DETERMINATION OF PHYTOCONSTITUENTS OF TWO HERBAL PRODUCTS (LONG JACK (*EURYCOMA LONGIFOLIA*) AND SPERM BOOM SOLD IN NIGERIAN MARKETS

BY

EGUN HOPE NDIDI

MATRIC NO: BMS1802445

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCE,

UNIVERSITY OF BENIN,

BENIN CITY.

APRIL, 2024

DETERMINATION OF PHYTOCONSTITUENTS OF TWO HERBAL PRODUCTS (LONG JACK (*EURYCOMA LONGIFOLIA*) AND SPERM BOOM SOLD IN NIGERIAN MARKETS

BY

EGUN HOPE NDIDI

MATRIC NO: BMS1802445

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN

THIS PROJECT IS SUBMITTED TO THE:

DEPARTMENT OF MEDICAL LABORATORY SCIENCES,

UNIVERSITY OF BENIN

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

SUPERVISED BY PROF M. A. EMOKPAE

APRIL, 2024

CERTIFICATION

This is to certify that this work was carried out by **EGUN HOPE NDIDI** with matriculation number **BMS1802445**,under the supervision of Prof. M.A Emokpae, is being submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree.

_____ _____

PROF. M. A. EMOKPAE **DATE**

Supervisor

_____ _____

DR (MRS) Z. OMORUYI **DATE**

Head of Department

_____ _____

EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to God Almighty for his love, grace, wisdom and knowledge he bestowed upon me throughout my stay in University of Benin.

AKNOWLEDGEMENTS

I give thanks to JEHOVAH GOD the great teacher, who guided me throughout all the stages of my academic pursuit. My profound gratitude goes to my supervisor PROF. M.A EMOKPAE, who through his vast experience and wealth of knowledge painstakingly guide me in the course of this work. Special thanks go to the Acting Head of Department, Medical Laboratory Science DR (MRS) Z OMORUYI for her concern, constructive and supportive idea which aided this project work. I sincerely appreciate the whole hearted cooperation and valuable help rendered by the entire staff of the Department of Medical Laboratory Science. My appreciation also goes to my co-supervisor DR.OSAMUYI UWUMARONGIE for his guidance in the course of accomplishing this work

I sincerely appreciate my parents, MR & MRS SUNDAY EGUN for their continued support and love. I also want to appreciate my lovely sister MRS BLESSING COLLINS and MR CLEMENT OSAHON for their never ending assistance.

I am indeed grateful to my colleagues. You all are my heroes.

TABLE OF CONTENTS

Title page	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Table of content	vi
List of tables	ix
List of figures	x
Abstract	xi
CHAPTER ONE	**1**
1.0 Introduction	1
1.1 Background of Study	1
1.2 Statement of problem	4
1.3 Justification of Study	5
1.4 Aim of Study	5
1.4.1Specific Objectives	5
1.5Research Question	6
1.6Research Hypothesis	6
CHAPTER TWO	**8**
2.0 Literature Review	8
2.1 Phytoconstituents	8

2.1.1 Alkaloids 8

2.1.3 Terpenoids 10

2.2 Herbal Products 12

2.2.1 Significance of herbal products 12

2.3 Long Jack XXXL 12

2.3.1 Tongkat Ali 13

2.3.2 Maca extract 14

2.3.3 Fenugreek extract 15

2.3.4 Siberian Ginseng 17

2.3.5 Horny Goat weed 18

2.3.6 Bioactive compounds in Long Jack (*Eurycoma longifolia)* *18*

2.3.7 Toxic effect of Long Jack 21

2.4 Sperm Boom 22

2.4.1 *Sesamum radiatum* *23*

2.4.2 *Cissus populnea* *24*

2.4.3 *Lophira lanceolata* *26*

2.4.4 Bioactive compounds in Sperm Boom (*Lepidium meyenii*) 28

2.4.5 Toxic effect of Maca roots 28

CHAPTER THREE **29**

3.0 Materials and Method 29

3.1 Study design 29

3.2 Data collection 29

3.3 Sample collection 29

3.4 Sample Analysis 29

3.5 High performance liquid chromatography 30

3.5.1 HPLC principle 30

3.5.2 Procedure of HPLC analysis for Long Jack XXXL (*Eurycoma longifolia*) 31

CHAPTER FOUR **32**

4.0 Results 32

CHAPTER FIVE **36**

5.0 Discussion 36

5.1 Conclusion 39

REFERENCE 40

LIST OF TABLES

Table 4.1: Identification of the bioactive compound in Long Jack XXXL (*Eurycoma longifolia*)

Table 4.2 : Identification of bioactive compound in Sperm Boom(*Lepidium meyenii*)

LIST OF FIGURES

Figure 1: Classes of various Alkaloids

Figure 2 : The basic skeleton of flavonoids and their various classes

Figure 3: classes of Terpenoids

Figure 4: Chemical structures of various biological active constituents from *E.longifolia*

Figure 5: Phytoconstituent of maca root

ABSTRACT

Herbal products, derived from plant sources, have been integrated into traditional medicine systems and are gaining widespread popularity in contemporary health practices. This study seeks to examine the phyto-chemical compositions of Long Jack and Sperm Boom herbal products sold in Nigerian markets. This is important to identify the presence of synthetic adulterant in the products. The herbal products were dissolved in appropriate diluents and analyzed using High Performance Liquid Chromatographic technique with Ultra Violet radiation. Long Jack contains twelve phyto-chemical constituents (ppm) which are: Eurycomanone (255.3), Canthin-6-one (130.9), Niloticin (66.8), Scopoletin (266.7), Eurylene (98.4), Eurycomalide A(209.2), Pasakbumin A(466.4), Pasakbumin C (103.0), Longilactone (133.0), 4-hydroxyglaucarubol (74.3), Laurycolactone (116.1) and Beta-Carboline (136.9). Maca sperm boom contains eleven phyto-chemical constituents (ppm): Macaene (31.4), Macaridine (222.3), Gingerol (104.4), Beta-Sitosterol (197.4), Stigmasterol (248.8), Testosterone Decanoate (36.0), Paraxytriol (56.2), Rotundifolioside (56.0), Lanatoside (63.3), Kuwanon G (64.2) and Laricitrin (80.2). No synthetic chemical substance was identified using this method.These identified bioactive compounds are associated with various health benefits, such as enhancing sexual stamina, anti-inflammatory, antioxidant, antimicrobial, and adaptogenic properties. Quality assurance measures, including analytical techniques like High-Performance Liquid Chromatography (HPLC), are crucial in ensuring the authenticity and safety of herbal products. Understanding their phytoconstituent composition and implementing robust quality control measures are imperative for ensuring efficacy and safety.

CHAPTER ONE

1.0 INTRODUCTION

1.1Background of Study

According to a global survey published by the World Health Organization (WHO) in 2018, 88% of all member states acknowledged the use of complementary and alternative medicine. Approximately 75% of Nigerians use traditional medicine (Ohemu *et al.,* 2023).This is due to the fact that in most places the access to modern medicine is limited, therefore traditional medicine serves as the primary form of care (Guler *et al.,* 2021).Because of this, more attention should be paid on the use of local and natural resources as pharmaceutical raw materials to create novel medications.

It has been suggested that Long Jack (*Eurycoma longifolia*) and sperm boom improves male sexual health. In Southeast Asian traditional these herbal medicines are widely used and recognized for their aphrodisiac properties. This is because these plant species are found in parts of Thailand, Cambodia, and Myanmar. In Malaysia, this plant is often called Tongkat Ali, which translates to "walking stick." Some call it tho nan (Laotian), Ian-don (Thailand), Cay ba benh (Vietnam), Pasak Bumi or Bedara Pahit (Indonesia), and Ali's Umbrella in Malaysia (Rehman *et al.,* 2016). Traditional medicine uses the root extract of *Eurycoma longifolia* (Long Jack) and sperm boom (maca root) plant to raise men's testosterone levels. Males who routinely take the herbal supplement were reported to expand their muscle mass and strength for physical body building and these have made these herbal drug to become more popular. Others use the plant (roots) in native traditional medicine as aphrodisiac, antipyretic, cytotoxic, antimalarial, and antiulcer effects (Bhat *et al*.,2010). According to Talbott *et al.,* (2013), *Eurycoma longifolia* is incorporated in contemporary dietary supplements to support weight loss and athletic

performance, boost libido and energy, and balance hormone levels especially testosterone and cortisol. A number of human clinical trials and in vivo animal research conducted recently have documented the potential benefits of Long Jack in the treatment of a range of male sexual diseases including poor libido, male infertility, erectile dysfunction, and downregulated testosterone levels (Thu *et al.,* 2017). Numerous bioactive substances from the canthin-6-one alkaloids, tirucallane triterpenes, squalene derivatives, quassinoids, and bioactive steroid families are suggested to have the stated effects. A wide array of phytoconstituents has been isolated and characterized from *E. longifolia*, particularly from the roots, and these include β-carboline alkaloids, canthin-6-one alkaloids, quassinoids, quassinoid diterpenoids, squalene derivatives, tirucallane-type triterpenes, biphenylneolignans, laurycolactone, eurycolactone, and eurycomalactone. (Rehman *et al.,* 2016). The most predominant of these phytoconstituents was quassinoids which was thought to acount for a large portion of *E. longifolia* root phytochemicals. Among these constituents, eurycomanone was the major quassinoid reported in *E. longifolia* extract accounting to a large extent for its health effects.

These herbal products are recognized as natural and mostly preferred over synthetic alternatives due to the belief that there are safe with little to no adverse effects by producers. Unfortunately, some have been reported to contain high rate of synthetic adulterants which may compromises their safety (Akuomoa *et al.,* 2022). It is of public health interest to identify the presence of adulterants in herbal medicines sold in Nigerian markets. Since some authors have demonstrated that most herbal aphrodisiacs sold in the markets are adulterated with either approved synthetic phosphodiesterase-5 inhibitors (PDE-5i) or their unapproved analogues (Bujang *et al.,* 2017). The use of approved PDE-5i strictly based upon medical recommendation with appropriate monitoring. Their use as adulterants in herbal products is not only illegal but a public health

concerns. This is particularly worrisome because of the presence of unapproved synthetic analogues (European Medicines Agency 2008). Studies have revealed that these analogues may perform similar function as the parent drug, they may have different chemical structures that may change their pharmacokinetic and pharmacodynamic properties as well as their toxicity (Akuamoa *et al.,* 2022). The safety and efficacy of the herbal product which may depend to a great extent on the concentration of their bioactive constituents is very much desirable. This may suggest the importance of using available analytical tools to determine the accurate quality control and adulteration detection for the herbal product. Despite the fact that the plant is utilized for a variety of medicinal purposes, the market is flooded with goods that have *E. longifolia* as their primary constituent. They are found in a variety of commercial goods, such as a powdered dark brown beverage called coffee flavored with *E. longifolia*. Additionally, Physta was the first *E. longifolia* extract to be patented in the US; it is also referred to as Long Jack (LJ100). It is a freeze-dried extract powder from roots that maintains the nutrients and inhibits deterioration during extraction (Rehman *et al.,* 2016; Farag *et al.,* 2023). It has been found to deliver the highest level of bioactive chemicals from the root, with maximal potency, safety, and health benefits (Akarali, 2021). It is said to contain 1.5% eurycomanone and provide the greatest concentration of bioactive compounds from the root, ensuring optimal potency, safety, and health advantages (Akarali, 2021). At a recommended daily dosage of one to two tablets, a comparable pharmaceutical containing 300 mg extract in tablet form is used to improve male fertility and testosterone levels, as well as to relieve stress, increase energy and muscle strength, and improve athletic performance (Farag *et al.,* 2022).

Maca (Lepidium meyenii) is a plant native to high Andes of Peru, known for its edible root (Zhou et al.,2017). While traditional uses of maca include is proported role as a fertility enhancer

and aphrodisiac properties (Uchiyama et al.,2014).Maca roots and leaves contains a diverse arrays of compound with both pharmacological and nutritional importance including non-starch polysaccharides, polyphenol such as flavonoligians, malamedas, macaenes, macamides, glucosinolates and macahydantoins, alkaloids,sterols (Carvalho *et al*.,2019) Modern infertility is a public health concern that can be viewed as a psychological crisis due to the hardship it places on infertile couples. It can pose a serious risk to mental health, which could ultimately result in domestic violence, divorce, and marital problems (Rahebi *et al.,* 2019; Shayesteh-Parto et al., 2023). The use of herbal products for the management of male infertility is increasing popular also, since its prevalence varies depending on the location in Nigeria, which may up to 45% in some parts of Nigeria (Uadia and Emokpae, 2015).

1.2Statement of Problem

In order for a man to procreate, he must be able to mate and ejaculate the semen into the birth canal of a healthy female partner. He must also be able to generate a good quantity and quality of semen. The primary causes of male infertility are anomalies in semen properties, such as low spermatozoa concentration, low sperm motility, or aberrant morphology (Kumar and Singh, 2015). Additional reasons include varicocele, genital duct blockage, genetic abnormalities, male impotence, and STDs (Idu *et al.,* 2016). It has been observed by certain authors that 2% of men may have inadequate semen parameters (Kumar and Singh, 2015). There are treatments for male infertility that can increase fertility, including surgery, medications (both synthetic and herbal), and laboratory procedures. Nevertheless, the safety of some herbal products is frequently jeopardized by the high rate of adulteration. Additionally, these treatment alternatives come with substantial side effects, are frequently difficult to get, are expensive and beyond the means of the bulk of the general people (Njagi *et al.,* 2020; Sharma and Shrivastava, 2022). The use of natural

herbal supplements has become more widespread as a result of the shortcomings of the current treatment. The majority of consumers of these herbal remedies are mostly unaware of the toxicity, phytoconstituents, and other potential hazards they may contain. While elevated PDE-5i levels in natural remedies or supplements might not be fatal, but it's possible that the effects of these substances will be amplified if there are pre-existing medical disorders like diabetes or hypertension, which might even result in death. The purpose of this study was to determine the phytoconstituents of sperm boom and Long Jack that are sold in Nigerian markets.

1.3Justification of Study

It is crucial for customers to understand the phytoconstituents of herbal goods. This is especially crucial since, in a blatant instance of dishonesty, some manufacturers of these herbal products might add other synthetic products as adulterants in order to increase their effectiveness. The situation become worse when PDE-5 inhibition is comparatively increased with unapproved synthetic analogs as adulterants. Consuming large amounts of these adulterants can be harmful to one's health. The study's findings could educate the public about the need of exercising caution when utilizing herbal products.

1.4Aim of Study

To determine the phytoconstituents of the herbal products (Long Jack (*Eurycoma Longifolia*) and Sperm Boom) sold in Nigerian markets.

1.4.1 Specific Objectives

The specific objectives of the study are to

1. determine the phytoconstituents of Long Jack XXXL and Sperm Boom herbal products sold in Nigerian market

2. determine the concentrations of aphrodisiac contents of the herbal products

3. examine whether other synthetic products were added to the herbal products as adulterants

1.5Research Questions

1. What phytoconstituents are present in Long Jack XXXL and Sperm Boom herbal products sold in Nigerian market?

2. What are the concentrations of aphrodisiac contents in the herbal products?

3. Are synthetic compounds added to the herbal products as adulterants?

1.6 Hypothesis

1.6.1 Null Hypothesis

1. Long Jack XXXL and Sperm Boom herbal products sold in Nigerian market do not contain unknown phytochemical constituents

2. The concentrations of aphrodisiac in the herbal products are low

3. The Long Jack XXXL and Sperm Boom do not contain synthetic compounds as adulterants

1.6.2 Alternate Hypothesis

1. Long Jack and Sperm Boom herbal products sold in Nigerian market contain known phytochemical constituents

2. The concentrations of aphrodisiac in the herbal products are high

3. The Long Jack XXXL and Sperm Boom do contain synthetic compounds as adulterants

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Phytoconstituents

Definition: Phytoconstituents are chemical compounds found in plants that contribute to their medicinal properties. These constituents can include various classes of compound such as alkaloid, flavonoids, terpenoid and phenolic compounds. Phytoconstituents are responsible for the therapeutic effects of many medicinal plant are often studied for their potential pharmacological benefits.

2.1.1Alkaloid

Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. Alkaloids are mainly biosynthetically derived from amino acids resulting in variety of chemical structures, mostly isolated from plants (Heinrich *et al.,* 2021) Alkaloids can be found in about 20% of plant species in small qualities (Srivastava and Srivastava 2013) and their production (including in biotechnology), extraction and processing remain major areas of research and development (Heinrich *et al.*,2021). Alkaloid biosynthetic pathways can be manipulated genetically for example in order to achieve higher production levels of alkaloids (Mah *et al.*,2021). In fact, as many as one-quarter of higher plants are estimated to contain alkaloids, of which several thousand different types have been identified**.** Alkaloids are often classified on the basis of their chemical structure. For example, those alkaloids that contain a ring system called indole are known as indole alkaloids. On this basis, the principal classes of alkaloids are the pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, and the terpenoids and steroids.

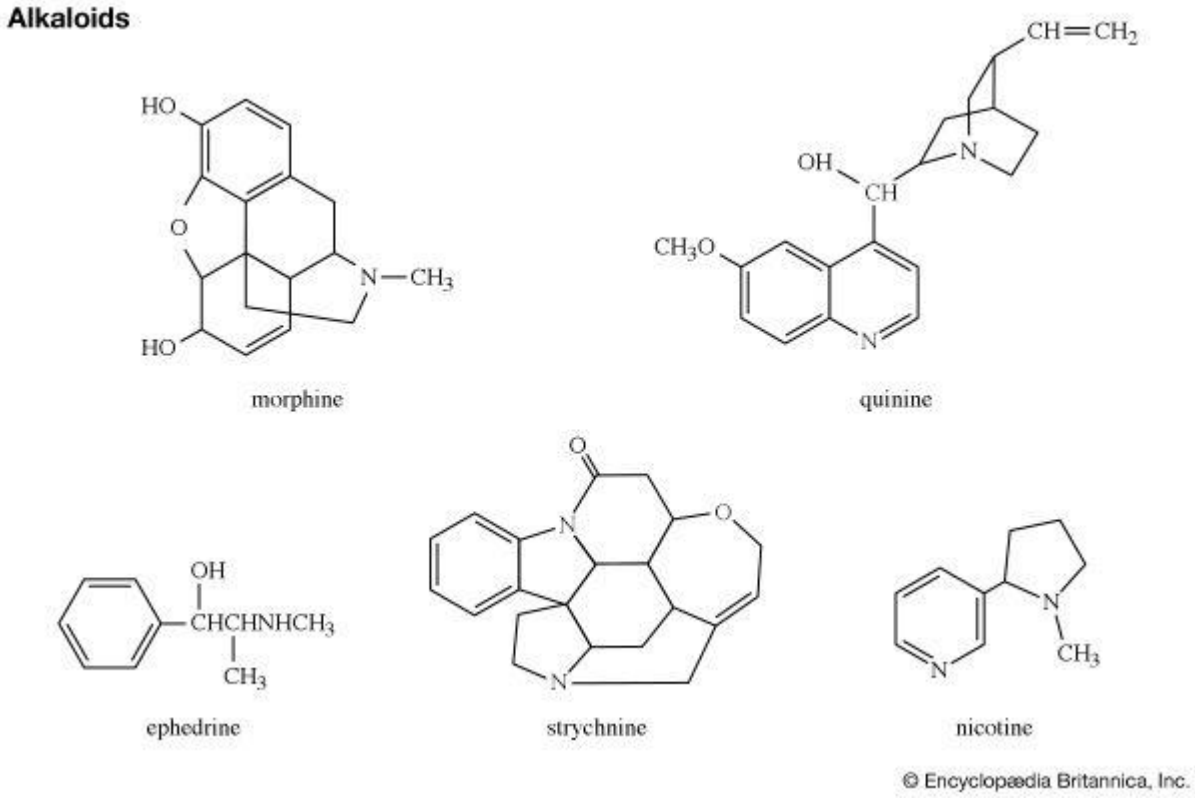


Fig 1: Classes of various Alkaloids (Heinrich *et al.*,2021)

2.1.2 Flavonoid

Flavonoids are phytochemicals responsible for the various colors in the seeds, flowers, fruits, leaves, and bark (Sangeetha *et al.,* 2016) Flavonoids are a large class of natural aromatic compounds as there are reported to be the most common plants' phenolics (Ekalu and Habila 2020). Over the years, flavonoids have represented a vast percentage of phytochemicals from natural sources. It has been reported that more than 10,000 different classes of flavonoids have been found in kingdom Plantae (Rudrapal and Chetia 2016). Flavonoids are secondary metabolites found in organs of these plants with different functions (Górniak *et al.,* 2019)

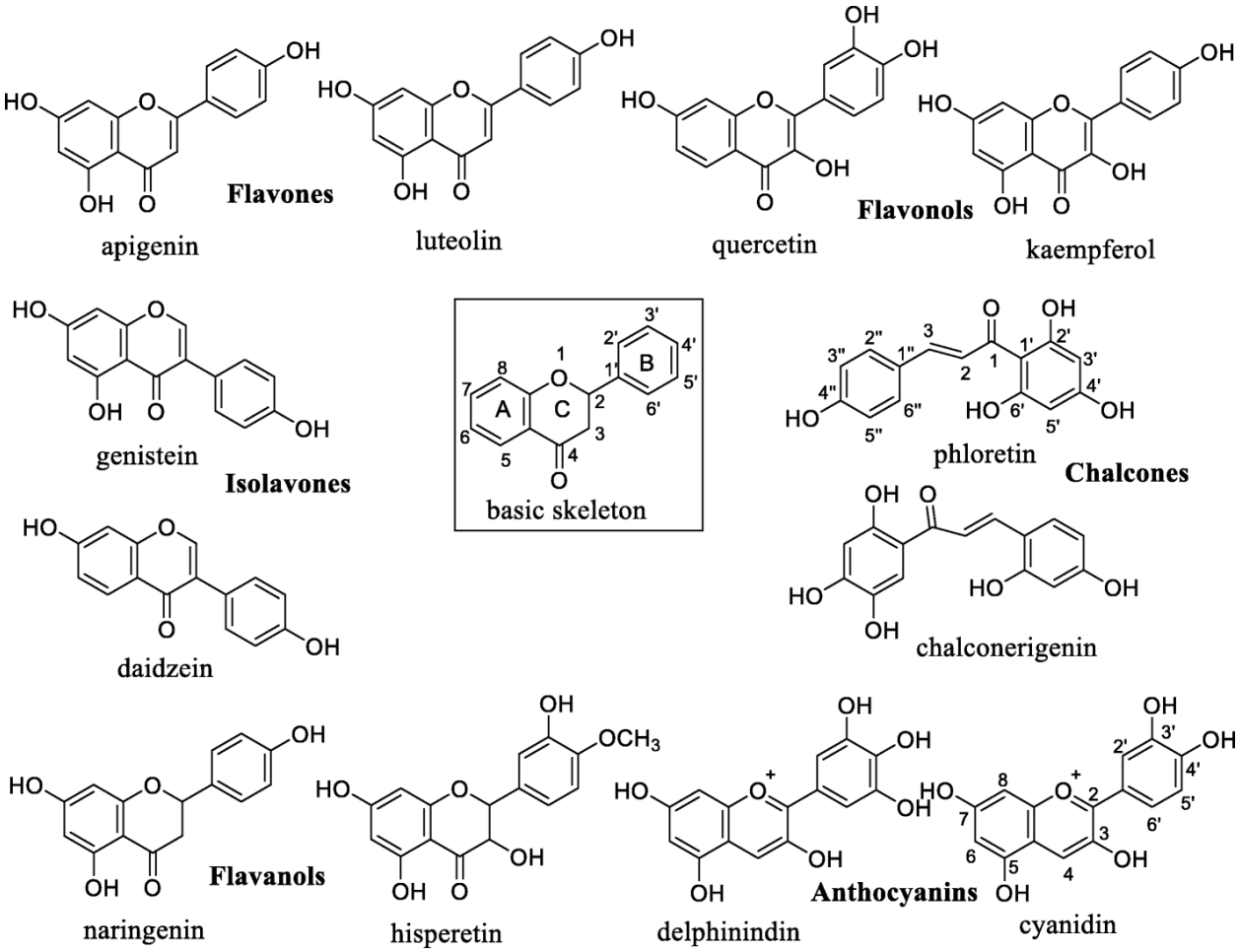


Fig 2: The basic skeleton of flavonoids and their various classes (Panche *et al.*,2016)

2.1.3 Terpenoid

Terpenoids are another type of terpenes containing oxygen molecules that are constructed via biochemical modifications (removal or addition of methyl groups) (Pandey et al., 2017). Terpenoids can be divided into alcohols, aldehydes, esters, ether, epoxides, ketones, and phenols. Examples of terpenoids are: carvacrol, citronellal, geraniol, linalool, linalyl acetate, piperitone, menthol, and thymol (Hyldgaard *et al.,* 2012) These bioactive compounds confer several biological activities such as anticancer (Potočnjak *et al* .,2018), anti-allergic (Kobayashi *et al.*,

2016), antibacterial (Guimarães et al., 2019), and antioxidant (Wang *et al* ., 2019).

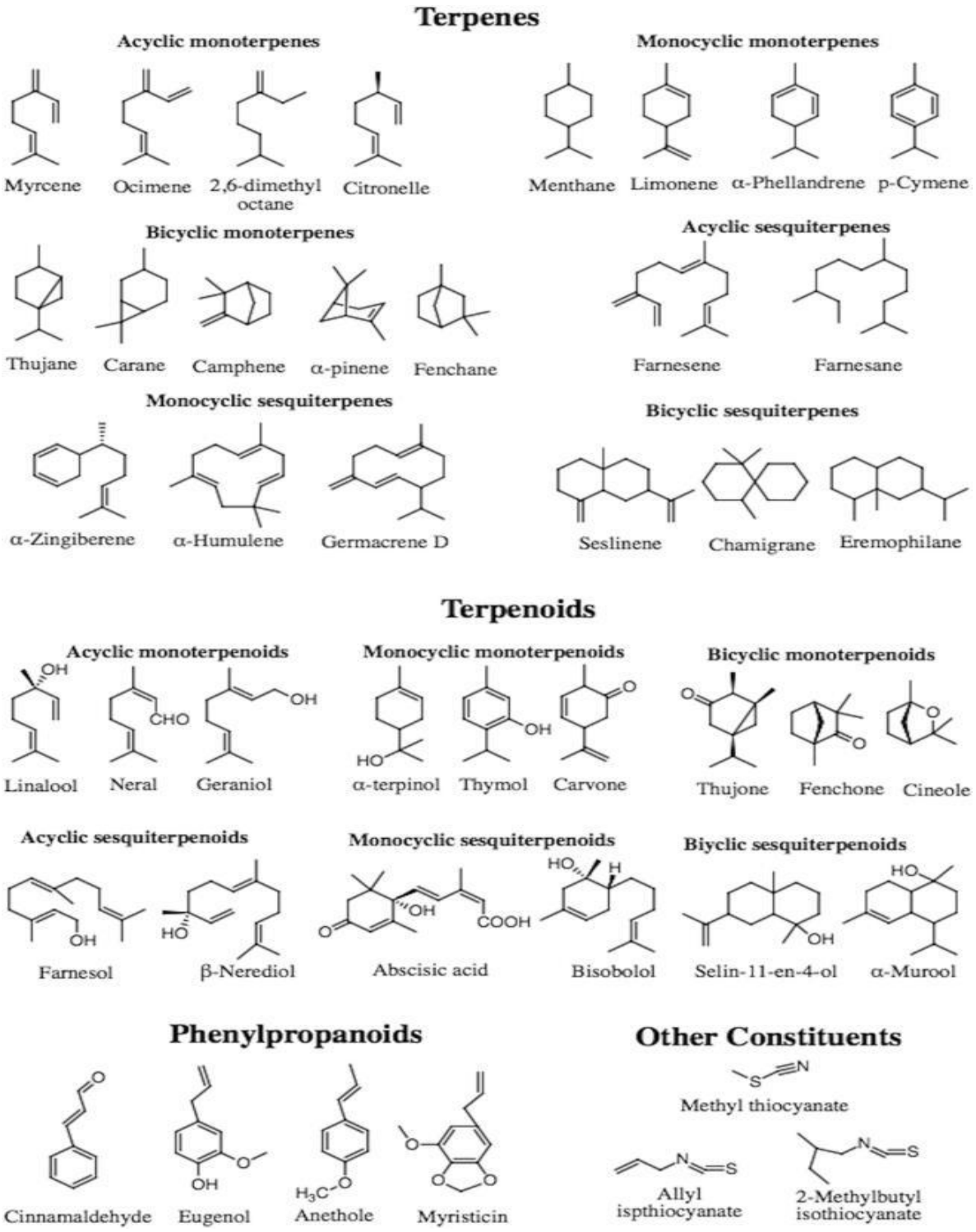


Fig 3: classes of Terpenoids (Masyita *et al.*, 2022)

2.2 Herbal products

Herbal products are substances derived from plant or plant extracts that are used for medicinal, therapeutic or health-promoting purposes. These products can include supplements or other formulations made from plant materials. People often use herbal products for various health reasons such as fertility complications. It is important to note that some herbal product have demonstrated health benefits, caution is advised and consulting healthcare professionals is recommended.

2.2.1Significance of herbal product

Herbal remedies have been a cornerstone of traditional medicine in many cultures in Nigeria. Traditional knowledge and practice have been passed through generations, forming the basis for using herbs in treating various health conditions. Herbs contain a plethora of bioactive compound including alkaloids, flavonoids, terpenoids and polyphenols. These compounds often possess medicinal properties, contributing to the effectiveness of herbal product in preventing or managing diseases. Herbal products are often utilized as part of complementary and alternatives medicine approaches, offering alternatives or adjuncts to conventional medical treatments. Herbal products are frequently used for treating common ailments and are sometimes used for preventive purpose. Herbal medicine often emphasizes a holistic approach addressing not only symptoms but also aiming to balance and support overall wellbeing.

2.3 Long Jack XXXL

Long Jack XXXL is a herbal product derived from various plant extracts such as Maca extract, Fenugreek extract, Siberian Ginseng, Tongkat Ali, Horny Goat Weed.

2.3.1 Tongkat Ali

Eurycoma longifolia Jack or "Tongkat Ali" is a herb which has been claimed to possess various medicinal properties. Traditionally, people believe that this herb can be used as remedies for sexual dysfunction, constipation, cancer, leukemia, exercise recovery, loss of libido, aging, stress, high blood pressure, malaria, osteoporosis, diabetes, fever, and glandular swelling (Ezzat *et al.,* 2019).

Eurycoma longifolia is a tall, slender tree which grows on sandy soil.It has compound leaves on branches that can grow up to 1cm long. The leaves are pinnate and green in colour. The numerous leaflets are opposite or sub-opposite, lanceolate to ovate-lanceolate, 5-20cm by 1.5-6cm, with smooth margins.

The flowers are tiny, reddish, unisexual and are densely arranged. The drupes are ovoid with a distinct ridge 1-2cm by 0.5cm-1.2cm and they turn dark reddish brown when ripe.

This plant is evergreen and tends to grow slowly bearing fruit 2-3 years after cultivation and 25years to mature. The roots are usually preferred for commercial use (Effendy *et al.,* 2012).

E.longifolia is a tropical plant that belongs to the family of simaroubaceae,which comprises of three species namely, *Eurycoma apiculate*,*Eurycoma harmandiana* and *E. longifolia* (Bhat and Karim,2010).It is mostly found in south-east countries like Malaysia, Indonesia and Vietnam, some of the plant species are also found in certain patches in regions of Cambodia, Myanmar, Laos and Thailand (Aziz *et al.,* 2012). In Malaysia, it was taken to improve strength and power during sexual activities (Mohamed *et al.,* 2019).

E. longifolia extracts lead to an increase in sexual arousal and motivation and frequency of sexual activity in both rats and mice (Hassan *et al.,* 2019). *E. longifolia* is famously known for its aphrodisiac effect, which is due to its ability to stimulate the production or action of androgen hormones, especially testosterone.

2.3.2 Maca Extract

Maca root comes from the maca plant. Also known as Peruvian ginseng and its scientific name *Lepidium meyenii*, maca is commonly grown in the mountains of Peru. Maca and its root has long been used by Peruvians for its nutritional and medicinal properties .

Macca belongs to Brassicaceae family and it thrives at altitude ranging from 2800 to 5000m above sea level. Maca demonstrates a remarkable ability to survive in challenging high- altitude conditions including intense UV radiation, low oxygen levels and unpredictable climates (Zhang *et al.,* 2016).

The edible components of Maca are vegetables, stems, hypocotyls (primary tap root) and infloresence (aerial parts) of maca are not fully utilized (Jin *et al.,* 2018).

The maca root is the most preferred cause exhibits significant genetic diversity in terms of morphology, including varying weight (1-5kg), shapes (spherical,oval,spindle-shaped) and a spectrum of colours for the skin and flesh (white, cream,yellow, orange, red, claret,and purple) (Jin *et al.,* 2018).The genetic variability may contribute to variations in the nutritional compostion of maca.

Maca roots and leaves contains a diverse arrays of compound with both pharmacological and nutritional importance including non-starch polysaccharides, polyphenol such as flavonoligians,

malamedas, macaenes, macamides, glucosinolates and macahydantoins individually or in various combinations, the components present in maca exhibit a diverse range of bioactivities in model systems. These include promoting reproductive health,providing neuroprotection acting as antioxidants combating fatigues, demonstrating anticancer properties protecting the liver, preventing osteoporosis, alleviating memory impairment and modulating the immune. The researchers observed that treatment with Maca increased the volume of ejaculate, sperm count, and motility of the sperm (Lee *et al.*, 2016).

2.3.3 Fenugreek Extract

Fenugreek is an annual legume which displays a morphological characteristic of an upright aromatic annual plant closely resembling large clover which is a member of Fabaceae family known as Trigonella (Flammang *et al.,* 2004) .This herb is commercially cultivated in countries such as India, Parkistan,Afghanistan,Iran,Nepal,Egypt,France,Spain,Turkey,Morocco,North Africa, the Middle East and Argentina(Flammang *et al.*, 2004).

The stem is lengthy and cylindrical ranging from 30-60cm in length with a pinkish hue while its roots manifest a robust fingerlike structures.Fenugreek features pinnates, trifoliate compound leaves with long stalk, exhibiting toothed, lanceolate leaflet with stipules that varies from triangular to obovate or oblanceolate.

The leaves is known to contain Beta-carotene,ascorbate,fiber,iron,calcium,and quantities that surpassing those found in typical food items(Thomas *et al.,* 2011)

This plant contains flowers which consist of five petals referred to as banner,wing and keel.The ovary appears deep green and glaucous and the pollen grains are oval to circular in shape.Fenugreek flowers give rise to pods approximately 15cm long, ranging in colour from

brownish to yellow-brown containing 2-8 seeds. Each seed is small about 5mm in length and is characterized by its hard smooth texture and dull yellow to brownish-yellow colour (Altuntas *et al.,* 2005)

The seed is the most valuable part of the plant, in their raw form its exhibits a golden colour with maple flavour but possess a bitter taste. However roasting mitigates the bitter taste. The seeds are characterized by their fibrous, sticky and gummy nature (Jani *et al.,*2019).As it contains bioactive compounds such as saponins and alkaloids(trigonelline).

Fenugreek is rich in flavonoids, alkaloids, saponins and other antioxidants. It contains a significant group of phenolics,including gallic acid, protocatechiu acid,catech, gentisic acid,chlorogenic acid, vanilic acid and syringic acid (Rababah *et al.,* 2011).These compound are biologically active and exhibit pharmacological effect when consumed. Studies have shown that fenugreek can significantly improve sperm count and motility in men with low sperm count (Maheshwari *et al.,*2017). The herb contains compounds such as saponins and flavonoids, which have been found to stimulate the production of testosterone, an essential hormone for male fertility .

Fenugreek has also been found to have other health benefits as it has been shown to lower blood sugar levels in people with diabetes, reduce inflammation which may help to reduce the risk of chronic diseases such as heart disease and cancer, and improve digestive health. Fenugreek also contains antioxidants, which have been found to protect sperm from damage (Tewari *et al.,*2020).

2.3.4 Siberian ginseng

Siberian ginseng or *Eleutherococcus senticosus* is a medicinal plant that has been used for centuries in traditional medicine to treat various conditions, including male infertility.

The plants fruit have been a traditional component in fermented wine, that contains compounds such as eleutherosides (eleutherosides B and E),flavonoids, phenolics and essential oil (0.3%,v/d.w), when consumed as food, the dried fruit are rich source of calcium,magnesium, manganese,zinc and copper

Ginseng leaves are used as tonic and functional beverages marketed for reducing liver damage and expediting alcohol detoxification (Yang *et al.*,2013).These leaves contains flavonoids such as quercetin,quercetrin and rutin (Zaluski *et al.,* 2012).The root is the most preferred part of the, because the ethanol gotten from the root is widely used for the treatment of weakness and conditions associated with inflammation such as rheumatism, hemorrhoids ,and impotence (Arouca and Grassi-Kassisse, 2013)

Research has shown that ginseng contains compounds known as ginsenosides, which have antioxidant and anti-inflammatory properties (Lee et al., 2020). These properties are believed to help improve sperm quality and motility, as well as increase testosterone levels due to be a cyclic adenosine monophosphate-responsive element modulator (Lee *et al.,* 2020).Ginseng has been found to have a positive effect on sexual function and libido in both men and women(Lee *et al.,*2020). Studies have also shown that ginseng can improve erectile dysfunction in men. This is because ginseng contains compounds that can increase blood flow to the penis, leading to stronger and longer-lasting erections (Lee *et al.,* 2020). Additionally, ginseng has been found to reduce stress and anxiety, which can also contribute to erectile dysfunction.

2.3.5 Horny Goat weed

Horny goat weed, also known as *Epimedium*, yin yang huo, or herbal "Viagra," is a plant used in traditional Chinese medicine (Zhang *et al.,* 2020).Horny goat weed belongs to Beriberidaceae family.The active compound identified in horny goat weed is icariin, a flavonol glycoside,(Liu *et al.,* 2006).

Icariin in horny goat weed, inhibits a protein linked with erectile dysfunction called phosphodiesterase type 5 PDE (Anand *et al.,* 2021). Phosphodiesterase-5 inhibitors, such as horny goat weed and Viagra, widen the blood vessels, increasing blood flow to the penis needed for an erection. Erectile dysfunction occurs when there is not enough blood flowing to the penis. A systematic review noted that horny goat weed was one of the most common ingredients in testosterone and erectile dysfunction supplements (Kuchakulla *et al.,* 2021).

2.3.6 Bioactive compounds of Long Jack (*Eurycoma longifolia)*

Long Jack (*E.longifolia)* has contributed good role as a complementary and alternative medicine in herbal therapies. Mostly the roots extract are being used in folk medicine for sexual dysfunction,aging,malaria,cancer,diabetes,anxiety,aches,exercise recovery, fever, increased energy, increased strength, leukemia, osteoporosis, stress, syphilis, and glandular swelling as well as aphrodisiac, antibiotic and health supplement (Miyake *et al.,* 2009).

Several biologically active chemicals found in the roots, stem, leaves and even the bark of *Eurycoma longifolia* were directly linked to the wide range of pharmacological effect of Long Jack.These biologically active compounds include biphenyl neolignans, quassinoids,alkaloids,triterpene-type tirucallane and squalene derivatives (Tran *et al.,* 2014).

Quassinoid phytoconstituents account for major portion in the *Eurycoma longifolia* root contents.Some of the quassinoids include eurycomanone (pasakbumin-A),euryocomanols, pasakbumin-B, hydroxyklaineanone and are involved in increased testosterone production and improved spermatogenesis (Meng *et al.,* 2014).Quassinoids are able to do this by suppressing alpha-2- Heremans Schmids (HS) glycoprotein expression which indirectly increases the testosterone level and insulin sensitivity (Chen et al., 2015)

Alkaloids are the second most abundant bioactive compounds in Long Jack.They represents a group of organic compounds such as 5,9-dimethoxycanthin-6-one, 9,10-dimethoxycanthin-6-one, 11-hydroxy-10-methoxycanthin-6-one, 10-hydroxy-9-methoxycanthin-6-one and 9-methoxy-3-methylcanthin-5,6-dione (Tran *et al.,* 2014).These alkaloids are involved in oxidative burst.They have been shown to provide cytotoxic activity human lung cancer (A-549) and human breast cancer (MCF-7).In addition they confer antimalarial action against *Plasmodium falciparium* (Miyake *et al.,* 2010).

The biphenyl neolignans class includes; 2-hydroxy-3,2,6-trimethoxy-4-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)-biphenyl; two isomeric 2,2-dimethoxy-4-(3-hydroxy-1-propenyl)-4-(1,2,3-trihydroxypropyl) diphenyl ethers; and 2-hydroxy-3,2-dimethoxy-4-(2,3-epoxy-1-hydroxypropyl)-5-(3-hydroxy-1-propenyl)biphenyl (Rehman *et al* ., 2016)

The squalene derivatives include teurilene, eurylene,14-deacetyleurylene and longilene perioxide (Rehman *et al.,* 2016).They have shown to provide cytotoxic activity against human epithelial carcinoma (KB) cells (Morimoto *et al.,* 2010).

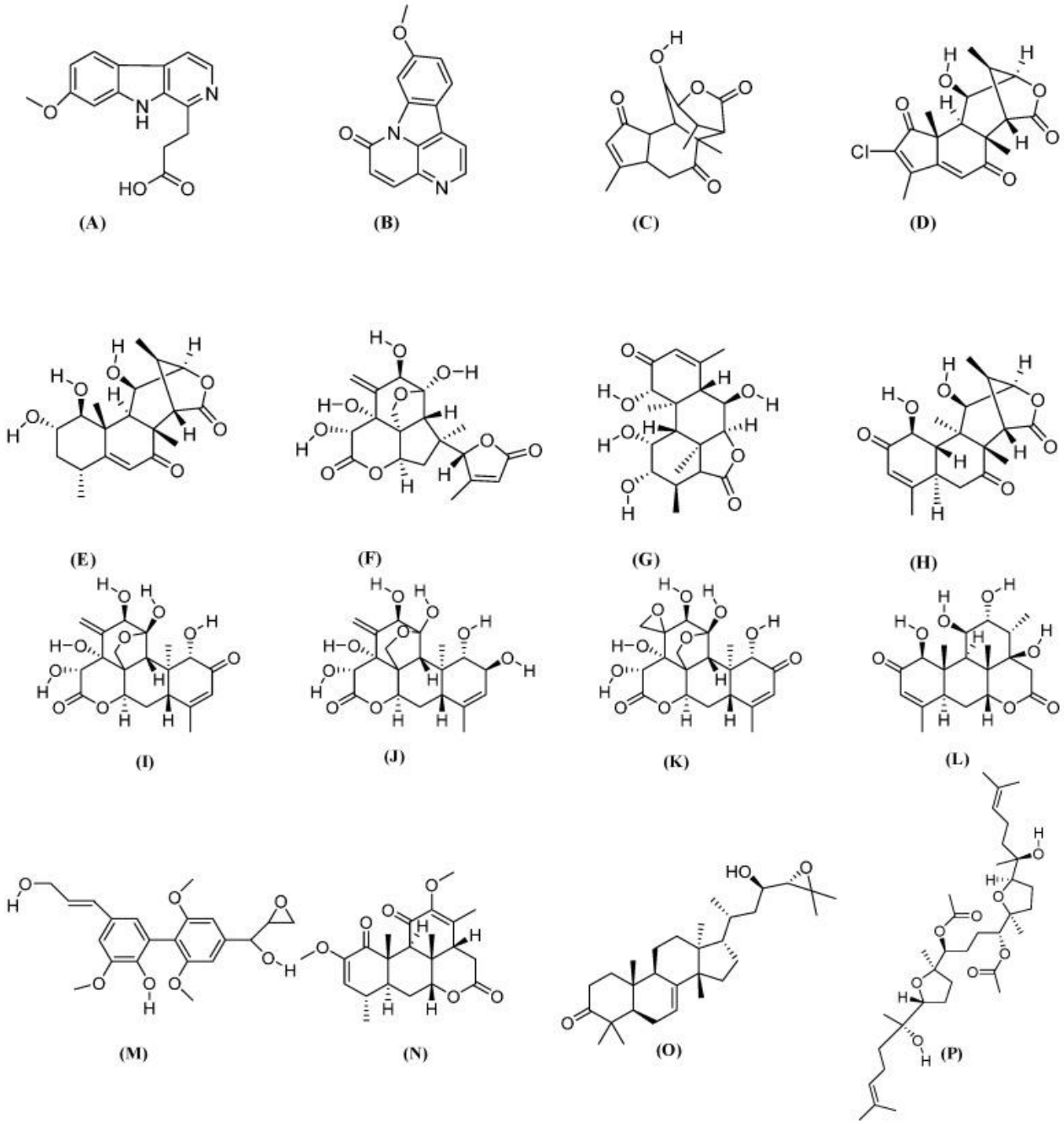


Fig 4:Chemical structures of various biological active constituents from *E.longifolia* (Rehman *et al.*, 2016)

Chemical structures of various biological active constituents from E. longifolia; (**A**) 7-Methoxy-beta-carboline-1-propionic acid (C15); (**B**) 9-methoxycanthin-6-one (C15); (**C**) Laurycolactone (C17);

(**D**) Eurycolactone B (C18); (**E**) Eurycomalide A (C19); (**F**) Eurylactone (C19); (**G**) Longilactone (C19); (**H**) Eurycomalactone (C19); (**I**) Eurycomanone (C20); (**J**) Eurycomanol (C20); (**K**) Pasakbumin B (C20); (**L**) Hydroxyklaineanone (C20); (**M**) Biphenyl-neolignan (C21); (**N**) Quassin (C22, basic ring of quassinoids); (**O**) Niloticin (C30); and (**P**) Eurylene (C34).

2.3.7 Toxic effect of Long Jack (*Eurycoma longifolia*)

While *Eurycoma longifolia* has been traditionally used in herbal medicine, excessive or prolonged use may lead to potential toxic effects such as;

Insomnia or Restlessness: *Eurycoma longifolia* is believed to have stimulant properties.These properties could lead to increased alertness or wakefulness by interacting with the neurotransmitters in the brain including serotonin and dopamine which plays a role in mood and sleep regulations

Increased heart rate: *Eurycoma longifolia* increases alertness and heightened physiological response by activating the sympathetic nervous system which is responsible for the "fight or flight" response that influences catecholamine release such as adrenaline and noradrenaline having stimulant effect on the cardiovascular system leading to an increased heart rate

Studies have suggested that Long Jack may have influence on testosterone production by stimulating the luteneinizing hormone that signals the testes to produce testosterone. Long Jack also have anti-estrogenic properties, meaning it may reduce the activity of estrogen,a hormone that counteract some effect of testosterone (Teh *et al.,* 2011).

2.4 Sperm Boom

Sperm Boom is a herbal product derived from various product such as *Sesamum radiatum* , *Cissus populnea* , *Lophira lanceolate*, Maca root powder .

2.4.1Sesamum radiatum

Sesamum radiatum is a perennial herb found in the tropical areas of Africa and belongs to the Pedaliaceae family (Ogunlesi *et al.*, 2010). One of the local names in South-Western Nigeria is "Ewe atura" which means 'leaves that bring relaxation and health to the body' possibly because they relieve consti-pation and cure other ailments on ingestion (Odugbemi, 2008). The leaves, seeds and oil serve as food especially in farming communities in Nigeria (Akpan-Iwo et al., 2006). Potential health benefits of sesame include anti oxidative, anticancer, anti-hypersensitive, and anti-immunoregulatory actions (Ogunlesi *et al.*,2010).

Sesame extract enhances the process of spermatogenesis through a complex of hormonal pathway or increase LH and improves spermatogenesis (Mahabadi *et al.*, 2013).

Sesame leaves intake has been found to improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rat (Shittu et al., 2007).

Sesame lignans have been shown to increase tissue tocopherol levels by inhibition of cytochrome P450 3A-dependent n-hydroxylase. Consequently, they potentiate the antioxidants activity of tocols in lipid peroxidation system (Khani *et al.*, 2013). Therefore, sesame lignans improve the quality of the produced sperm (Khani *et al.*, 2013)

Furthermore, sesame seed intake causes an improvement in the testicular parameters, fertility, and sperm production in adult male rats which may be attributed to the antioxidant properties of sesame (Mahabadi *et al.,* 2013**).**

Sesame is one of the richest dietary sources of lignan, Phytoestrogens which exist in it were known to human from the beginning of civilization and they are mixed with human food because of having many benefits for health. Sesame lignan, such as: Sesamin, Sesamolin, Sesaminol, Sesamolinol, Pinorsinol, Sesamol and gammaTocopherol which are isolated from Sesamum indicum and Sesamum radiatum seeds and they have more tumorigenic, estrogenic or anti-estrogenic and antioxidant features compare with other plant species (Shittu; and Hou, 2005)

In terms of phytochemical, this plant has phenolic compounds (Phenols, Sterols, Flavonoids and lignans), non-protein amino acids, cyanogenic glucoside, alkaloids, unsaturated fats and lipids with multiple double bonds, glazes, phospholipids and E, B1 and B2 vitamins(Ogunlesi *et al.,* 2010)

Minerals or trace elements such as calcium, iron, magnesium, zinc, copper and phosphorus exist in this plant (Konan *et al.,* 2008).

2.4.2 Cissus populnea

Cissus polpulnea Guill and Perr (Vitaceae) is a thick woody climber with a perennial rootstock and jointed stems that often drip watery fluid native to west tropical Africa (Aguoru et al., 2014). It is widely distributed throughout Nigeria, with common names such as ‘Okoho’ (Idoma and Igala), ‘Ogbolo or ajara’ (Yoruba) and ‘Dafaaraa or Latutuwa’ (Hausa) (Achikanu and Ani, 2020).

The plant which grows in valley beds and the surrounding area measures about 8–10 m in length and 7.5 cm in diameter. *Cissus polpulnea* is a gel-forming plant with an intrinsic ability to absorb water which indicates that it can stay fresh in both dry and wet seasons respectively. When young, the bark is creamy and smooth, but as it matures, it becomes grey and rough.

The branch is slightly hairy and then becomes hairless, green with a branching tendril on the opposite side of the leaf. It bears creamy flowers with blackish-purple fruits (Aguoru *et al.,* 2014).

The roots are fleshy in nature, and when dried, they can be utilized for construction. Most sections of the plant are mucilaginous, producing a highly viscous sap when freshly cut from stems. The leaves, when squeezed, become gelatinous and slimy (Soladoye and Chukwuma, 2012).

The plant is also used in the treatment of sore breast, indigestion, veneral diseases, sexual dysfunction intestinal parasites, oedema and eye problems (Uleh *et al.*, 2018)

C. populnea aqueous stem bark extract have been reportedly used as aphrodisiac/fertility enhancer for males among the Yoruba in South Western part of Nigeria (Ojekale *et al.,* 2006). It increases libido (Olaolu, 2018).

The bioactive compounds of *Cissus populnea* are anthraquinone, flavonoids,steroids,cyanogenic glycoside, tannins,saponins,carotenoids,triterpenoids and ascorbic acid (Jain et al., 2019).

2.4.3 Lophira lanceolate

Lophira lanceolata is a small deciduous tree growing to a height of 16 m (52 ft) or more. The tree has a narrow crown and steeply ascending branches, and forms suckers readily (Dicko *et al.,*

2022). The trunk is usually unbranched to around 8 m (26 ft) and can reach a diameter of about 70 cm (28 in). The bark is grey and corky, coming away in coarse flakes. The leaves are clustered at the end of the twigs. They are alternate, narrowly oblong, simple and entire, with rounded or notched apexes. The new foliage is pink or red at first. The inflorescence take the form of a loose panicle at the end of the shoot.

The individual flowers are bisexual, scented and white, with parts in fives. This is followed by a partially woody, conical achene surrounded by a winged calyx, containing a single, large seed (Dicko *et al.,* 2022). The tree is very similar to the closely related *Lophira alata* but is smaller, has narrower leaves with longer stalks, and larger seeds (Dicko *et al.*,2022).

Iron wood (*Lophira lanceolata*) is a tree that is socioeconomically and culturally valuable to inhabitants of Africa and it is a traditional medicine recipe that has been used to treat several disorders (Agu *et al.,* 2020).

Some researchers reported fertility-enhancing effects of aqueous stem bark extract of *L. lanceolata* in male rats model, sperm count was significantly increased, but motility and morphology were not affected (Fischer *et al.,* 2020) .

There was a decrease in the blood level of Follicle stimulating hormone (FSH) but no significant increase in the levels of Leutinizing hormone (LH) and Testosterone. Testicular histology examination revealed increased spermatogenesis (Fischer *et al.,* 2020).

The bioactive compound present in leaf extract of L. *lanceolata* are polyphenols, tannins, flavonoids, saponins, alkaloids, sterols and polyterpenes, and quinones.

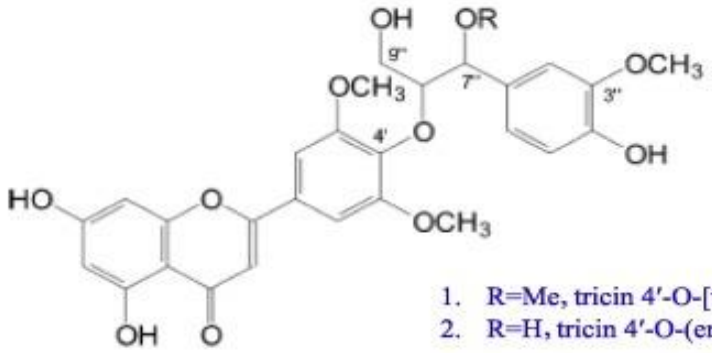
2.4.4 Bioactive compounds of Sperm Boom (*Lepidium meyenii*)

Maca (*Lepidium meyenii*) is a plant native to high Andes of Peru, known for its edible root(Zhou *et al.*,2017). While traditional uses of maca include is proported role as a fertility enhancer and aphrodisiac properties (Uchiyama *et al.*,2014).

Maca roots and leaves contains a diverse arrays of compound with both pharmacological and nutritional importance including non-starch polysaccharides, polyphenol such as flavonoligians, malamedas, macaenes, macamides, glucosinolates and macahydantoins, alkaloids,sterols (Carvalho *et al.*,2019)

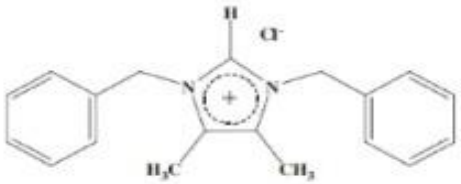
Macamides and macaenes interacts with the hypothalamus- pituary-gonadal axis by interacting with androgen receptors that binds to testosterone,this enhances the target tissues to testosterone.

Alkaloids can influence neurotransmitters and hormonal pathway by acting as enzyme inhibitor or activators which plays a role in the synthesis, metabolism and degradation of neurotransmitters and hormone. Glucosinolates are sulfur containing compound found in maca that have antioxidant properties, which is beneficial in reproductive health (Huang *et al.*,2019). Sterols, including beta-sitosterol are present in maca. These have potential benefits for prostrate health and hormonal balance. Polyphenols such as flavonoligians with antioxidant properties in reducing oxidative stress which can impact sperm quality

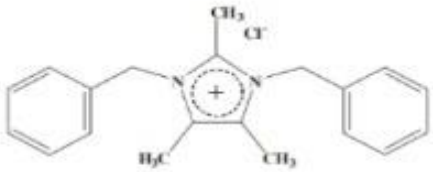


1. R=Me, tricin 4′-O-[threo-β-guaiacyl-(7″-O-methyl)-glyceryl] ether
2. R=H, tricin 4′-O-(erythro-β-guaiacyl-glyceryl) ether

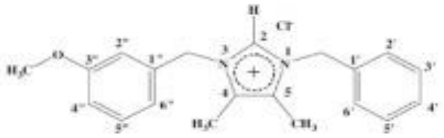
Flavonolignans (Bai et al., 2015)



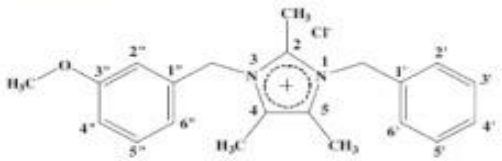
Lepidiline A



Lepidiline B

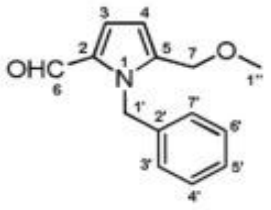


Lepidiline C

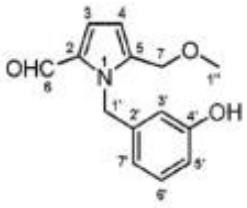


Lepidiline D

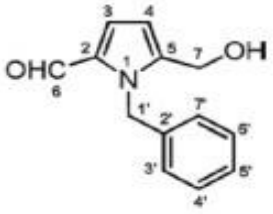
Imidazole alkaloids (Cui et al., 2003; Jin et al., 2016)



Macapyrrolin A



Macapyrrolin B



Macapyrrolin C

Pyrrole alkaloids (Zhou et al., 2018)

Fig 5:Phytoconstituent of maca root(Zhou *et al*.,2018;Jin *et al*., 2016)

2.4.5Toxic effect of Maca (*Lepidium meyenii*)

While maca root is often marketed as natural supplement with potential benefit for fertility and sexual health, its crucial to recognize that scientific evidence supporting its efficacy and safety especially as sperm booster is limited.

Common Allergic reaction

Maca roots causes common allergic reactions such as itching, redness ,rash or hives, respiratory symptoms like sneezing, runny nose,gastrointestinal symptoms including nausea, vomiting,or abdominal pain

Hormonal Effects

Maca is often promoted as an adaptogen ,suggesting that it can help the body adapt to stress and potentially regulate hormonal balance.Some studies have explore its effect on sex hormones like testosterone but result are in consistent.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

An experimental and descriptive study of two herbal products (Long Jack XXXL and Sperm boom) sold in Nigerian market was aimed to identify their phytoconstituents . This study was conducted at the University of Benin, Benin city.

3.2 Data Collection

Data were obtained by careful analysis of sample within the laboratory.

3.3 Sample Collection

Herbal supplements were obtained from a reputable Pharmaceutical outlet in Benin City.

3.4 Sample Analysis

Solutions of the herbal products were prepared by dissolving each herbal products in an appropriate solvent for use in HPLC with UV. The herbal products was dissolved in acetonenitrite/water (70:30). Exactly 10.00g Sample extracted with Acetonitrile, the extract was stabilized with Ethyl Acetate, and then introduced into 25 mL standard flask , and made up to the mark. Then, 5 µL was injected at 2mL / min flow rate. The prepared solutions upon aspiration by the nebulizer into the High performance liquid chromatography analysis (HPLC) and identified and characterized.

3.5High performance liquid chromatography

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.

The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.

In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns. HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres (Aryal, 2023).

3.5.1HPLC Principle

The purification takes place in a separation column between a stationary and a mobile phase.

The stationary phase is a granular material with very small porous particles in a separation column. The mobile phase, on the other hand, is a solvent or solvent mixture which is forced at high pressure through the separation column via a valve with a connected sample loop, i.e. a small tube or a capillary made of stainless steel, the sample is injected into the mobile phase flow from the pump to the separation column using a syringe. Subsequently, the individual components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase. After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer. At the end of this operation/run, a chromatogram in the HPLC software on the computer is obtained. The chromatogram allows the identification and quantification of the different substances (Aryal, 2023).

3.5.2Procedure of HPLC analysis for Long Jack XXXL (*E.longifolia)*

Column- uBONDApak C18

Mobile phase- Acetonitrile/water (70:30)

HPLC analysis was done using HPLC Shimadu (Nexera Mx) type equipment with column (uBONDAPak C18,100nm length,4.6mm interval diameter and 7µm thickness),UV 254nm detector and 15mPa pump pressure. Exactly 10g of the sample was extracted using acetonitrile and the extract was stabilized with ethyl acetate. It was put in 25mL standard flask and made up to mark with the diluent. Then, 5µL of stabilized extract was injected at flow rate of 2ml/min. The phytochemical constituents were identified using appropriate standards.

The same procedure was repeated for Sperm boom (maca root) but the sample extracted was maca roots

CHAPTER FOUR

4.0 RESULT

Table 4.1 shows 12 phytoconstituents present in Long Jack XXXL(*Eurycoma longifolia)* after HPLC analysis was carried out using column-uBONDpak,mobile phase-acetonitrile

Component	Retention	Area	Height	External	Units
Eurycomanone	1.1160	255.3410	22.1550	25.5341	ppm
Canthin-6-one	2.7160	130.9665	6.3340	13.0967	ppm
Niloticin	3.4330	66.8790	4.0380	0.0000	ppm
Scopoletin	4.2500	226.7320	10.5050	22.6732	ppm
Eurylene	6.2500	98.4535	4.6620	0.0000	ppm
Eurycomalide A	7.3660	209.2445	5.9720	0.0000	ppm
Pasakbumin A	8.8000	466.4985	7.6320	0.0000	ppm
Pasakbumin C	10.1830	103.0640	4.6840	10.3064	ppm
Longilactone	11.6000	133.0355	6.9550	13.3036	ppm
4-hydroxyglaucarubol	14.5500	74.3660	5.0820	0.0000	ppm
Laurycolactone	14.8830	116.1210	5.0590	0.0000	ppm
Beta-Carboline	16.3660	138.9290	4.8580	13.8929	ppm

Figure 4.1.1: HPLC chromatogram showing 12 separated components in the acetonitrile extract of Long Jack

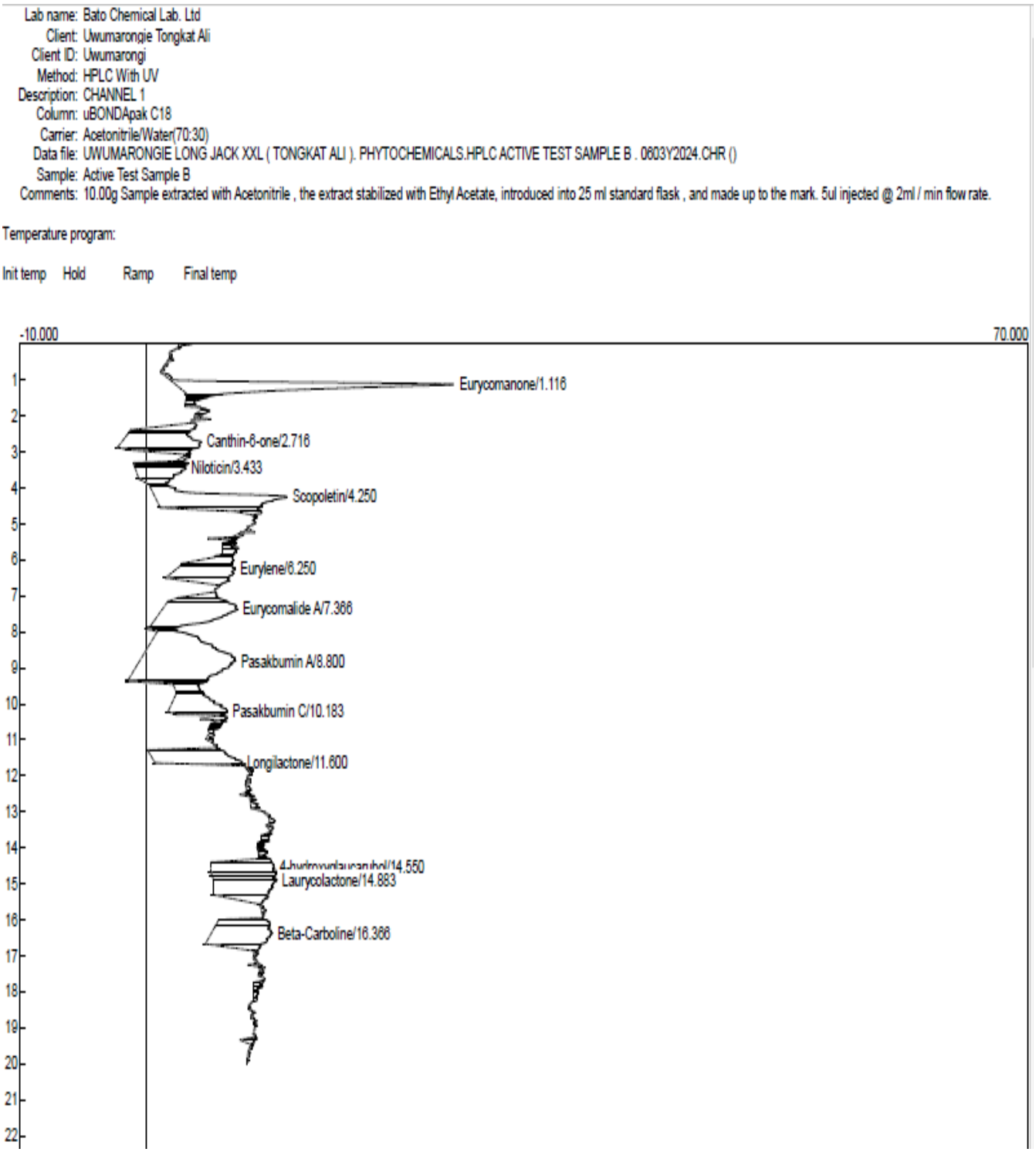
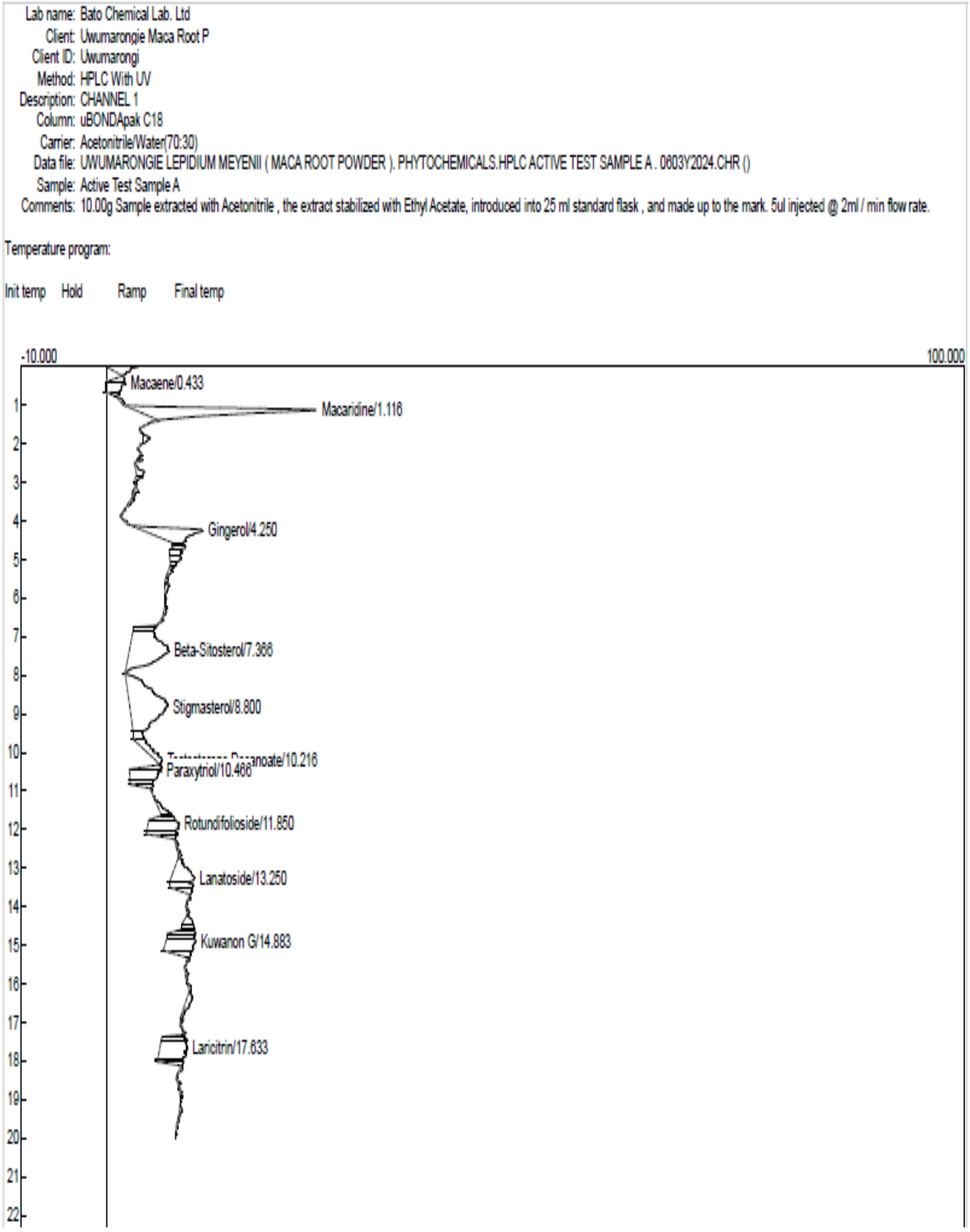


Table 4.2 shows 11 phytoconstituents present in Sperm boom (Maca root) after HPLC analysis was carried out using column-uBONDpak,mobile phase-acetonitrile/water

Component	Retention	Area	Height	External	Units
Macaene	0.4330	31.4710	2.437	3.1471	ppm
Macaridine	1.1160	222.365	21.239	22.2365	ppm
Gingerol	4.2500	104.4785	6.9600	0.0000	ppm
Beta-Sitosterol	7.3660	197.4080	4.6730	19.7408	ppm
Stigmasterol	8.8000	248.8820	4.4880	0.0000	ppm
Testosterone Decanoate	10.2160	36.0855	0.7540	0.0000	ppm
Paraxytriol	10.4660	56.2015	3.7890	0.0000	ppm
Rotundifolioside	11.8500	56.0770	3.6910	5.6077	ppm
Lanatoside	13.2500	63.3805	2.8010	6.3380	ppm
Kuwanon G	14.8830	64.2975	3.5140	0.0000	ppm
Laricitrin	17.6330	80.2490	3.2240	0.0000	ppm

Figure 4.2.1: HPLC chromatogram showing 12 separated components in the acetonitrile extract of Maca root



CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

Herbal products have been shown to have to arrays of biological activities including antimicrobial, ant-inflammatory, antioxidant, anti-aging biological properties and others. Herbal product (Long Jack and Sperm boom) contains various phytoconstituents that illicit the various biological activities especially in fertility. However, the question of whether herbal product such as Long Jack XXXL and Sperm boom contain unapproved synthetic Phosphodiesterase inhibitor which may be have adverse effect or cause pathological conditions which would make possible their preventive usage rather than their potential remediation of fore stated conditions remains to be answered

This study was design to determine the phytoconstituents of two herbal products Long Jack XXXL (*Eurycoma longifolia*) and Sperm boom. Long Jack XXXL(*Eurycoma* longifolia)contains 12 bioactive compounds which are Eurycomanone, canthin-6-one, Niloticin, Scopoletin, Eurylene, Eurycomalide A, Pasakbumin A, Pasakbumin C,Longilactone,4-hydroxyglaucarubol,Laurycolactone, Beta- carboline.

Eurycomanone is the major components of quassinoids which increases testosterone production in the isolated rat testicular Leydig cell-rich interstitial cells by inhibiting aromatase conversion of testosterone to oestrogen, and at a high concentration may also involve Phosphodiesterase inhibition (Rehman *et al.,* 2016).

Canthin-6-one is an alkaloid which illicit its aphrodiasic activities in *E.longifolia* inhibiting phosphodiesterase type 5(Choonong *et al.,*2022). In addition, HCO may be associated with penile erection and delayed ejaculation in rats, by interfering with Ca^{2+} mobilization and, thus,

antagonistically toning the smooth muscles of the corpus cavernosum and seminal vesicle (Chiou, 2012).

Niloticin is a tirucallane-type triterpenes derivative known for Anti-cancer activity against ovarian leukemia and renal cell lines (Rehman *et al.,* 2016).

Scopoletin is known for its antioxidant and anti-inflammatory properties by neutralizing the reactive oxygen species (ROS) and protect cells from oxidative stress. It is also known as a low molecular weight compounds that are biosynthesized in response to microbial attack over the plant. Scopoletin has been used as antibacterial and antifungal compound in traditional medicine (Gao et al., 2024).

Eurylene is a Squalene-type triterpenes involved in Cytotoxicity Cytotoxic activity against KB cells (Rehman *et al.*, 2016).

Eurycomalide A is a bioactive compound found in root of *E.longifolia* involvedCytotoxicity against human lung cancer (A-549), and human breast cancer (MCF-7) cell lines NF-κB inhibitor (Rehman *et al.,* 2016).

Pasakbumin A and C are quassinoids are involved in increased testosterone production and improved spermatogenesis (Meng *et al.,* 2014).They are Anti-ulcer Cytotoxicity against human lung cancer (A-549) and human breast cancer (MCF-7) cell lines (Rehman *et al.,* 2016). 4-hydroxyglaucarbol are quassinoids are involved in increased testosterone production and improved spermatogenesis (Meng *et al.,* 2014). Longilactone are involved in Cytotoxicity against human HT1080 Cytotoxicity against human lung cancer (A-549), and human breast cancer (MCF-7) cell lines

Compounds possess anti-tumor promoting, antischistosomal and plasmodicidal activities NF-κB inhibitor (Rehman *et al.,* 2016).

Laurycolactone is involved in Cytotoxicity against human HT1080, Beta-carboline is an alkaloid involved in improved sexual function (Rehman *et al.,* 2016).The quassinoids and alkaloids in *E.longifolia* increases testosterone production and improved spermatogenesis(Yang *et al.,* 2021).They are the most abundant while others are known to elicit anti-cancer activities

Sperm boom (Maca root) contains 11 bioactive compounds which are Macaene, Macaridine, Gingerol, Beta-sitosterol, Sigmasterol, Testosterone decanoate, Paraxytriol, Rotundifolioside, Lanaside, Kuwanon G, Laricitrin

Macaenes are polyunsaturated fatty acid interacts with the hypothalamus- pituary-gonadal axis by interacting with androgen receptors that binds to testosterone, this enhances the target tissues to testosterone. Macaridines are the most abundant alkaloid involved in fertility enhancing properties, together with the macamides and macaenes. (Zhou *et al.,* 2017). Gingerol and Kuwanon G are phenols with antioxidant properties in reducing oxidative stress which can impact sperm quality and anti-atherosclerotic effect (Talhaoui *et al.,* 2015)

Beta-Sitosterol, Stigmasterol and testosterone decanoaate are sterols which have potential benefits for prostrate health and hormonal balance improves the functions of male hormones such as sexual development at puberty, sexual function, maintenance of mass and strength of the bone and muscle, and maintenance of the body composition in males (Nique *et al.,* 2012)

Paraxytriol, Rotundfolioside, Lanastoside are saponins involved in anti inflammatory and antioxidant effect(Lee *et al.,* 2011). Lactrin are flavonoid which also involved in anti inflammatory and antioxidant effect.In Maca roots the alkaloids and sterols are involved in

fertility enhancing properties while others are involved in antioxidant effect.Findings in this study has shown no presence of unapproved synthetic adulterants and are natural remedies.In addition the phytoconstituent shown are in line with previous studies (Rehman *et al.*, 2016;Zhou *et al.*,2017).

There was no synthetic phosphodiesterase 5 inhibitor was found in the two herbal products. The inability to identify synthetic compound may be due to the method used in this analysis. In contrast to previous study done in Accra, Ghana, in 35 supplements using Liquid-Chromatography- Mass Spectrophotometry and Proton nuclear magnetic resonance (LC–MS/MS and 1H-NMR), it was found that hydroxythiohomosildenafil, a sildenafil analogue which has not been approved as PDE-5 inhibitor was identified (Akuamoa *et al*., 2022).

CONCLUSION

In conclusion, the application of High-Performance Liquid Chromatography (HPLC) in the analysis of phytoconstituents within a herbal product represents a significant step toward ensuring its quality and authenticity. The was absence of adulterants, signifying the purity and integrity of the herbal product using this method. The meticulous screening for adulterants is imperative to guarantee the safety and efficacy of the herbal supplement.

REFERENCES

Abiche E., and James D. H. (2020) Flavonoid:isolation,characterisationand heaith benefits:Beni-suef University.Journal of basic and applied sciences,9:45

Achikanu C. E., and Ani O. N. (2020). Nutritional and Phytochemical Content of Cissus populnea (Okoho) Stem Bark. Asian Journal of Research in Biochemistry, 7(3):8–15

Adebayo E.M, Adeyemi A.A, Omotade O.O,Fasola F.A,Famojuro T.I,Adebisi L,Moody J.O (2015) Antisickling Activity of the Fresh and Dried Roots of Cissus Populnea Guill. Et Perr (Vitaceae) Nigerian journal of herbal product and medicine,19.

Agu T.S, Ezihe C. O, Itodo F.P, and Abu H. A(2020).Lophira lanceolata protects testicular and spermatological damages induced by cisplatin in male wistar rats.Clinical Phytoscience, 6: 81

Aguoru C.U, Ameh S.J and Olasan (2014).Comparative phytochemical studies on the presence and quantification of various bioactive compounds in the three major organs of okoho plant (cissus populnea guill and perr) in benue state north central Nigeria, western Africa. European Journal of Advanced Research in Biological and Life Sciences,2:2

Akarali. World's first patented Tongkat Ali extract, Physta. https://akarali.com/physta-tongkat-ali/, 2021

Akuamoa F, Boveea T. F .H, van Dama R, Marod L, Wesseling L, Vervoorte J, Rietjens IMCM, Hoogenboom LAP (2022). Identification of phosphodiesterase type-5 (PDE-5) inhibitors in herbal supplements using a tiered approach and associated consumer risk. Food Addictives and Contaminants: Part A, 39(6): 1021–1032

Arouca A and Grassi-Kassisse D.M (2013) Eleutherococcus senticosus: Studies and effects.Scientifc Research Health,5:9

Attia, S. M. (2015). Eurycoma longifolia, a promising suppressor of RANKL-induced differentiation and activation of osteoclasts: An in vitro mechanistic evaluation. Journal of Applied Pharmaceutical Science, 5(7), 065-070.

Ayad B, Omolaoye T S, Louw N, Ramsunder Y, Skosana B T, Oyeipo P I, Du Plessis SS. (2022). OxidativeStress and Male Infertility: Evidence From a Research Perspective. Frontier Reproduction Health,4:822257.

Aziz N. A. A, Hasham R, Sarmidi M .R, Suhaimi S .H, Idris M.K.H. (2021). A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on Orthosiphon aristatus, Eurycoma longifolia and Andrographis paniculata. Saudi Pharmacology Journal 29 (2): 143-165

Bhat R., Karim A (2010) Tongkat Ali (Eurycoma longifolia Jack): A review on its ethnobotany and pharmacological importance. Fitoterapia. 81:669–679.

Bujang B, Chin F.C, Choon H.H, Noorsaadah A, Micheal B (2017).Phosphadiesterase-5-inhibitors and their analogues as adultery of herbal and food products .Retrieved from https://www.researchgate.com

Carvalho F V.,Paulo R., Ribeiro (2019)Structural diversity, biosynthetic aspects, and LC-HRMS data compilation for the identification of bioactive compounds of Lepidium meyenii. Food Research International volume 125, 108615

Chan K. L, O'Neill M J, Phillipson J D, Warhurst DC. (2009) Plants as sources of antimalarial drugs. Part 3 Eurycoma longifolia. Planta Medication, 52, 105–107.

Chen Y., Phang W.M., Mu A.K.W., Chan C.K., Low B.S., Sasidharan S., Chan K.L.(2015). Decreased expression of alpha-2-HS glycoprotein in the sera of rats treated with Eurycoma longifolia extract. Frontiers in Pharmacology.00211.

Choonong R. Chaingam J. Chantakul R. Mukda S. Temkitthawon P. Ingkaninan K. Juengwatanatrakul T. Yusakul G. Kanchanapoom T. Putalun W (2022). Chemical Biodiversity,19:e202200121.

Chua L, Kamarudin S, Markandan M, Hamidah M. A (2005) preliminary checklist of vascular plants from the Machinchang Range, Pulau Langkawi, Peninsular Malaysia. Malaysia Nature Journal,57:155–172

Cooper T.G., Noonan E., Von Eckardstein S., Auger J., Baker H.G., Behre H.M., Haugen T.B., Kruger T., Wang C., Mbizvo M.T. (2010). World Health Organization reference values for human semen characteristics. Human Reproduction Update, 16:231–245

Damayanti, A., and Sukesi, T. W. (2014). Effects of Eurycoma Longifolia Root Extract on Larvae Aedes aegypti mosquito. Journal of Public Health, 7(2): 259-267

Damilare E. R,IkponwosaO. E,Mattew E I,Tomilola D Olaolu O,Adeleke O (2023).Pharmacological activities of Cissus populnea in human disease.Biocatalysis and Agricultural biotechnology volume 50:101271

Danial M., Keng C.L., Alwee S.S.R.S., Subramaniam S.(2005) Seed histology of recalcitrant Eurycoma longifolia plants during germination and its beneficial attribute for hairy roots production. Journal of Medicinal Plants Research,5:93–98

Dicko A, Fandohan B, Armand K .N (2022).Ecophysiology of Lophira lanceolate Seeds Germination and Conservation Perspective.American Journal of Plant Science,13:9

Dietary Effect of Cissus Populnea and Securidaca Longepedunculata Aqueous Leave Extracts on Reproductive, Haematological and Biochemical Parameters of African Catfish, Clarias gariepinus (Burchell, 1822) Broodstocks

Duraz A.Y, Khan S.A (2011) Knowledge, attitudes and awareness of community pharmacists towards the use of herbal medicines in muscat region. Oman Medical Journal ,26

Effendy M N., Mohamed N., Muhammad N., Mohamad I., Shuid A N. (2012). Eurycoma longifolia: Medicinal plant in the prevention and treatment of male osteoporosis due to androgen deficiency. Evidence Based Complementary Alternative Medicine: 125761.

Farag, M. A., Ajayi, A. O., Taleb, M., Wang, K., and Ayoub, I. M. (2022). A Multifaceted Review of Eurycoma longifolia Nutraceutical Bioactives: Production, Extraction, and Analysis in Drugs and Biofluids. ACS Omega, 8(2), 1838-1850.

Fischer, V. A.; Effiom-Ekaha, O. O.; Fischer, C. E (2020) Morphological and Histological Alterations in the Testes of Wistar Rats Administered Extracts of Monodora Myristica

Seeds. Egyptian Academic Journal of Biological Sciences. Department of Histology and Histochemistry, 12:9

Flammang A. M,Cifone M.A,Erexson G L,Stankowski L F.Genotoxicity testing of fenugreek extract.Food Chemical Toxicology,42(11):1769-1775

Gao X-Y,Li X-Y,Zhang C-Y and Bai C-Y(2024)Scopoletin:a review of its pharmacology, pharmacokinetics and toxicity Frontier pharmacology 15:1268464

George A., Henkel R. (2014). Phytoandrogenic properties of Eurycoma longifolia as natural alternative to testosterone replacement therapy. Andrologia ;46:708–721

Górniak I, Bartoszewski R, Króliczewski J (2019). Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemical Reviews, 18:241–272

Guimarães A.C., Meireles L.M., Lemos M.F., Guimarães M.C.C., Endringer D.C., Fronza M., Scherer R (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules,24:2471.

Güler O , Polat R , Mustafa K , Uğur Ç , Akbulut S (2021) An ethnoveterinary study on plants used for the treatment of livestock diseases in the province of Giresun (Turkey).South African Journal of Botany,142:5362

Heinrich M, Mah J, Amirka V (2021) Alkaloids used as medicine structural phytochemistry meets biodiversity-An update and forward look.Molecules,26 (7):1836

Hyldgaard M., Mygind T., Meyer R.L. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology. 2012;3:12.

Ismail, S. B., Wan Mohammad, W. M., George, A., Nik Hussain, N. H., Musthapa Kamal, Z. M., and Liske, E. (2012). Randomized Clinical Trial on the Use of PHYSTA Freeze-Dried Water Extract of Eurycoma longifolia for the Improvement of Quality of Life and Sexual Well-Being in Men. Evidence Based Complement Alternate Medicine, 429268.

Jain S., Akur V.,Shah K., Durgesh N.,Nagendra S.C (2019) Anti-sickling herbs. Plant and Human Health volume 3

Jesuniyi, F. I,Aderolu, A.Z,Lawal, M.O,Okoronkwo L.I,Awobajo, F.O(2017)

Jin W. X. Chen, Q. Huo, Y. Cui, Z. Yu, L. Yu (2018). Aerial parts of maca (Lepidium meyenii Walp.) as functional vegetables with gastrointestinal prokinetic efficacy in vivo. Food and Function: 3456-3465

Jothi, S., Parumasivam, T., and Mohtar, N. (2023). Eurycoma longifolia: an overview on the pharmacological properties for the treatment of common cancer. Journal Public Health Africa, 16;14(1), 2495.

Jun-Hyun O,Enkhbolor B , Hong H-S , Lee K-W , and Huang Y.W (2019) Antioxidant Activity, Macamide B Content and Muscle Cell Protection of Maca (Lepidium meyenii) Extracted Using Ultrasonification-Assisted Extraction.Microbiology and Biotechnology, 48(2): 129-137

Khani B, Bidogoli S R, Fariborz M and Hassan H (2013). Effect of sesame on sperm quality of infertile men.Journal of research in medical sciences 18(3):184-187

Khanijo, T., and Jiraungkoorskul, W. (2016). Ergogenic Effect of Long Jack, Eurycoma Longifolia. Pharmacognosy Reviews, 10(20), 139-142

Kobayashi Y., Sato H., Yorita M., Nakayama H., Miyazato H., Sugimoto K., Jippo T. (2016).Inhibitory effects of geranium essential oil and its major component, citronellol, on degranulation and cytokine production by mast cells. Bioscience, Biotechnology, and Biochemistry,80:1172–1178.

Kumar N and Singh A.K (2015).Trends of male factor infertility an important causes of infertility:A review of literature.Journal of Human Reproductive Science,8(4):191-196

Lee J.H, Jeon J.K, Kim S.H, Chun T and Imm J.Y (2011b).Comparative analyses of total phenol, flavonoids, saponins and antioxidant activities in yellow soy, beans.International journal of food science and technology,46(12):2513-2516

Lin K. J. Huang, D. Sun-Waterhouse, M. Zhao, K. Zhao, J. Que (2018). Maca (Lepidium meyenii) as a source of macamides and polysaccharide in combating of oxidative stress and damage in human erythrocytes. International Journal of Food Science and Technology: 304-312

Low B .S, Das P .K, Chan K.L. (2013). Standardized quassinoid-rich Eurycoma longifolia extract improved spermatogenesis and fertility inmale rats via the hypothalamic-pituitary-gonadal axis. Journal Ethnopharmacology 145 (3): 706-714.

Maheshwari A,Narsingh V, Anand S,Manashi Bagchi, Harry G P,Kiran T and Bagchi D (2017) Efficacy of Furosap, a novel Trigonella foenum graceum seed extract in Enhancing Testosterone Level and improving sperm profile in male volunteer.International Journal of Medical Science,14(1):58-66

Meng D., Li X., Han L., Zhang L., An W., Li X. (2014). Four new quassinoids from the roots of Eurycoma longifolia Jack. Fitoterapia,92:105–110.

Miyake K., Tezuka Y., Awale S., Li F., Kadota S. (2010) Canthin-6-one alkaloids and a tirucallanoid from Eurycoma longifolia and their cytotoxic activity against a human HT-1080 fibrosarcoma cell line. Natural Product Communications ,5:17–22.

Morimoto Y., Iwai T., Yoshimura T., Kinoshita T. (2010). Diastereoselective two-directional synthesis and cation transport ability of the central tristetrahydrofuranyl unit of meso polyether glabrescol as naturally occurring podand. Bioorganic and Medicinal Chemistry Letters;10.1016

Nique F,Hebbe S, Peixoto C, Annoot D, Lefrancois J. M,Mollat P.Thauvin M, Prange T,Miret D, Clememt-Lacroix P,Robin-Jagreschmichidt,Fleury D,Guedin D and Deprez P (2012).Discovery of diarylhydantoins as new selective androgen receptor modulators.Journal of medicinal chemistry,55(19):8225-8235

Njagi P, Groot W, Arsenijevic J, Dyer S, Mburu G, Kiarie J (2020) Economic costs of infertility care for patients in low-income and middle-income countries: a systematic review protocol. British Medical Journal,10:e042951

Ogunlesi M,Okei W, Osibote A.E. (2010) Analysis of the essential oil from leaves of Sesame radiatum,a potential medication for male fertility factor by gas chromatography- mass spectrometry.African Journal of Biotechnology,9:1060-1067

Ohemu T.L, V.A. Okwori , J.J. Azila, C.A. Nwobodo (2023) Ethnobotanical Survey of Medicinal Plants Used to Treat Male Infertility in Jos North Local Government Area of Plateau State, Nigeria. Journal of Herbal Medicine, 43: 100825.

Pandey A.K., Kumar P., Singh P., Tripathi N.N., Bajpai V.K.(2010) Essential oils: Sources of antimicrobials and food preservatives. Frontiers in Microbiology,7.

Potočnjak I., Gobin I., Domitrović R (2018) Carvacrol induces cytotoxicity in human cervical cancer cells but causes cisplatin resistance: Involvement of MEK-ERK activation. Phytotherapy Research,32:1090–1097.

Rababah T.M, Majdi A. A, Kilani I, Yang W, Mohammad N. A, Khalil E, Muhammad A.Effect of jam processing and storage on total phenolics, antioxidant activity, and anthocyanins of different fruits.Journal of the Science of Food and Agriculture.91:6

Rahebi S.M, Rahnavardi M, Sedighe-chamani, Nazari M,Sabetghadam S (2019).Relationship between domestic violence and infertility.East mediterrean heaith journal,25(8):539-542

Rehman S. U., Choe K., Yoo H.H. (2016). Review on a traditional herbal medicine, Eurycoma longifolia Jack (Tongkat ali): Its traditional uses, chemistry. Molecules 21 (3): 331.

Rudrapal M, Chetia D (2016) Plant flavonoids as potential source of future antimalarial leads. Systemic Review Pharmacology 8(1):13–18.

Sagar Aryal (2023) HPLC: Principle, Parts, Types, Uses, Diagram.Microbe Notes

Sangeetha K. S. S, Umamaheswari S, Reddy C. U. M, Kalkura S N (2016) Flavonoids: Therapeutic potential of natural pharmacological agents. International Journal , 7(10):3924–3930.

Shittu LA, Adesite SO, Ajala MO, Bankole MA, Benebo AS, Tayo AO (2008) Sesame Radiatum Phytoestrogenic Lignans Enhances Testicular Activity In Adult Male Sprague Dawley Rat Testis. International Journal Morphology,26:643–52.

Srivastava S., Srivastava A.K (2013) Biotechnology and Genetic Engineering for Alkaloid Production. In: Ramawat K.G., Mérillon J.-M., editors. Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes. Springer; Berlin/Heidelberg, Germany, 213–250.

Talbott S.M,Talbott J.A, George A,Pugh,M.(2013)Effect of Tongkat Ali on stress hormone and psychological mood state in moderately stressed subject.Journal international of Social Sport Nutrition,10(1):28

Talhaoui N, Taamali A, Gomez-caravaca A.M, Fernadez-Gutierrez A and Segura-Carreto A (2015). Phenolic compound in olive leaves.Analytical determination, biotic and Abiotic influence and health benefits.Food research internal,77:92-108

Tambi M., Kadir A (2006) Eurycoma Longifolia jack: A potent adaptogen in the form of water-soluble extract with the effect of maintaining men's health. Asian Journal Andrologia,8:49–50.

Teh C.H., Abdulghani M., Morita H., Shiro M., Hussin A.H., Chan K.L. (2011). Comparative X-Ray and Conformational Analysis of a New Crystal of 13α,21-Dihydroeurycomanone with Eurycomanone from Eurycoma longifolia and Their Anti-Estrogenic Activity Using the Uterotrophic Assay. Plantae Medication, 77:128–132.

Teweri D, Artur J,Malgorzata L.G,Weronika G,Wioletta A.B,Jacek B,Strzalkowska N, Kaminska A,Olaf K.Horbanczuk and Atanas G Atanasov (2020).Fenugreek(Trigonella foenum-graecum L) Seeds Dietary Supplementation Regulates Liver Antioxidant Defense System in Aging Mice.Nutrients,12(9):2552

Thu E.H, Mohamed N.I, Zahid H,Ahmad N.S (2017).Eurycoma longifolia as a potential alternative to testosterone for the treatment of osteoporosis: Exploring time-mannered proliferative, differentiative and morphogenic. modulation in osteoblasts.Journal of Ethanopharmacology,195:143-158

Tran T.V.A., Malainer C., Schwaiger S., Atanasov A.G., Heiss E.H., Dirsch V.M., Stuppner H. (2014) NF-κB Inhibitors from Eurycoma longifolia. Journal of Natural Products ,77:483–488.

Uadia P.O and Emokpae M.A(2015).Male infertility in Nigeria:Aneglected reproductive health issue requiring attention.Journal of Basic and Clinical Reproductive Science ,4:2

Uchiyama F., Jikyo T.,Takeda R.,Ogata M (2014),Lepidium meyenii (Maca) enhances the serum levels of luteinizing hormone in female rats.Journal of Ethnopharmacology,151:897-902

Wang C.-Y., Chen Y.-W., Hou C.-Y (2019) Antioxidant and antibacterial activity of seven predominant terpenoids. International Journal of Food Properties,22:230–238.

Zaluski D, Oleh M, Galanty A, Robert V,Rafal K,Nowak R and Bogucka-Kocka (2016) Phytochemical Content and Pharma-Nutrition Study on Eleutherococcus senticosus Fruits Intractum.Oxidative Medicine and Cellular Longetivity, 2016: 9270691

Zhang L, G. Li, S. Wang, W. Yao, F. Zhu (2017). Physicochemical properties of maca starch Food. Chemistry: 56-63.

Zhou Y., Peng Li, Brantner A., Wang H., Shu X, Yang J, Si N ,Han L, Zhao H and Bian B (2017) Chemical profiling analysis of Maca using UHPLC-ESI-Orbitrap MS coupled with UHPLC-ESI-QqQ MS and the neuroprotective study on its active ingredients) Scientific Reports volume ,7 : 44660