPH can disrupt hormonal balances, including testosterone levels, which can contribute to ED.

e.) Surgical and medical treatments:

Certain treatments for BPH, such as surgery or medications, can also contribute to ED.

1.4.0 DEPRESSION;

Depression is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that depression can impair erectile function by affecting the physiological processes involved in erection. According to Kessler, *et al.* (1994), mechanisms linking depression to ED include:

a.) Neurotransmitter imbalance:

Depression can disrupt the balance of neurotransmitters, such as serotonin and dopamine, which play a crucial role in erectile function.

b.) Hormonal imbalance:

Depression can lead to hormonal imbalances, including decreased testosterone levels, which can contribute to ED.

c.) Reduced libido:

Depression can decrease libido, making it harder to achieve and maintain an erection.

d.) Anxiety and stress:

Depression can lead to anxiety and stress, which can impair erectile function by affecting the physiological processes involved in erection.

1.4.1 PRESCRIPTION MEDICATION SIDE EFFECTS

Certain antidepressant medications can contribute to ED as a side effect.

Prescription medications are believed to contribute to about 25% of all ED cases. Among the 12 most commonly prescribed drugs in the U.S., 8 list erectile dysfunction as a potential side effect, These medications include most antidepressants (particularly selective serotonin reuptake inhibitors), cimetidine, ketoconazole, spironolactone, certain sympathetic blockers (like methyldopa, clonidine, and guanethidine), thiazide diuretics, and other antihypertensive.

Angiotensin-converting enzyme (ACE) inhibitors and calcium channel blockers are less likely to cause ED. Beta-blockers have a minor impact on ED, while alpha-blockers may actually improve erectile function, (Kirby *et al*, 2005).

1.4.2 LOWER URINARY TRACT SYMPTOMS

Lower urinary tract symptoms (LUTS) are a significant risk factor for erectile dysfunction (ED). Research has consistently shown that LUTS can impair erectile function by affecting the physiological processes involved in erection. According to Jacobsen, *et al*. (1999), mechanisms linking LUTS to ED include:

a.) Nocturia and sleep disturbances:

LUTS can cause nocturia, leading to sleep disturbances, fatigue, and decreased libido, all of which can contribute to ED.

b.) Urinary frequency and urgency:

LUTS can cause urinary frequency and urgency, leading to anxiety and stress, which can impair erectile function.

c.) Pelvic floor dysfunction:

LUTS can lead to pelvic floor dysfunction, which can impair erectile function by affecting the muscles involved in erection.

d.) Bladder outlet obstruction:

LUTS can cause bladder outlet obstruction, leading to increased pressure on the bladder and urethra, contributing to ED.

1.4.3 PREMATURE EJACULATION

Premature ejaculation (PE) is a significant risk factor for erectile dysfunction (ED). Research has consistently shown that PE can impair erectile function by affecting the physiological and psychological processes involved in erection. According to Giuliano *et al*. (2008), mechanisms linking PE to ED include:

a.) Performance anxiety:

PE can lead to performance anxiety, which can impair erectile function by causing stress and distraction.

b.) Reduced confidence:

Premature ejaculation can reduce confidence in one's ability to perform sexually, leading to anxiety and ED.

c.) Conditioned response:

PE can create a conditioned response, where the individual becomes accustomed to ejaculating quickly, leading to difficulty in maintaining an erection.

d.) Neurological factors:

PE can be related to neurological factors, such as serotonin and dopamine imbalances, which can also contribute to ED.

Treatment side effects:

Certain treatments for PE, such as selective serotonin reuptake inhibitors (SSRIs), can contribute to ED as a side effect.

1.4.4 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 conditions. They are;

1.4.5 Drug management of erectile dysfunction (orthodox therapy).

Orthodox Drug therapy for the management of ED includes;

a.) Oral medications;

Phosphodiesterase Type 5 Inhibitors (PDE5 Inhibitors)

According to Rosen, (2004), these drugs enhance erectile function by inhibiting the enzyme PDE5, which breaks down cGMP (cyclic guanosine monophosphate) in the smooth muscle of the penis. Increased cGMP levels lead to relaxation of the smooth muscle and improved blood flow, resulting in an erection. Common drugs in this class include; sildenafil (Viagra), tadalafil (Cialis), vardenafil (Levitra), avanafil (Stendra).

Alpha-Blockers

Alpha-blockers (alpha-adrenergic antagonists) can help with ED by relaxing the smooth muscles of the prostate and bladder neck, improving urine flow, and possibly enhancing blood flow to the penis.Common Drugs**:** Tamsulosin (Flomax), Alfuzosin (Uroxatral), Doxazosin (Cardura), (*Kohan,* 2003).

b.) Other medications

Alprostadil (Prostaglandin E1)

Alprostadil is a vasodilator that increases cyclic AMP (Camp) levels in the smooth muscle of the penis, leading to muscle relaxation and enhanced blood flow. It can be administered via injection or urethral suppository. Common forms: Intracavernosal injection; (Caverject, Edex), Urethral suppository; (Muse), (Wang, 2007).

Testosterone Replacement Therapy (TRT)

Testosterone therapy is used for men with ED caused by hypogonadism (low testosterone levels). Increasing testosterone levels can restore libido and erectile function. Common forms: Testosterone enanthate, Testosterone cypionate, Transdermal testosterone (Androgel, Testim), Testosterone pellets (Testopel), (Corona, 2014).

Dopamine Agonists

Dopamine plays a crucial role in the brain's reward and pleasure centers. Dopamine agonists work by stimulating dopamine receptors, which can enhance libido and erectile function. Common Drugs: Apomorphine (Uprima), (Montorsi, 2002).

Yohimbine (Alpha-2 Adrenergic Antagonist)

Yohimbine works by blocking alpha-2 adrenergic receptors, increasing the release of norepinephrine, which enhances penile blood flow and improves erectile function. Common Drugs: Yohimbine hydrochloride, (Macdonald, 2005).

1.4.6 Non – Drug therapy for Erectile Dysfunction

Non – pharmacological treatment for erectile dysfunction includes; sex therapy, the use of vacuum erection devices, penile prosthesis implantation and penile vascular surgery. Sex therapy can be an effective treatment for erectile dysfunction (ED), particularly when the underlying causes are psychological or relational. ED can result from a combination of factors such as stress, anxiety, depression, relationship issues, and negative body image, among others. Sex therapy focuses on addressing these emotional, mental, and relational aspects. According to Wincze *et. al*. (2001), here is how sex therapy can help:

a.) **Psychological Support**: Sex therapists help individuals and couples identify and address the psychological factors that may contribute to ED, such as performance anxiety, stress, or depression. Therapy often includes techniques to reduce anxiety and improve self-esteem and body image.

b.) Communication and Relationship Issues: Sometimes, relationship problems such as lack of intimacy, poor communication, or unresolved conflicts can contribute to ED. Therapy can help improve communication between partners, enhance emotional connection, and build trust, which may alleviate ED symptoms.

c.) Cognitive Behavioral Therapy (CBT): This type of therapy can help break the cycle of negative thoughts and behaviors associated with ED. For example, CBT may focus on changing the way a person thinks about sex and their ability to perform, replacing negative thoughts with healthier, more realistic ones.

d.) Behavioral Techniques: Sex therapists may also teach practical techniques to improve sexual function, such as the use of sensate focus (a method where the couple focuses on non-sexual touch and increases intimacy over time) or exercises that help with relaxation and managing anxiety.

e.) Education and Reframing Expectations: Sometimes, men with ED develop unrealistic expectations about sex or performance. Sex therapy can help reframe these expectations and focus on the emotional and physical aspects of intimacy, rather than just performance.

f.) Couples Counseling: If ED is affecting a relationship, couples therapy can help partners navigate the challenges of intimacy and sexual health together. This can be especially helpful when one partner's ED is affecting the other's emotional well-being.

Vacuum Erection Devices (VED)

A vacuum device is a non-pharmacological treatment that creates a vacuum around the penis, drawing blood into the penile tissue to induce an erection. A constriction ring is placed at the base of the penis to maintain the erection. Devices: Vacuum pumps, (Eardley, 2005).

Penile Prosthesis Implantation

Penile prosthesis implantation is a surgical treatment option for erectile dysfunction (ED) when other less invasive treatments, such as medications or therapy, have not been effective. It is often considered for men with severe ED, particularly when the condition is due to physical causes like nerve damage, blood vessel issues, or other medical conditions that prevent normal erectile function, (Hatzichristou, *et al*. 2002).

Types of Penile Prostheses:

1. **Inflatable Prosthesis**: The most commonly used type. It consists of a pump, a reservoir, and two cylinders that are implanted into the penis. The pump, usually placed in the scrotum, is used to inflate the cylinders, creating an erection. The system is deflated after intercourse.
2. **Malleable (Semi-rigid) Prosthesis**: This type involves two flexible rods that are inserted into the penis. These rods allow the penis to be manually adjusted into an erect or flaccid position, but they don't offer the natural feel of an erection like inflatable models do.

1.4.7 ALTERNATIVE MANAGEMENT OF ERECTILE DYSFUNCTION

The majority of men with erectile dysfunction (ED) in both developed and developing countries rely primarily on psychotherapy and pharmacological treatments. However, many medications used to treat ED, such as sildenafil, tadalafil, and vardenafil, have drawbacks, including limited effectiveness, availability issues, high costs that may be unaffordable for the average African man, unpleasant side effects, and contraindications in certain medical conditions. For instance, sildenafil alters the hemodynamics in the penis but is associated with side effects such as headaches, flushing, dyspepsia, and nasal congestion, (Lue, 2002).

To address the limitations of conventional ED treatments, there is a need to explore natural supplements derived from medicinal plants and conduct further research on plants with aphrodisiac properties, (Yakubu *et al*. 2007).

1.4.8 USE OF MEDICINAL PLANTS IN MANAGEMENT OF ERECTILE DYSFUNCTION.

a**.)** ***TRIBULUS TERRESTRIS***

Tribulus terrestris, also known as puncture vine, has been traditionally used in Ayurvedic medicine to enhance libido and improve erectile function. The plant is native to the Mediterranean region and has been used for centuries to treat various health conditions, including ED. *Tribulus terrestris* has been shown to increase testosterone levels, which can help improve erectile function, improve blood flow to the penis, which is essential for achieving and maintaining an erection. The plant also has antioxidant properties, which can help reduce oxidative stress and improve overall health, (Li, *et al*. 2010).

b.) PANAS GINSENG

Panax ginseng, also known as Asian ginseng or Chinese ginseng, has been traditionally used in Chinese medicine to improve erectile function and enhance libido. The plant is native to the mountains of East Asia and has been used for centuries to treat various health conditions, including ED. *Panax ginseng* has been found to improve blood flow to the penis, which is essential for achieving and maintaining an erection, increases nitric oxide production, which can help relax blood vessels and improve erectile function, possesses antioxidant properties, which can help reduce oxidative stress and improve overall health, (Li, *et al*. 2010).

c.) GINKGO BILOBA

Ginkgo biloba, an ancient plant extract, has been used in traditional medicine for centuries to improve blood flow and cognitive function. Its potential benefits for erectile dysfunction (ED) have also been explored. *Ginkgo biloba's* flavonoids and terpenoids may help relax blood vessels, improving blood flow to the penis and potentially alleviating ED symptoms. It also has antioxidant properties, which may help reduce oxidative stress and inflammation, which can contribute to ED. *Ginkgo biloba* may help increase nitric oxide production, which can help relax blood vessels and improve erectile function, (Paick, et al. 2006).

d.) SAW PALMETTO

Saw palmetto (*Serenoa repens*) is a plant that has been used in traditional medicine for centuries to treat various health conditions, including erectile dysfunction. *Saw palmetto* may help block the conversion of testosterone to dihydrotestosterone (DHT), a hormone that can contribute to ED, it does this by the inhibition of 5-alpha-reductase. *Saw palmetto's* anti-inflammatory properties may help reduce inflammation and improve blood flow to the penis. The plant also possesses antioxidant properties, (Paick, et al. 2006).

1.4.9 *MORMORDICA CHARANTIA* (BITTER MELON)

Medicinal plants are used to used prevent, treat and manage different diseases, (Schulz *et al*. 2001). Bitter gourd, (*Mormordica charantia* L.) is also called bitter melon or bitter apple or balsam pear. It is a vine that grows in tropical countries. Tropical vines are a type of climbing or trailing plant that grows in warm and humid environments. *Mormordica charantia* is cultivated for various purposes. It is used for medicinal purposes, as a vegetable in delicacies for making several dishes in countries like China, India, (Behera *et al*. 2008). The whole plant is luscious in nature, it is grown for its leaves, fruits and flowers. The fruits, flowers, and young shoot gives a slight bitter taste, making it suitable as a flavouring agent in various Asian dishes, (Saeed *et al*. 2018). *Mormordica charantia* has been used for a long time by Asians for the management of diabetes, treatment of malaria parasite, GIT disorders and menstrual disorders. There are also claims that the stem and leaf of *Mormordica charantia* is also used in the treatment of erectile dysfunction. Researches carried out has shown that bitter melon contains an insulin like property which is often referred to as plant insulin, it has a positive effect in lowering the blood and urine glucose content, (Janagal *et al*. 2018). Bitter melon has also proven to have anti-cholesterol, anti-cancer, anti-dementia, anti-bacterial and anti-fungal, antioxidant and anti-inflammatory activities, (Bortolotti *et al*. 2019). All part of the plants mainly the fruits and seeds, contain more than 60 phyto-medicines active against more than 30 diseases including cancer and diabetes, (*Kole et al*. 2020).

1.5.0MORMORDICA CHARANTIA

1.5.1 TAXONOMIC CLASSIFICATION OF MORMORDICA CHARANTIA

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Violales

Family: Cucurbitaceae

Genus: Mormordica

Specie: *Mormordica charantia* L.

Figure 1.1 Picture of *Mormordica charantia,* leaf, stem, flower, showing the plant in its natural habitat.

1.5.2 *MORMORDICA CHARANTIA* (BITTER MELON): PLANT DESCRIPTION

Bitter melon can be described as a slender plant that can produce male and female gametes (monoecious plant). It is a tendril climbing annual vine of almost 2 to 4 m high. The features of bitter melon include: sharp tooth-like projections pointing towards the tip of the leaf, separate yellow coloured male and female flowers. The different varieties of bitter melon have different shapes of fruits, being ovoid or ellipsoid or discoid to oblong and pointed towards the end, (Kole *et al*. 2020). The fruits are usually 2 to 10 cm long.

The exterior of the fruits are warty and the cross section is hollow with a thin layer of fleshy flattened seeds and pith are seen in the central cavity which is surrounded by the thin flesh layer, The immature fruits are pale green or whitish in colour whereas the mature ones can be seen in dark green, green and light green depending on the varieties. While on ripening the colour turns to orange yellow. The fruit of bitter melon can take 45 to 80 days to get mature. The seed of bitter gourd is 8 to 15 mm long which are straw coloured and they are covered with flesh. White in unripe fruits and red in ripened ones, (Sorifa, 2018).

1.5.3 NUTRITIONAL PROFILE

Bitter melon is a vegetable that is often underrated and thrown away, because of its bitter taste, not regarding the fact that it is a source of several key nutrients. It is highly nutritious, even more nutritious than other cucurbits such as squash, pumpkin, cucumber and zucchini due to its high mineral and vitamin content, (Krawinkel and Keding, 2006). The fruit, leaves and stem is highly rich in vitamins namely vitamin A, vitamin E, thiamine, riboflavin, niacin, folate and vitamin C. It also contains a high amount of potassium, iron, calcium, magnesium, phosphorous and zinc. It is a good source of dietary fibre. The table below shows the detailed nutritional composition of bitter gourd leave.

TABLE 1: Nutritional composition of *Mormordica charantia* leaves.

Constituents	**Amount**
Water (%)	83.2 -92.4
Lipids (%)	0.1 – 1
Carbohydrates (%)	4.2 - 9.8
Proteins (%)	1.6 - 2.9
Fiber (%)	0.8 - 1.7
Ash (%)	7 – 18
Calcium (mg/100mg)	20 – 50
Phosphorus (mg/1000mg)	70 – 140
Iron (mg/100mg)	2.2 - 9.4
Magnesium (mg/100)	16
Sodium (mg/100mg)	3 – 40
Potassium (mg/100mg)	8 – 170
zinc (mg/100mg)	0.1
Manganese (mg/100mg)	0.08 - 0.32
Copper (mg/100mg)	0.18 – 5
Vitamin A as carotenes	210 - 220 IU
Vitamin C	70 - 120 mg
Thiamine (mg)	0.05
Riboflavin (mg)	0.03
Niancin (mg)	0.4

Adapted from (Behera *et al*. 2008).

The detailed amino acid composition of bitter melon fruit and seed protein is given in Table 2. The bitter melon seeds contain 35 to 40% of oil with fatty acid profile containing monounsaturated fatty acids (3.33%), saturated fatty acids (36.71%) and poly unsaturated fatty acids (59.96%). Bitter melon is one of the few edible fruit which contains conjugated α-linolenic acid in its seeds. The presence of α-eleostearic acid has been reported in bitter melon seed oil. They are one of the naturally best sources of chromium (5.65 mg / 100 g) and zinc (45.45 mg / 100 g), (Behera *et al*. 2008.).

Table 2. Bioactive compounds present in *Mormordica charantia.*

Amino acid	***Mormordica charantia* mature**	***Mormordica charantia* mature**
	fruit (mg/g protein)	**seed (mg/g) protein**
Cysteine	22.3	16.5
Aspartic acid	93.8	78
Threonine	25.2	17.4
Serine	55	43.5
Glutamic acid	96	124
Proline	54.4	49.7
Glycine	44.9	39.9
Alanine	51.2	46.7
Valine	42.2	36.7
Isoleucine	30.8	30.7
Leucine	64.9	60.5
Tyrosine	59.4	44.7
Phenylalanine	40.2	34.5
Methionine	27.6	23.6
Histidine	72.8	40.9
Lysine	101	98.7
Arginine	45.6	80.8

Adapted from (Behera et al. 2008.)

1.5.4 REPORTED BIOLOGICAL ACTIVITIES OF *MORMORDICA CHARANTIA*

Various researches has been carried out and proved the nutraceutical properties attributed to the bitter melon.

1.5.5 ANTIOXIDATIVE PROPERTY

Oxidative stress is the main cause for the development of various life style diseases including hypertension, diabetes and obesity. Researches are being conducted on the effect of *Mormordica charantia* and specific compounds in it against oxidative stress, most of them showing bitter melon has potential antioxidant properties. Bitter melon showed good anti-oxidant capacity in comparison with colocasia, (*Colocasia esculenta*) and pumpkin (*Curcubita pepo*), Various in vitro studies have been carried out to establish the anti-oxidative activity of *Mormordica charantia* whole fruit pulp, extracts, seed powder, leaves and stem, (Kubola & Siriamorn- pun, 2008).

1.5.6 ANTIDEMENTIA ACTIVITY

Neurodegenerative diseases are illnesses which affect the brain cells causing miscommunication and there by leading to irreversible effects in movement, memory, speech and intelligence. These diseases are untreatable and are described by a degeneration of certain neurons in a progressive manner occurs due to certain metabolic or toxic stress, (Valarmathi *et al.* 2020). Some examples for neurodegenerative diseases are dementias, Parkinson's disease, prion disease, motor neuron diseases (MND), Huntington's disease (HD) and spinal muscular atrophy (SMA). Dementia is the term used to depict a group of neurodegenerative disorders which affect the memory keeping power of the brain.

Charantin is a steroidal glycoside which exist as a mixture of sigmasterol gucoside and β sitosterol glucoside. *Mormordica charantia* ethanol extract significantly reduced the H_2O_2 induced cell death in human neuroblastoma SK-N-MC cells, (Desai and Tatke 2015)

1.5.7 HYPOLIPIDEMIC AND HYPOTENSIVE ACTIVITY

Hyperlipidaemia is a condition in which blood has abnormally high levels of lipids namely cholesterol and triglycerides mainly occur due to unhealthy food choices, chronic stress and obesity. It is considered as a potential risk factor for cardiovascular diseases. Researches are undergoing in exploring the role of bioactive ingredients from *Mormordica charantia* fruit and its parts against this condition. The possibility of using of *Mormordica charantia* juice as a hypolipidemic agent was investigated by, (Valarmathi *et al.* 2020). Norwegian rats were fed with high fat diet and the hypolipidemic effect of *Mormordica charantia* juice was compared to atorvastatin, a commonly used hypolipidemic drug.

A reduction of serum total cholesterol, low density lipoprotein cholesterol and triglycerides was observed in bitter gourd juice fed group which was similar to those fed with the drug. (Arshad *et al*. 2018), compared the hypolipidemic effect of ethanolic extract of over dried peel, flesh and seeds powder and concluded that bitter melon seed is helpful in controlling the hyperlipidaemia more than other bitter gourd components through rat model. Another such comparative study showed bitter gourd whole fruit powder had the highest hypolipidemic activity, when the bitter gourd skin, flesh and whole fruit powders were fed to rats. They also reported a slight increase in high density lipoprotein, (Kubola & Siriamorn- pun, 2008).

1.5.8 ANTI-MICROBIAL AND ANTHELMINTIC ACTIVITY

Bitter gourd is a folk lore medicine for various skin and stomach ailments owing to anti-microbial activities and the potential of bitter gourd as an antimicrobial agent is proven, (Kole *et al*. 2020), The ethanol extract of leaves of *Mormordica charantia* was found effective against proliferation of *Eschericia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*.

Phytochemistry

Numerous bioactive chemicals in *M. charantia* have only recently been discovered and reported. Terpenoids, tannins, saponins, flavonoids, and steroids were discovered by phytochemical investigations, (Desai et al. 2015).

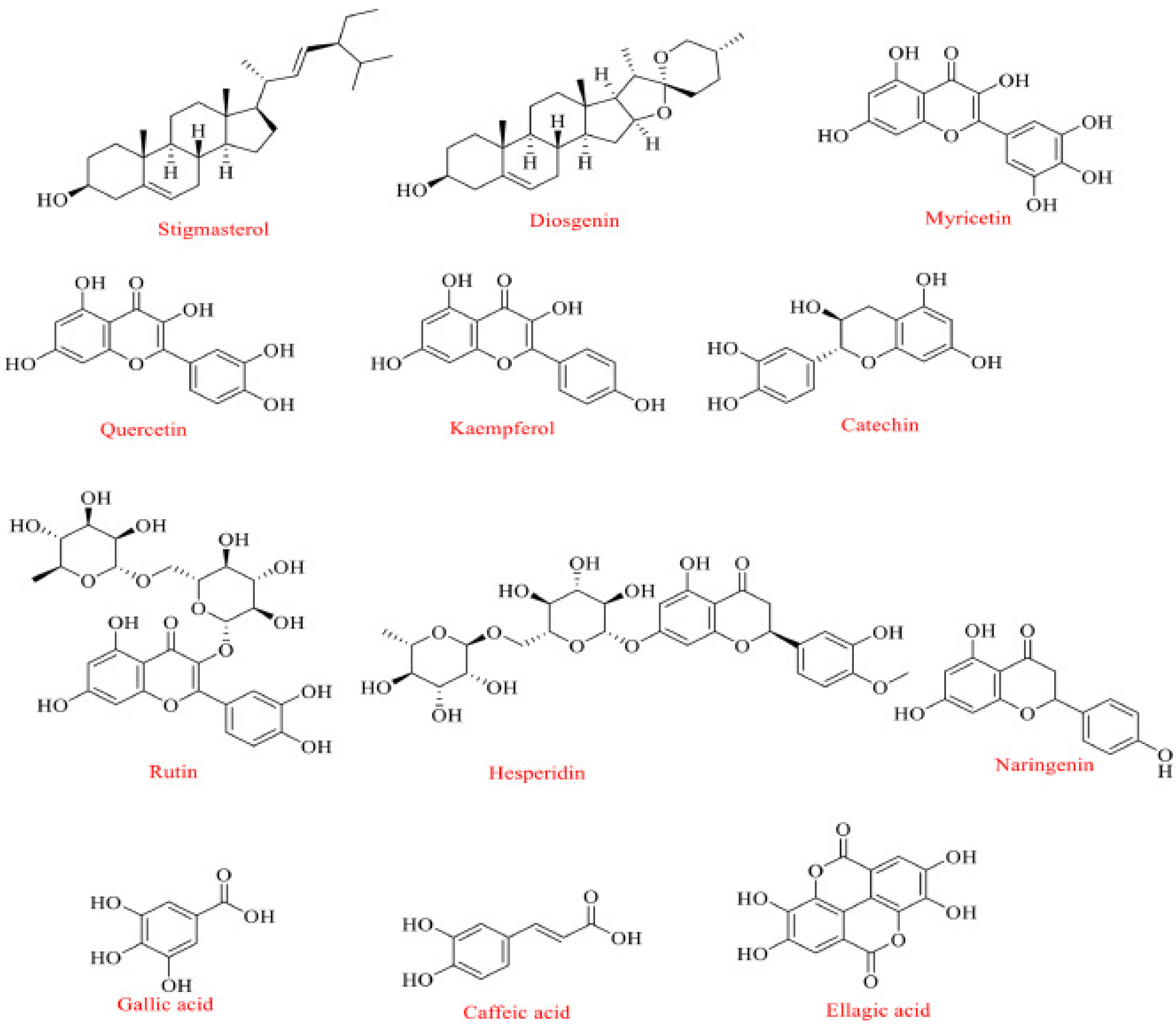

Fig 1.2 Some compounds present in *Mormordica charantia*

1.5 .9 Justification for the Study

Erectile dysfunction (ED) is a common health issue affecting millions of men worldwide, with signific ant impacts on quality of life, relationships, and overall well-being. Despite the availability of various treatments, including phosphodiesterase type 5 inhibitors, vacuum erection devices, and penile implants, many men seek alternative therapies due to concerns about safety, efficacy, and cost, (Thompson et. al, 2005).

1.6.0 AIM AND OBJECTIVES OF THE STUDY

Aim of the study

The aim of this study is to investigate the modulatory effects of ethanol extract and fractions of *Mormordica charantia* on corpus cavernosum smooth muscle of albino wistar rats.

Objectives of the study

The objectives of this study are:

1. To extract and fractionate the leaves of *Mormordica charantia.*

2. To evaluate the relaxant effects of ethanol extract and fractions of Mormordica charantia on corpus cavernosum smooth muscle in vitro.

3. To determine the phytochemical constituents of Mormordica charantia responsible for its smooth muscle modulatory effects on corpus cavernosum.

CHAPTER TWO

2.0 MATERIALS AND METHODS

Materials used in the laboratory are as follows.

2.1.1 PLANT COLLECTION AND PREPARATION

The leaves of *Mormordica charantia* was freshly plucked on the 20th of June, 2024, from a field at the university of Benin teaching hospital. The plant was identified and authenticated at the department of Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, Benin City, Nigeria by Prof. Akinnibosun Henry Adewale. A voucher number was deposited and the number UBH-M617 issued.

The leaves were spread on the work bench in the laboratory for 10 days to allow it to dry properly at room temperature. The dried leaves were then pulverized.

2.1.2 SOLVENTS/REAGENTS USED FOR EXTARCTION

Ethanol, n-hexane, chloroform, ethyl acetate, water.

2.1.3 APPARATUS USED FOR EXTRACTION

Jar bottles, conical flask, beaker, retort stand, fractionating flask, rod, stirrer, porcelain dish, water bath, sample bottle.

2.1.4 EXTRACTION PROCEDURE

A measured weight of 500 g of powdered the plant material (*Mormordica charantia*), was then extracted with 1.5 litres of ethanol using the maceration method. The filtrate from the extraction process was then concentrated using a water bath to give rise to the concentrate which weighed 77.85 g (15.57%).

2.1.5 FRACTIONATION OF THE EXTRACT

The extract (50 g) was dissolved in a mixture of ethanol : water 1:9, in a conical flask. Each of the solvents: n-hexane (470 ml), chloroform (650 ml), ethyl acetate (150 ml), were added to the content of the separating funnel in successive and aliquot amounts of 200 ml to achieve the different solvent fractions as well as the residual aqueous fraction. Each fraction was collected separately and dried in the hot water bath maintained at 40 °C. Dried fractions were weighed and stored in sample glass bottles and then kept in the refrigerator at 4 °C, till further use.

2.2.0 ANIMAL CARE

Eighteen male albino wistar rats with an average weight of 125 g were sourced from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin. The albino wistar rats, were housed in the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin city. They were maintained under standard conditions of humidity (63% ± 5%), temperature (26 – 28 °C), and natural day / night cycle. The rats were housed in ventilated polypropylene cages and provided with pelleted animal feed, (Chikun finisher® pellet) as required. A two – week acclimatization period was given to all before the start of the experiment.

2.3.0 PREPARATION OF STOCK SOLUTION

A stock solution of 1.5625 mg/mL, 3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL and 50 mg/mL was prepared using the crude extract of *mormordica charantia*, residual aqueous fraction of *mormordica charantia*, ethyl acetate fraction, chloroform fraction, n-hexane fraction and sildenafil citrate extract. For the stock solution of 50 mg/mL, 0.050 g was dissolved in 1ml of distilled water, for the stock solution of 25 mg/mL, 0.025 g was dissolved in 1 mL of distilled water. Same method was applied for other concentration for other extracts and fraction too.

2.4.0 PREPARATION OF KREBS SOLUTION (PHYSIOLOGICAL SALINE SALT).

Krebs solution (1000 mL) was prepared by dissolving the following reagents in 1000 mL of distilled water:

a.) Sodium chloride (NaCl) - 6.9 g

b.) Potassium chloride (KCl) - 0.35 g

c.) Calcium chloride (CaCl2) - 0.28 g

d.) Magnesium sulfate (MgSO4) - 0.29 g

e.) Sodium phosphate (NaH2PO4) - 0.16 g

f.) D-Glucose - 2.0 g

2.5.0 PREPARATION OF CORPUS CAVERNOSUM SMOOTH MUSCLE

The male albino wislar rats were sacrificed using the cervical dislocation method. The penis of the rat was dissected to isolate the corpus cavernosum smooth muscle. The smooth muscle was placed in a physiological saline salt before it was then mounted on the organ bath. The organ bath was then calibrated to suit the experiment that was carried out. Volumes of 25 uL, 62.5 uL, 125 uL, 250 uL of various stock solutions (1.5625 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml) for various extracts and fractions were then administered on the corpus cavernosum smooth muscle that was mounted on the organ bath, giving a contact time of four minutes between each administration for volumes 25 uL, 62.5 uL, 125 uL, 250 uL. The corpus cavernosum muscle isolated from one rat was used to test for just one extract/fraction. The test for each extract/fraction was done in triplicate to get the best result. The result of the experiment was then recorded and interpreted.

2.6.0 PHYTOCHEMICAL TESTS

2.6.1 TEST FOR ALKALOIDS

The pulverized Mormordica charantia (0.5 g) was added to a test tube containing about 20ml of water. The mixture was then heated over a water bath for about 15 minutes. The mixture was filtered and the filtrate was tested with alkaloidal reagents. Another 0.5 g of the pulverized plant material was extracted using dilute acid (10% H_2S0_4). The purpose of extracting the powdered plant in dilute acid was to convert the alkaloidal base in the plant to salt.

Dragendoff"s Test: Three drops of Dragendoff"s reagent was added to 2 ml of aqueous extract of *Mormordica charantia* in a test tube and observed for formation of a precipitate and colour change.

Hager's Test: Three drops of Hager's reagent was added to 2ml of aqueous extract of *Mormordica charantia* and observed for the formation of precipitate and colour change.

Mayers Test: Three drops of Mayer's reagent was added to 2ml of aqueous extract of *Mormordica charantia* and observed for the formation of precipitate and a colour change.

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

The same procedure was repeated using the acidic solution of the extract. Observation of the color change was noted and a blank test was carried out for each of the reagents.

2.6.2 TEST FOR SAPONINS.

The powdered plant (0.5 g) was extracted in 2.5 ml of water and was shaken vigorously.

To another test tube, 5 ml of the extract was added, followed by the addition of an equal volume of 90% alcohol and was shaken.

2.6.3 TEST FOR CARBOHYDRATES.

The powdered plant of *Mormordica charantia* (0.2 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 2 ml of concentrated H_2SO_4, with the test tube inclined at an angle of 45°.

2.6.4 TEST FOR ANTHRACENE DERIVATIVES.

The powdered plant material (0.5 g) was extracted in 20 ml of water. The resultant solution was used for the various tests for anthracene derivatives.

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

The aqueous solution of the powdered plant (1 ml) was placed into a test tube. 2.5 ml of 10 % H_2SO_4 was added and heated on a water bath for 5 minutes and then allowed to cool. 5 ml of chloroform was added and shaken gently. About 3 ml of the Chloroform layer was pipetted and shaken gently with 1.5 ml of dilute ammonia solution.

Exactly 2.5 ml of 10% H_2SO_4 and 2.5 ml of H_2O_2 were added to 3 ml of the aqueous extract. The resultant solution was heated on a water bath for about 5 minutes and then allowed to cool. The solution was made up 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.

2.6.5 TEST FOR PHENOLIC COMPOUNDS (TANNINS).

A drop of solution of Ferric chloride was added to 2 mL of the aqueous extract. Iron complex test: About 2 drops of 0.25 % ferric ammonium citrate was added to the extract, followed by the addition of 1 g of sodium acetate, after which the mixture was boiled on a water bath and allowed to cool.

Modified iron complex test: A drop of 33% acetic acid and 1 g of sodium potassium tartrate was added to 5 ml of the extract and then warmed. The mixture was then filtered and washed. The washings were added to the filtrate, after which 0.25 % of ferric ammonium citrate was added to the resultant solution and was boiled for 5 minutes, (*Wadood et al. 2013*).

2.6.5 TEST FOR FLAVONOIDS (Alkaline reagent or NaOH test)

The powdered plant (0.5 g) was extracted in 2 mL of water. To this, three drops of 20 % sodium hydroxide was added. An intense yellow color was formed that turned colorless with the addition of three drops of 20 % hydrochloric acid.

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*).

2.6.6 TEST FOR CYANOGENIC GLYCOSIDES.

Test for Cardiac Glycosides

The powdered plant extract (0.5 g) was extracted with 5 ml of water. About a drop of Ferric chloride was added to 1 ml of glacial acetic acid, and the solution was added to 2 ml of the plant extract in a test tube, followed by the addition of concentrated H2SO4by the side of the test tube without mixing.

2.6.7 TEST FOR REDUCING SUGARS

The powdered plant (0.5 g) was extracted in 5 ml of water. 2 mL of the extract was added to about 4 mL of Fehling's solution A and B and heated for 5 minutes. It was then observed for color change and formation of precipitate.

2.6.8 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 of dichloromethane to obtain a dilute solution. To this solution, 0.5 mL of acetic anhydride was added, followed by the addition of three drops of concentrated sulfuric acid (*Yadav et al.*, 2014).

2.6.9 STATISTICAL ANALYSIS

Graph pad Instant version 2.0.5 software (UK) was used. The values were expressed as Mean ± Standard Error of mean. Statistial analysis was performed by one way analysis of variance (ANOVA), followed by Dunnett comparison tests. P values < 0.05 were considered significantly different from the control.

CHAPTER THREE

3.0 RESULTS

3.1.1 The percentage yield of ethanol extract obtained from 500 g of dried and pulverized leaves of *Mormordica charantia* was 15.57 %.

3.1.2 The weight of *Mormordica charantia* extract that was fractionated was 50 g.

3.1.3 Percentage yield of n-hexane fraction was 19.96 % (9.8 g).

3.1.4 Percentage yield of Chloroform fraction was 27.46 % (13.73 g)

3.1.3 Percentage yield of Ethyl acetate fraction was 5.88 % (2.94 g)

3.1.6 Percentage yield of Residual aqueous fraction was 46 % (23.0 g)

3.2.0 CUMULATIVE EFFECT OF ETHANOL EXTRACT OF MORMORDICA CHARANTIA ON SMOOTH MUSCLE OF CORPUS CAVERNOSUM.

The result obtained in this regard shows that the cumulative doses of *Mormordica charantia* extract produced a dose – dependent decrease in the mean response. This indicates that cumulative dose of the extract caused relaxing effects on the corpus cavernosum smooth muscle. (Figure 3.1)

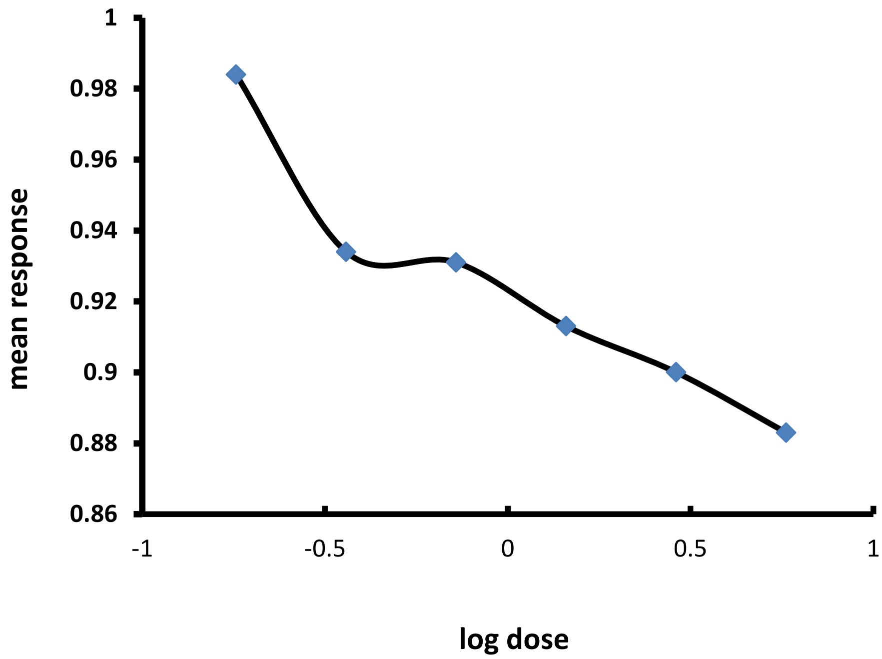

Figure 3.1 Dose – Response curve showing cumulative effect of ethanol extract of *Mormordica charantia* leaves on smooth muscle of corpus cavernosum.

3.2.1 CUMULATIVE EFFECT OF n - HEXANE FRACTION OF *MORMORDICA CHARANTIA* ON SMOOTH MUSCLE OF CORPUS CAVERNOSUM.

The findings indicate that as the cumulative doses of n-hexane fraction of *Momordica charantia* extract increased, the mean response decreased in a dose-dependent manner, suggesting a relaxing effect on the corpus cavernosum smooth muscle. (Figure 3.2)

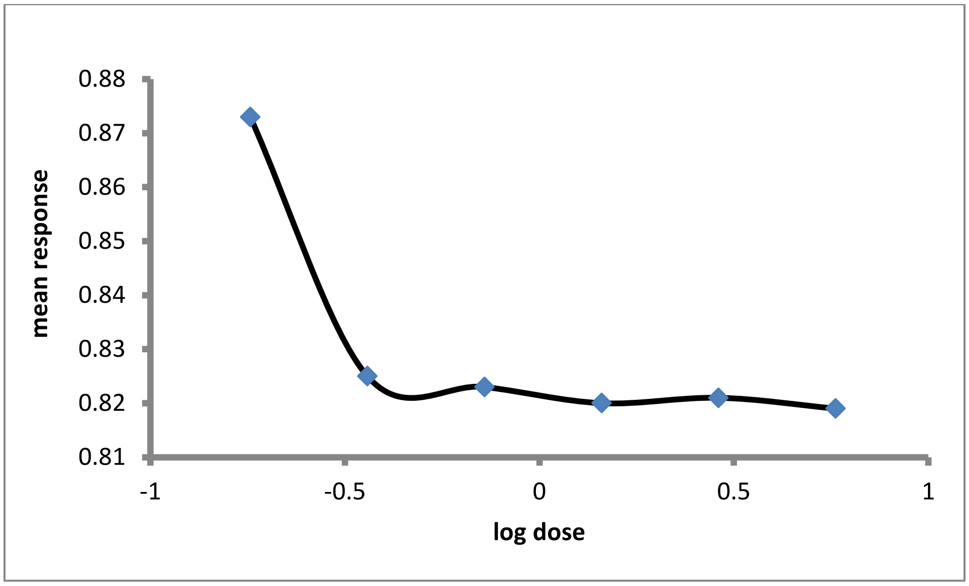

Figure 3.2 Dose – Response curve showing cumulative effect of n – hexane fraction of *Mormordica charantia* leaves on smooth muscle of corpus cavernosum.

3.2.2 CUMULATIVE EFFECT OF CHLOROFORM FRACTION OF *MORMORDICA CHARANTIA* ON SMOOTH MUSCLE OF CORPUS CAVERNOSUM.

The results indicate that higher cumulative doses of chloroform fraction of *Momordica charantia* extract led to a proportional, dose-dependent reduction in the mean response, suggesting that the chloroform fraction exerted a relaxing effect on the corpus cavernosum smooth muscle. (Figure 3.3)

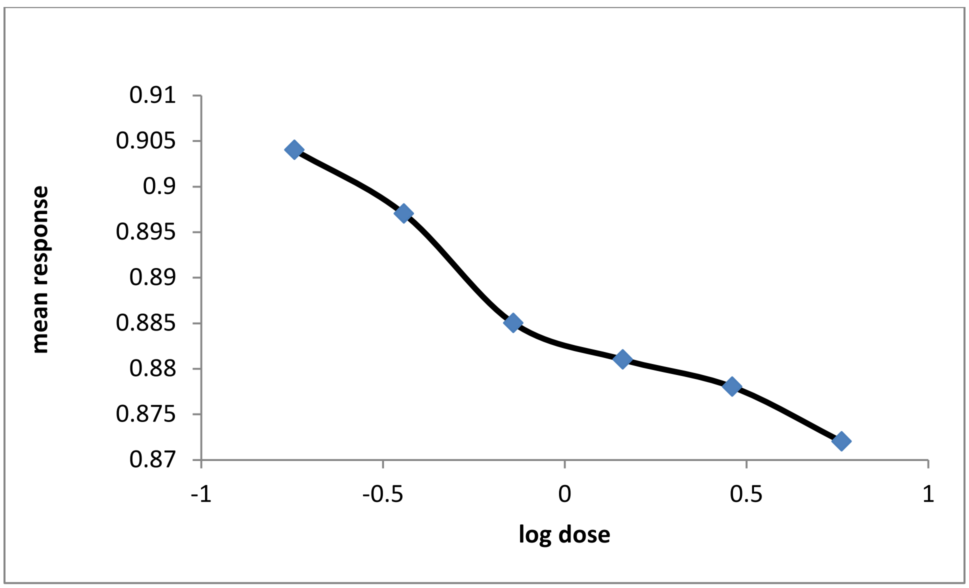

Figure 3.3 Dose – Response curve showing cumulative effect of chloroform fraction of *Mormordica charantia* leaves on smooth muscle of corpus cavernosum.

3.2.3 CUMULATIVE EFFECT OF ETHYL ACETATE FRACTION OF MORMORDICA CHARANTIA ON SMOOTH MUSCLE OF CORPUS CAVERNOSUM.

The findings suggest that as the cumulative doses of ethyl acetate fraction of *Momordica charantia* increased, there was a corresponding dose-dependent decrease in the mean response. This implies that the extract induced a relaxing effect on the corpus cavernosum smooth muscle. (Figure 3.4)

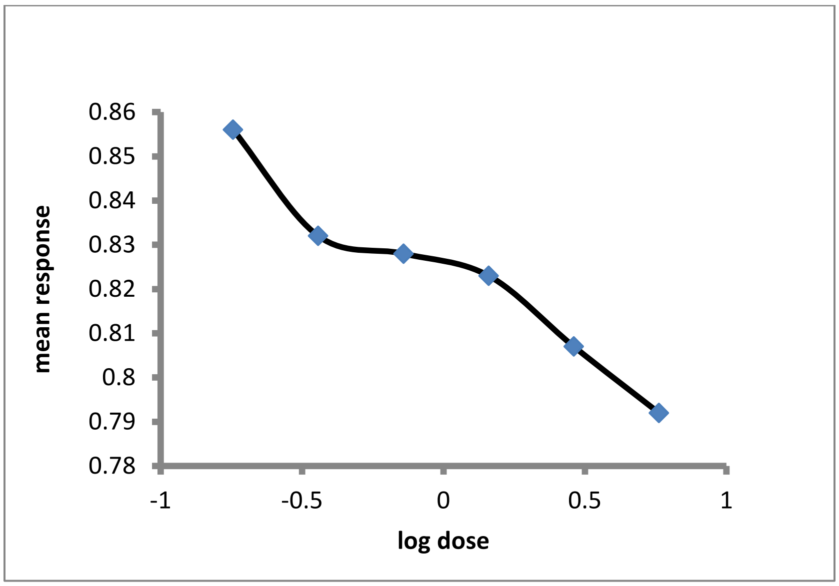

Figure 3.4 Dose – Response curve showing cumulative effect of ethyl acetate fraction of *Mormordica charantia* leaves on smooth muscle of corpus cavernosum.

3.2.4 CUMULATIVE EFFECT OF RESIDUAL AQUEOUS FRACTION OF MORMORDICA CHARANTIA ON SMOOTH MUSCLE OF CORPUS CAVERNOSUM.

The results indicate that increasing cumulative doses of residual aqueous fraction of *Momordica charantia* extract led to a dose-dependent reduction in the mean response. This suggests that the extract exerted a relaxing effect on the corpus cavernosum smooth muscle. (Figure 3.5).

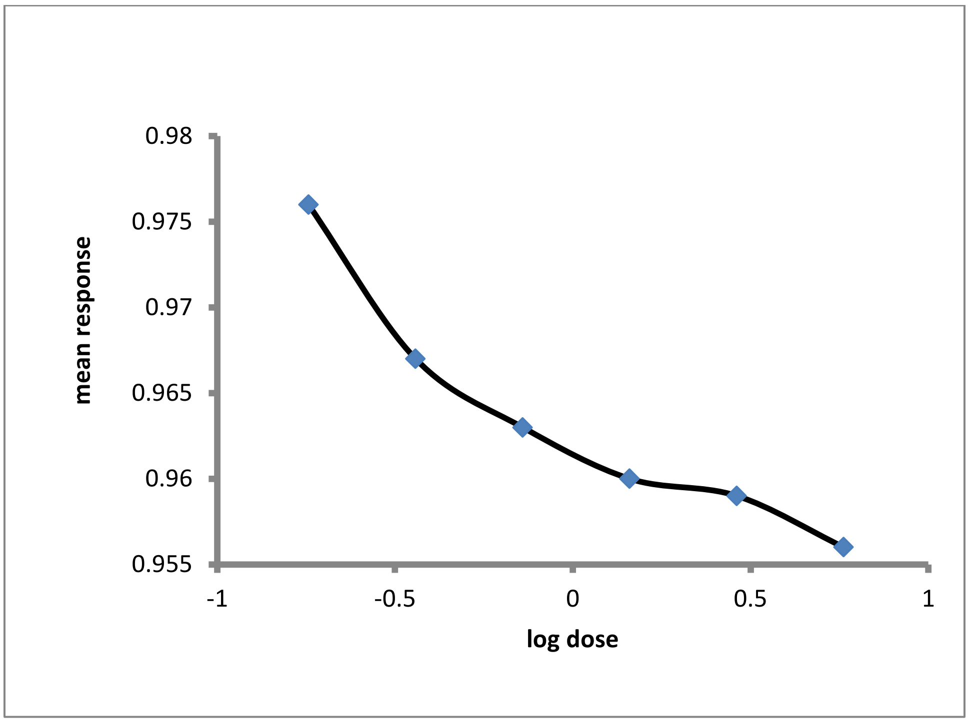

Figure 3.5 Dose – Response curve showing cumulative effect of residual aqueous fraction of *Mormordica charantia* leaves on smooth muscle of corpus cavernosum smooth muscle.

3.2.5 CUMULATIVE EFFECT OF SILDENAFIL EXTRACT.

The findings suggest that higher cumulative doses of sildenafil extract led to a dose-dependent reduction in the mean response, indicating a relaxing effect on the corpus cavernosum smooth muscle. (Figure 3.6)

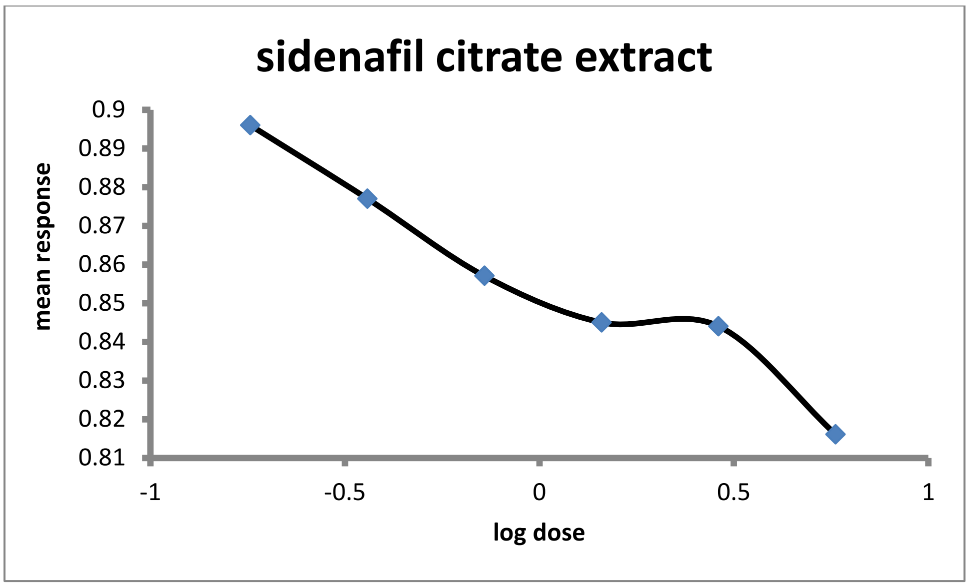

Figure 3.6 Dose – Response curve showing cumulative effect of Sildenafil extract on smooth muscle of corpus cavernosum.

Table 4. EC50 of Extracts and Fractions of *Mormordica charantia*.

EXTRACT / FRACTION	**EC50 Dose (mg)**
Mormordica charantia (crude exrract)	0.661
Residual aqueous fraction	0.363
Ethyl acetate fraction	1.585
Chloroform fraction	0.562
n-Hexane fraction	0.257
Sildenafil extract	0.794

3.3.0 PHYTOCHEMICAL TEST RESULTS.

Table 5. Phytochemical test results carried out for *Mormordica charantia.*

TEST	OBSERVATION	INFERENCE
Alkaloids		
Dragendorff	Reddish-brown precipitate observed	Alkaloidal salt present
Hager	Yellow precipitate observed	Alkaloidal salt present
Mayer	Dark cream colouration with no precipitate observed	Alkaloidal salt absent
Wagner	Dark brown precipitate observed	Alkaloidal salt present
Anthraquinone	Brown coloration observed	Anthraquinone absent
Tannins	Bluish coloration observed	Tannin present
Saponins	Formation of frothing	Saponins present
Reducing sugars	Brick red precipitate observed	Reducing sugar present
Cardiac glycosides	A brick red interface observed	Cardiac glycosides present

Phytochemical evaluation of the leaves of *Mormordica charantia* revealed the presence of Alkaloids, Tannins, Reducing sugars, Saponins, Cardiac glycosides.

CHAPTER FOUR

4.0 DISCUSSION

Momordica charantia, commonly known as bitter melon, is a medicinal plant widely used in traditional medicine for its diverse pharmacological properties, including anti-diabetic, anti-inflammatory, and cardiovascular effects. The study was carried out based on recent claims that *Mormordica charantia* plant had aphrodisicac properties for treating erectile dysfunction (ED), but there was no substantial scientific proof to back up this claim. This study evaluates the smooth muscle modulatory effects of the ethanol extract and different fractions of *Momordica charantia* by analyzing their phytochemical composition and their possible mechanisms of action. The effectiveness of different fractions of *Momordica charantia* in modulating smooth muscle function can be inferred from their EC_{50} values. Lower EC_{50} values indicate higher potency in inducing relaxation. The most potent fraction: n-Hexane fraction *(EC_{50} = 0.257 mg)* suggests strong smooth muscle relaxation effects, possibly through lipophilic bioactive compounds. Residual aqueous fraction (EC_{50} = 0.363 mg): indicates moderate potency, likely due to water-soluble flavonoids and tannins. Chloroform fraction (EC_{50} = 0.562 mg): suggests some degree of activity, potentially through alkaloidal compounds. Crude extract (EC_{50} = 0.661 mg): shows an intermediate effect, which may be due to the combined actions of various phytochemicals. Sildenafil extract (EC_{50} = 0.794 mg): Serves as a comparative standard, known for its PDE5 inhibitory activity. The least potent fraction: ethyl acetate fraction *(*EC_{50} = 1.585 mg) suggests weak activity, possibly due to poor solubility or lower bioavailability of active compounds.

The phytochemical screening of *Momordica charantia* revealed the presence of several bioactive compounds, including alkaloids, tannins, saponins, reducing sugars, and cardiac glycosides. These compounds are known to have various physiological effects that can contribute to smooth muscle relaxation or contraction, influencing erectile function. Alkaloid were detected in Dragendorff, Hager and Wagner tests. Alkaloids may act as phosphodiesterase (PDE) inhibitors or nitric oxide (NO) donors, contributing to vasodilation and smooth muscle relaxation. Tannins known for their antioxidant and vascular-protective properties, tannins may enhance endothelial function, thereby improving nitric oxide-mediated smooth muscle relaxation. Saponins exhibit vasodilatory effects, possibly through NO modulation or direct calcium channel inhibition. The presence of reducing sugars, suggests potential antioxidant activity, which may prevent oxidative stress-induced smooth muscle dysfunction. Cardiac glycosides are associated with cardiovascular effects, these compounds may influence vascular tone through sodium-potassium ATPase modulation. The ability of *Momordica charantia* extracts to influence corpus cavernosum smooth muscle function may be attributed to several mechanisms.

Nitric Oxide (NO)-cGMP Pathway activation. NO is a critical mediator of penile erection, leading to smooth muscle relaxation via cyclic guanosine monophosphate (cGMP) activation. Alkaloids and saponins in *Momordica charantia* may enhance NO production by stimulating endothelial nitric oxide synthase (eNOS). Increased NO levels lead to cGMP accumulation, causing smooth muscle relaxation and increased blood flow to the corpus cavernosum. \

Phosphodiesterase Type 5 (PDE5) Inhibition: Sildenafil, a standard ED drug, works by inhibiting PDE5, which degrades cGMP. The presence of alkaloids suggests that some fractions of *Momordica charantia* may act similarly to sildenafil by inhibiting PDE5, thereby prolonging cGMP-mediated smooth muscle relaxation. The lower EC_{50} values of certain fractions (n-Hexane and aqueous) compared to sildenafil suggest potential competitive inhibition.

Calcium Channel Blockade (CCB) and Smooth Muscle Relaxation: Tannins and saponins are known to inhibit calcium influx, preventing smooth muscle contraction. The presence of cardiac glycosides suggests Na^+/K^+-ATPase modulation, which may indirectly reduce intracellular calcium levels, promoting relaxation.

Antioxidant and Endothelial Protective Effects: Oxidative stress impairs NO bioavailability and contributes to erectile dysfunction. The presence of tannins and reducing sugars suggests significant antioxidant activity, protecting endothelial function and enhancing NO-mediated vasodilation.

Adrenergic Receptor Modulation: Catecholamine (norepinephrine) release contributes to corpus cavernosum contraction. Some alkaloids can act as adrenergic blockers, reducing sympathetic-induced vasoconstriction and facilitating erection.

Comparative Analysis with Sildenafil: while sildenafil directly inhibits PDE5, *Momordica charantia* fractions may exert broader effects, including NO release enhancement and antioxidant activity. This suggests a multi-target mechanism that could be beneficial for individuals with ED linked to oxidative stress, endothelial dysfunction, or neurogenic causes.

Potential Applications and Future Research Directions:

Alternative ED Treatment: Given the low EC_{50} values of certain fractions, *Momordica charantia* could serve as a natural alternative or adjunct to PDE5 inhibitors.

Vascular Health: Its endothelial protective effects could extend to cardiovascular disease management.

Clinical Trials: Further studies are needed to confirm its efficacy and safety in human subjects, including pharmacokinetic profiling and toxicological assessments.

Bioactive Compound Isolation: Identifying the specific molecules responsible for smooth muscle relaxation could lead to novel drug discoveries.

4.1 CONCLUSION

The ethanol extract and fractions of *Momordica charantia* exhibit significant corpus cavernosum smooth muscle modulatory effects through multiple mechanisms, including NO-cGMP pathway activation, PDE5 inhibition, calcium channel blockade, and antioxidant effects. The n-Hexane and aqueous fractions demonstrated the highest potency, suggesting their potential as natural therapeutic agents for erectile dysfunction. Future studies should focus on clinical validation and bioactive compound isolation to further explore its pharmacological potential.

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APPENDIX 1

Table 1. MEAN RESPONSE FOR DATA OBTAINED FOR ALL EXTRACTS AND FRACTIONS

MC-CE	RA-F	EA-F	C-F	Nh-F	S-E	$(X1-\bar{X}1)^2$	$(X2-\bar{X}2)^2$	$(X3-\bar{X}3)^2$	$(X4-\bar{X}4)^2$	$(X5-\bar{X}5)^2$	$(X6-\bar{X}6)^2$	
0.984	0.976	0.856	0.904	0.873	0.896	0.0036	0.000144	0.001089	0.000324	0.0044	0.000289	
0.934	0.967	0.832	0.897	0.825	0.877	0.0001	0.0174	0.001089	0.000121	0.000025	0.000441	
0.931	0.963	0.828	0.885	0.823	0.857	0.000049	0.000001	0.000025	0.000001	0.00049	0.000001	
0.913	0.96	0.823	0.881	0.821	0.845	0.000121	0.002601	0	0.000025	0.000081	0.000081	
0.9	0.959	0.807	0.878	0.82	0.844	0.000576	0.004096	0.000256	0.0044	0.0001	0.0001	
0.883	0.956	0.792	0.872	0.819	0.816	0.001681	0.000064	0.000961	0.000196	0.000121	0.000121	
						0.006127	0.0244	0.00241	0.005067	0.00477	0.007603	∑X
6	6	6	6	6	6							ng
						36						G
0.924	0.96	0.82	0.88	0.83	0.85							$\bar{X}$

	4	3	6		6							g
						0.88						X̄ G
						6						k

$p < 0.0.5$

Table 4 Key:

MC-CE: *mormordica charantia* crude extract

RA-F : residual aqueous fraction

EA-F : ethyl acetate fraction

C-F: chloroform fraction

Nh-F : n-hexane fraction

S-E : Sidenafil extract.

ng : number in each group

G : total number in each group

X̄g : mean of each group

X̄G : grand mean

APPENDIX 2:

k : total number of groups

MEAN RESPONSE CALCULATION FOR DATA OBTAINED FOR ALL EXTRACTS AND FRACTIONS USING ONE-WAY ANOVA

Ho : $M1 = M2 = M3 = M4 = M5 = M6$ (null hypothesis)

H1 : $M1 \neq M2 \neq M3 \neq M4 = M5 \neq M6$ (alternate hypothesis)

Using $\alpha = 0.05$

$F = MSS_B/MSS_E$

Where F : F statistics

MSS_B = mean sum of squares between the group

MSS_W = mean sum of squares within the group

$MSS_B = \sum g_{EG}\ ng\ (\bar{X}g - \bar{X}_G)^2 / k - 1$

$MSS_W = \sum g_{EG}\ (X - \bar{X}_G)^2 / n - k$

$\sum$Geg : sum of value of response within the group, for each number of group

X : response value in each group

$\bar{X}$: mean response for each group

n : total number of variables

APPENDIX 3

k : total number of groups.

n1 = $/\bar{X}_1 - \bar{X}_G/$ = 6 (0.924 – 0.880) ² = 6 X 0.001936 = 0.011616

n2 = $/\bar{X}_2 - \bar{X}_G/$ = 6 (0.964 – 0.880) ²= 6 X 0.007056 = 0.0423

n3 = $/\bar{X}_3 - \bar{X}_G/$ = 6 (0.823 – 0.880) ² = 6 X 0.001936 = 0.0195

n4 = $/\bar{X}_4 - \bar{X}_G/$ = 6 (0.886 – 0.880) ² = 6 X 0.000036 = 0.000216

n5 = $/\bar{X}_5 - \bar{X}_G/$ = 6 (0.830 – 0.880) ² = 6 X 0.0025 = 0.015

n6 = $/\bar{X}_6 - \bar{X}_G/$ = 6 (0.856 – 0.880) ² = 6 X 0.000576 = 0.003456

n1 + n2 + n3 + n4 + n5 + n6 = 0.011616 + 0.0423 + 0.0195 + 0.000216 + 0.015 + 0.003456 = 0.09208.

$MSS_B = \sum g_{EG}\, ng\, (\bar{X}g - \bar{X}_G)^2 / k - 1$ = 0.0928 / K-1 = 0.0928 / 6 – 1 = 0.09208 / 5 = 0.0184

MSS_B = 0.0184

$MSS_{W} = \sum g_{EG} (X - \bar{X}_G)^2 / n - k$

$\sum g_{EG} (X - \bar{X}_G)^2$ = 0.006127 + 0.0244 + 0.00241 + 0.005067 + 0.00477 + 0.007603 = 0.050377

MSS_W = 0.050377 / 36 – 6 = 0.050377/30 = 0.00168

APPENDIX 4;

$F = MSS_B/MSS_E = 0.0184 / 0.00168 = 10.9523.$

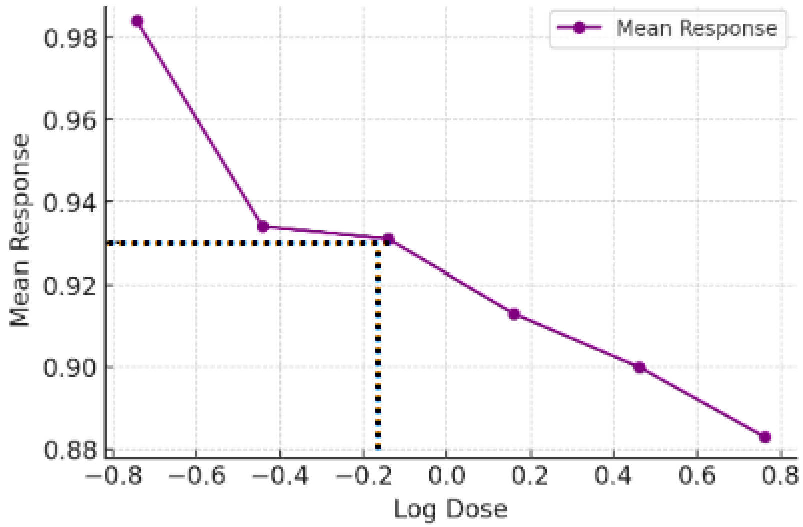


Fig. 1a Sigmoid dose response curve for *Mormordica charantia* crude extract

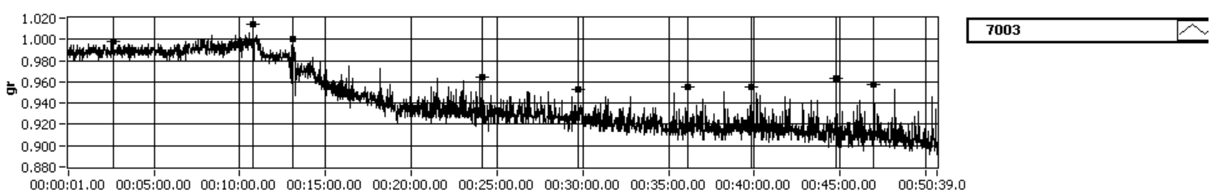


Fig. 1b Isometric recording for *Mormordica charantia* crude extract

APPENDIX 5:

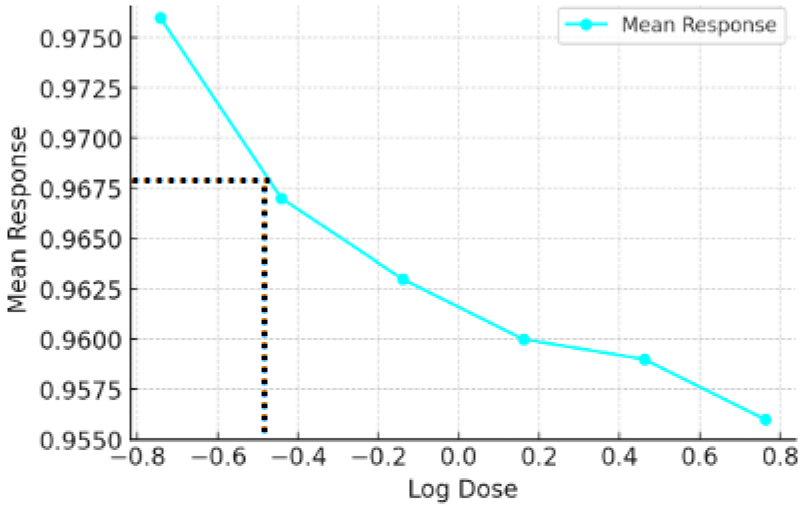


Fig. 2a Sigmoid dose response curve for residual aqueous fraction

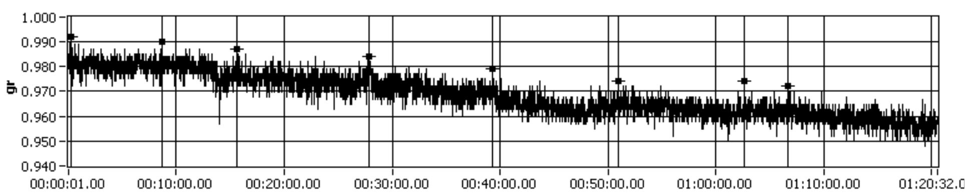
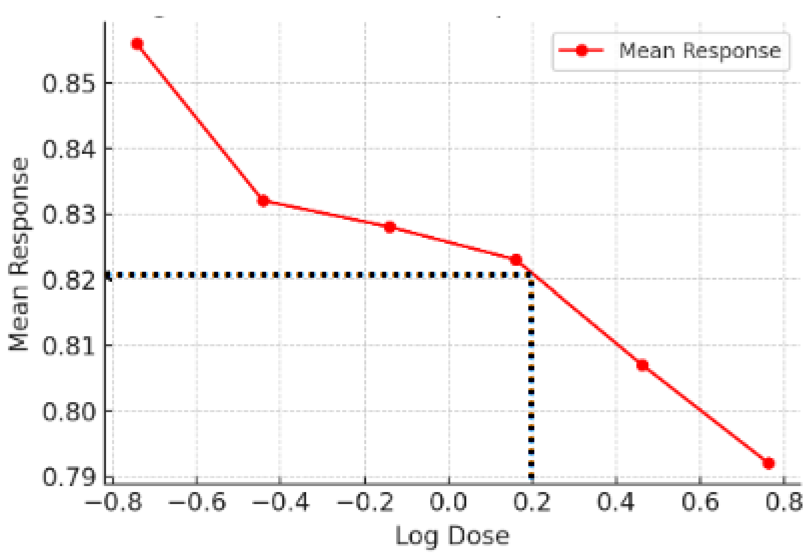


Fig. 2b Isometric recording for residual aqueous extract



APPENDIX 6:

Fig. 3a Sigmoid dose response curve for ethyl acetate fraction

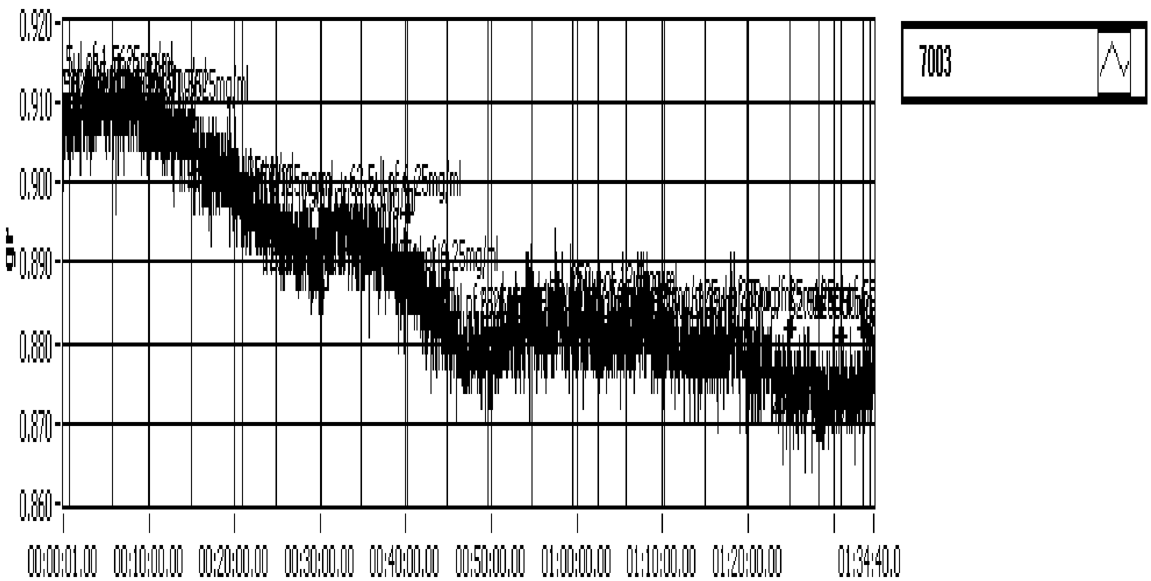


Fig. 3b Isometric recording forethyl acetate fraction

APPENDIX 7:

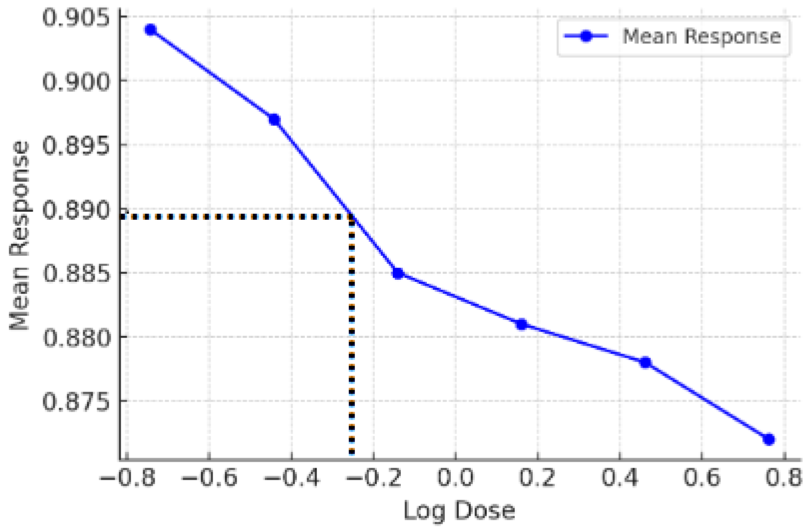


Fig. 4a Sigmoid dose response curve for chloroform fraction

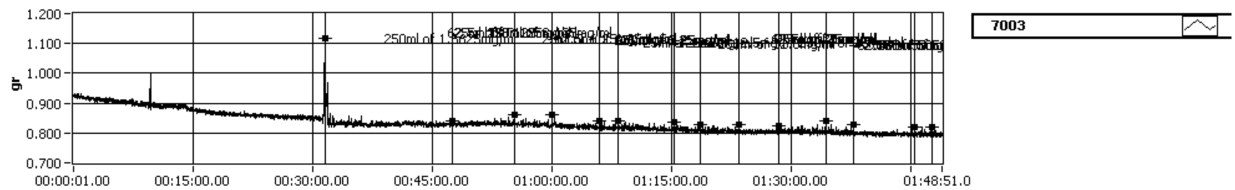


Fig. 4b Isometric recording for chloroform fraction

APPENDIX 8:

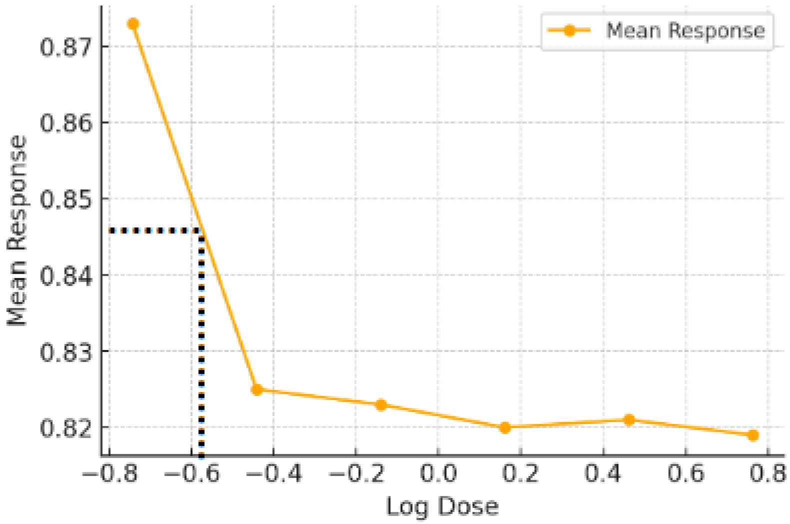


Fig. 5a Sigmoid dose response curve for n-hexane fraction

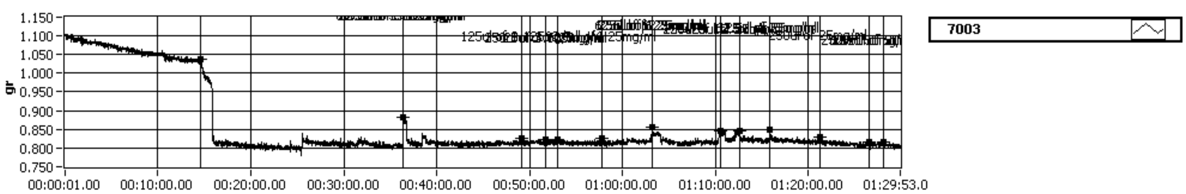


Fig. 5b Isometric recording for n – hexane fraction

APPENDIX 9:

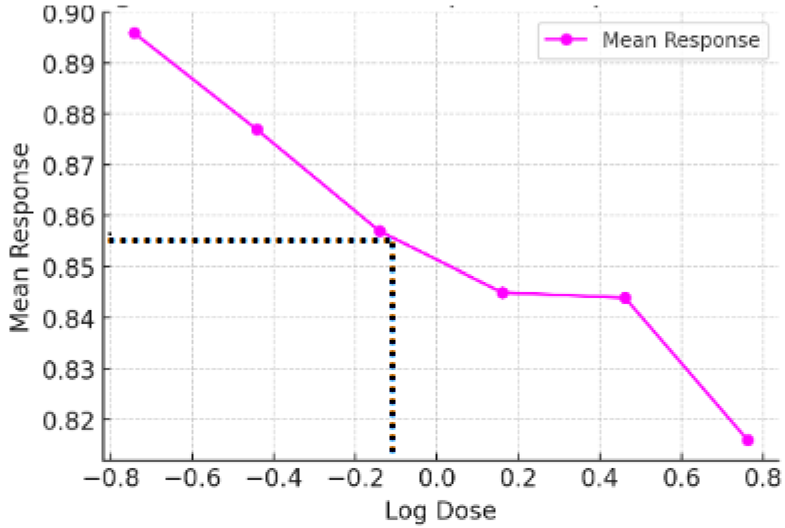


Fig. 6a Sigmoid dose response curve for sildenafil fraction

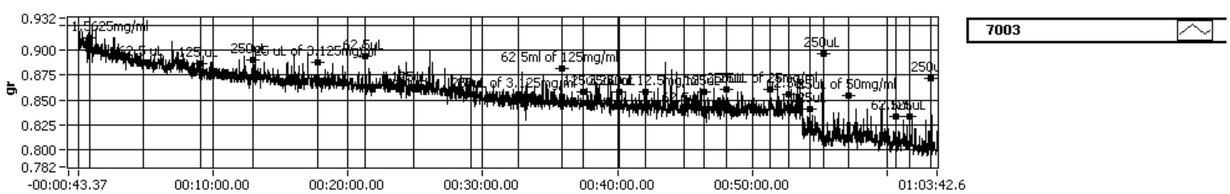


Fig. 6b Isometric recording for sildenafil fraction

APPENDIX 10:

3.2.0 EC50 Doses of Different Extracts and Fractions of *Mormordica charantia*

MORMORDICA CHARANTIA (CRUDE EXTRACT)

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.93).
- The vertical line intersects the log dose axis at approximately -0.18

Thus, the EC50 log dose is around **-**0.18

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 0.661 mg.

RESIDUAL AQUEOUS FRACTION

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.97).

APPENDIX 11:

- The vertical line intersects the log dose axis at approximately -0.44

Thus, the EC50 log dose is around -0.44

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 0.363 mg

ETHYL ACETATE

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.82).
- The vertical line intersects the log dose axis at approximately 0.20.

Thus, the EC50 log dose is around 0.20.

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 1.585 mg

CHLOROFORM

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

APPENDIX 12:

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.89).
- The vertical line intersects the log dose axis at approximately -0.25

Thus, the EC50 log dose is around **-**0.25

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 0.562 mg

N-HEXANE FRACTION

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.85).
- The vertical line intersects the log dose axis at approximately -0.59

Thus, the EC50 log dose is around **-**0.59

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 0.257 mg

APPENDIX 13:

SILDENAFIL EXTRACT

From the graph, the EC50 value corresponds to the log dose at which the mean response reaches 50% of its maximum effect.

Observing the black dotted lines in the image:

- The horizontal line marks the EC50 response level (~0.85).
- The vertical line intersects the log dose axis at approximately -0.1

Thus, the EC50 log dose is around **-**0.1

Actual Dose = $10^{\text{Log Dose}}$

The estimated EC50 dose is approximately 0.794 mg