HPLC ANALYSIS AND EFFECT OF A POLYHERBAL REMEDY ON BIOCHEMICAL PARAMETERS OF MALE WISTAR RATS

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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.

DEPARTMENT OF PHARMACOGNOSY,

FACULTY OF PHARMACY,

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CERTIFICATION

We certify that this work was carried out by ONOCHIE JOY EKENE with matriculation number PHA1603519, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria.

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DEDICATION

I dedicate this work to my siblings for their unending support throughout my stay in university of Benin.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Nature has long been a source of medicine, with traditional plant-based remedies playing a key role in modern healthcare. About 80% of the global population relies on traditional medicine. A polyherbal remedy from plant leaves including; *Moringa oleifera, Anacardium occidentale, Carica papaya, Azadirachta indica, Mangifera indica, Cymbopogon citratus, Justicia carnea, Ocimum gratissimum* and *Psidium guajava* is gaining popularity for their use in the treatment of various ailments including malaria. Although many phytochemicals present in these plants have beneficial effects, studies however need to be carried out to verify their safety and efficacy. This study aims to evaluate the safety potential of the polyherbal remedy on male Wistar rats.

Fifty (50) grams of each plant were weighed and chopped into smaller pieces and placed in a pot. Three (3L) liters of water was added and boiled for 30 minutes. This procedure was repeated for multiple extraction. After boiling, the extracts were cooled, filtered and the filtrates evaporated to dryness in porcelain dishes, over a boiling water bath. HPLC analysis was performed using a uBondapak C18 column and a UV 254nm detector. The effect of the extract on biochemical parameters was determined following a 14-day daily administration of the extract via the oral route, on male Wistar rats.

Upon concentration of the extract, a yield of 39.21g of the plant extract was obtained. The results of HPLC analysis revealed the presence of twenty (20) compounds with the most abundant being quercetin, chlorogenic acid, apigenin, mangiferine and guaijaverin. They have antioxidant, antimicrobial and anti-inflammatory properties which establish their use in the treatment of various ailments. The result of biochemical analysis showed that there was no significant difference between the control experimental rats which received distilled water (10 mL/kg) and the rats that received varying doses of the extract (200, 400 and 800 mg/kg) respectively except

for total bilirubin which was significantly lower at 800 mg/kg dose and urea which was significantly lower at 400 mg/kg and 800 mg/kg doses.

In conclusion, the presence of useful compounds in the polyherbal remedy have been established while the results of the toxicity study and effects of the extract on biochemical parameters showed that the extract maybe relatively safe on short term use. However, caution should be exercised during the use of the extract for long term purpose.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 HERBAL MEDICINE

Nature has been a primary source of medicine for as long humans can remember, providing a large portion of drugs which have been isolated from plants and other natural sources. This traditional system based on plants have continued to play a key role in modern healthcare. This is supported by statistics which estimate that 80% of the world's inhabitants rely majorly on traditional medicine for their primary healthcare (Owolabi *et al*., 2007).

Herbal remedies have been used since ancient times and their use has continued to be passed down through the generations. Nowadays, there is growing interest in traditional medicine and alternative therapies as people look for natural ways to promote health and treat illness. Herbal remedies have continued to gain popularity in recent years due to their potential benefits and fewer side effects than conventional medicine (Akinola and Obembe 2019).

One of the most widely used herbal remedies is the polyherbal remedy made from a combination of leaves of *Moringa oleifera, Anacardium occidentale, Carica papaya, Azadirachta indica, Mangifera indica, Cymbopogon citratus, Justicia carnea, Ocimum gratissimum and Psidium guajava.* This polyherbal remedy has been used by herbal practitioners for the treatment of malaria and other related illnesses in West Africa. It is believed to have potent antimalarial properties due to the presence of bioactive compounds such as flavonoids, alkaloids, and terpenoids. This study aims to investigate the effect of this herbal remedy on the biochemical parameters of male Wistar rats.

1.2 MEDICINAL PLANTS

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora 1982). Medicinal plants, for many years in the past, have been used in the treatment of many kinds of diseases. Over 5000 plants are known to be in use for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al*., 2001). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000). Natural products from plants can be another potent source for the discovery of excellent biological activities, that is: anticancer, antidiabetic, and antioxidant activities (Adebayo *et al*., 2012). Studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines (Sofowora *et al*., 2013).

Medicinal properties derived from plants can come from many different parts of a plant including leaves, roots, bark, fruit, seeds and flowers. The different parts of plants can contain different active ingredients within one plant. Thus, one part of the plant could be toxic while another portion of the same plant could be harmless. Research into the chemical constituents of plant parts has resulted in significant improvement in phytomedicine as well as medicine as a whole (Sofowora *et al*., 2013).

To ascertain safety and efficacy, modern research is focused on validating claims made about the medicinal benefits of plants used traditionally; as well as searching for newer alternatives with significant benefits over existing alternatives. Certain medicinal plants have reportedly been screened phytochemically and tested pharmacologically; however, the traditional uses of a large number of plants remain to be validated (Aziz *et al*., 2018).

The medicinal plants used for this study includes;

1.2.1 Moringa oleifera

M. oleifera Lam. belongs to the family Moringaceae and it is known by several different names in various languages such as Okwe oyibo (Igbo), Ewe ile (Yoruba), Bagaruwar maka (Hausa), Eweka (Bini) and Uhiokpa (Esan). In English, the common name for this plant is the Drumstick tree (Akinola and Obembe, 2019).

The classification of *M*. *oleifera* includes:

Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Dilleniidae, Order: Brassicales, Family: Moringaceae, Genus: *Moringa* and Species: *M. oleifera*.

Moringaceae is a family of flowering plants that includes 13 different species of Moringa. *M*. *oleifera* is the most commonly cultivated species. The family is native to tropical and subtropical regions of South Asia and Africa. *M. oleifera* is popularly known as the tree of life due to its numerous health benefits and uses in traditional medicine. It is a fast-growing, drought-resistant tree that can grow up to 10 meters tall and is grown for its leaves, pods and seeds.

Ethnomedicinal uses of Moringa leaves in community settings

This refers to the traditional ways in which people use these leaves for medicinal purposes. These traditional practices may be based on cultural beliefs, personal experiences or passed down from generation to generation.

1. Treating fever, cold and respiratory tract infections: Moringa leaves are boiled in water and the decoction is consumed to alleviate symptoms of fever and cold (Akinola and Obembe, 2019)

2. Wound healing and skin infections: Leaves of Moringa are made into paste and applied topically on wounds to promote healing and treat certain skin infections (Akinola and Obembe, 2019)

3. Digestive disorders: Moringa leaves are believed to improve digestion and are used to treat constipation, colitis and diarrhea.

4. Anemia and blood disorders: The high iron content in Moringa leaves makes it a popular remedy for anemia (Akinola and Obembe, 2019)

5. Hypertension and Diabetes: Moringa leaves are used to control blood pressure levels in people with hypertension and lower blood sugar level in people with diabetes (Akinola and Obembe, 2019)

Pharmacological uses of Moringa leaves

This refers to the scientific and medical uses of these leaves.

1. Antioxidant activity: The leaves of Moringa are rich in antioxidants which have been shown to protect against oxidative stress and damage caused by free radicals (Akinola and Obembe, 2019)

2. Anti-inflammatory properties: Moringa leaves have potent anti-inflammatory properties that can potentially help reduce inflammation-related illnesses (Akinola and Obembe, 2019)

3. Anti-diabetic effects: Moringa leaves have been shown to help lower blood sugar levels, making it a useful herbal remedy for type 2 diabetes patients (Akinola and Obembe, 2019)

4. Cholesterol-lowering effects: Moringa leaves may help decrease levels of harmful LDL cholesterol, reducing the risk of heart disease and stroke.

5. Anti-cancer properties: Recent studies have shown that Moringa leaves have anti-cancer properties that can help prevent or control the growth of cancer cells.

Figure 1: *M. oleifera* growing in its natural habitat at Isiohor community in Ovia North East Local Government Area of Edo state.

1.2.2. *Justicia carnea*

J. carnea Lindi. belongs to the Acanthaceae family. It is commonly known as the Brazilian Plume Flower. *J. carnea*, commonly referred to as Blood Justicia or Jacob's Coat, is a species of flowering plant with various regional names including Ogwu obara (Igbo), Shinfida (Hausa), Ewe ajeri (Yoruba) and Uhinze (Bini) (Akinola and Obembe, 2019).

Ethno medicinal uses of *J. carnea*

1. In India, the plant is traditionally used for the treatment of cough, bronchitis, asthma, and other respiratory problems.

2. The leaves and roots of the plant are used externally in the treatment of wounds, boils, and skin infections (Akinola and Obembe, 2019)

3. The plant is also used as a diuretic and for the treatment of gastric disorders, menstrual disorders and stomach ulcers

Pharmacological uses of *J. carnea*

The plant contains several bioactive compounds such as flavonoids, alkaloids and phenolic acids, which have demonstrated various pharmacological activities such as anti-inflammatory, antidiabetic, antimicrobial, antioxidant, antihypertensive, hepatoprotective, analgesic, immune-boosting and antifungal activities. The plant has been found to be effective in the treatment of asthma, bronchitis, and other respiratory problems due to its bronchodilator and anti-inflammatory properties.

The plant also exhibits anti-cancer potential due to the presence of several bioactive compounds that have been shown to inhibit the growth and proliferation of cancer cells (Akinola and Obembe, 2019)

J. carnea has been found to be effective against microbial pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* due to its antimicrobial properties.

Figure 2: *J. carnea* growing in its cultivated habitat at Isiohor community in Ovia North East Local Government Area of Edo state.

1.2.3. *Cymbopogan citratus*

C. citratus (DC.) Stapf. belongs to the family, Poaceae; which is also known as the grass family. The English name is Lemon grass. It is known in different cultures as Achara ehi (Igbo), Kooko oba (Yoruba), Tsauri (Hausa), Usiobun (Esan) and Usi (Bini).

Ethnomedicinal uses of *C. citratus*

1. Fever: Lemon grass tea is used traditionally for fever as it promotes sweating to reduce body temperature.

2. Headaches: Lemon grass is used to treat headaches, migraines and other forms of head pain (Akinola and Obembe, 2019).

3. Digestive issues: Lemon grass tea is used to improve digestion and to treat stomach cramps, bloating, and constipation.

4. Respiratory problems: Lemon grass has anti-inflammatory properties that make it useful in treating respiratory problems like asthma, bronchitis, and cough (Akinola and Obembe, 2019).

5. Anxiety and depression: Lemon grass tea is said to have calming effects and help treat anxiety, depression, and other mood disorders.

Pharmacological uses of *C. citratus*

1. Antimicrobial properties: *C. citratus* essential oil has been shown to have strong antibacterial and antifungal properties. It can be used to treat skin infections and respiratory infections (Akinola and Obembe, 2019).

2. Anti-inflammatory properties: It contains a compound called citral, which has anti-inflammatory properties that can help reduce inflammation and pain (Akinola and Obembe, 2019).

3. Antioxidant properties: It contains antioxidants, which help protect against oxidative stress and free radical damage in the body.

4. Anticancer properties: Some studies suggest that lemon grass may have anticancer properties and be effective against certain types of cancer (Akinola and Obembe, 2019).

5. Analgesic properties: *C. citratus* essential oil has been shown to have analgesic properties, which can help reduce pain and discomfort.

Figure 3: *C. citratus* growing in its cultivated habitat at Isiohor community.

1.2.4. *Carica papaya*

C. papaya Linn. belongs to the Caricaceae family. The English name is papaya or pawpaw while it is known as 'Mkpuru oyibo' (Igbo), 'Gwanda' (Hausa), 'Ibepe' (Yoruba), 'Agbayun' (Bini) and 'Udugbogu' (Esan).

Ethnomedicinal uses of *C. papaya* in community settings

It is used in treating digestive problems such as constipation, bloating, and inflammation, as well as menstrual cramps, skin problems, and infections. The leaves and seeds of the pawpaw plant are often used in traditional medicine to help with digestion, reduce inflammation, and alleviate pain (Farombi *et. al.,* 2014)

Pharmacological uses of pawpaw are also numerous.

Papaya contains many essential vitamins and minerals, including vitamins A, C, and E, as well as potassium, calcium, and magnesium. It also contains enzymes that help break down proteins, making it a useful digestive aid. The papaya fruit has also been shown to have antioxidant and anti-inflammatory properties, potentially making it useful for preventing or treating chronic diseases such as cardiovascular disease, cancer, and arthritis. Additionally, pawpaw has been studied for its potential use in wound healing, and its seeds have shown antifungal and antibacterial properties (Akinola and Obembe, 2019).

Overall, pawpaw has a variety of uses in both traditional and modern medicine settings, and its pharmacological properties continue to be studied for their potential health benefits (Akinola and Obembe, 2019).

Figure 4: *C. papaya* growing in its cultivated habitat at Isiohor community.

1.2.5. *Anacardium occidentale*

A. occidentale L. belongs to the Anacardiaceae family. It is a popular tropical fruit commonly referred to as Cashew. It is known in various cultures as Kachu (Igbo), Kaju (Yoruba), Kụkwà (Hausa) and Ebeahor (Bini).

Ethno medicinal uses of *A. occidentale* in community settings

This includes the following;

1. Treatment of diarrhea and dysentery: The leaves of *A. occidentale* are boiled and the resulting decoction is used to treat diarrhea and dysentery (Akinola and Obembe, 2019).

2. Management of high blood pressure: The leaves are boiled in water and the resulting infusion is used to manage high blood pressure.

3. Treatment of skin infections: The leaves are crushed and applied topically to treat skin infections such as rashes and itchiness (Akinola and Obembe, 2019).

4. Management of diabetes mellitus: The leaves are boiled in water and the resulting infusion is used to manage diabetes mellitus.

Pharmacological uses of cashew

This includes the following;

1. Antimicrobial properties: The extract from *A. occidentale* leaves and bark exhibits antimicrobial properties. The extract is effective against various pathogens such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* (Akinola and Obembe, 2019).

2. Anti-inflammatory properties: The extracts exhibit anti-inflammatory properties and can be used to manage inflammatory conditions such as arthritis. (Akinola and Obembe, 2019).

3. Antioxidant properties: The extract from the leaves and nuts contains antioxidant properties that help protect the body against damage from free radicals. *(*Akinola and Obembe, 2019).

4. Wound healing properties: The extracts have been shown to promote wound healing due to their antioxidant and anti-inflammatory properties. (Farombi *et. al.*, 2014)

Figure 5: *A. occidentale* growing in its cultivated habitat at Isiohor community.

1.2.6. *Ocimum gratissimum*

O. gratissimum L. belongs to the Lamiaceae family. It is commonly known as Scent leaf. It is known in various cultures as Nchanwu (Igbo), Efinrin (Yoruba), kamshi ganye (Hausa), Ebe-ahinhiowan (Bini) and Aramogbo (Esan).

Ethnomedicinal uses of scent leaves in community settings.

The following are some of the ethnomedicinal uses of scent leaves:

1. Relieving headaches: *O. gratissimum* is believed to have pain-relieving properties that can help alleviate headaches (Akinola and Obembe, 2019).

2. Treating fever and malaria: They are used to treat fever and malaria by boiling the leaves and drinking the water. The bitter taste of the water is believed to reduce fever and alleviate malaria symptoms.

3. Relieving digestive issues: The leaves are believed to have anti-inflammatory and anti-diarrheal properties that can help relieve digestive issues like stomach cramps, diarrhea and nausea (Akinola and Obembe, 2019).

4. Boosting appetite: The leaves are believed to have an appetite-stimulating effect and are sometimes used as a remedy for loss of appetite (Akinola and Obembe, 2019).

5. Treating respiratory issues: The leaves are believed to have anti-inflammatory and expectorant properties that can help treat respiratory issues like cough, asthma and bronchitis (Akinola and Obembe, 2019).

Pharmacological uses of scent leaves

Pharmacological studies have identified several active compounds in scent leaves that have potential therapeutic effects and include:

1. Antimicrobial activity: *O. gratissimum* have been found to have antimicrobial properties that can help fight against bacterial and fungal infections (Olaniyi *et al*., 2017).

2. Anti-inflammatory activity: The leaves contain compounds that have anti-inflammatory properties, which can help reduce inflammation in the body (Olaniyi *et al*., 2017).

3. Antioxidant activity: The leaves have been found to contain antioxidants, which can help protect cells from damage caused by free radicals (Olaniyi *et al*., 2017).

4. Anti-diabetic activity: The leaves have been found to have anti-diabetic properties, which can help regulate blood sugar levels in people with diabetes (Olaniyi *et al*., 2017).

5. Immune-boosting activity: Some compounds found in scent leaves have been found to boost the immune system, which can help fight against infections and diseases (Olaniyi *et al*., 2017).

Figure 6*: O. gratissimum* growing in its cultivated habitat at Isiohor community.

1.2.7. *Mangifera indica*

M. indica L. belongs to the family; Anacardiaceae. It is commonly known as mango in English. It is known in various cultures as Mangolo (Igbo), Mangoro (Yoruba), Maijaniya (Hausa), Eman (Bini) and Emwin (Esan).

Ethnomedicinal uses of Mango leaves in community setting

Some ethnomedicinal uses includes;

1. Treatment of Diabetes: *M. indica* are known to be an effective remedy for managing diabetes. The leaves contain a compound called tannins which help to regulate insulin levels in the body, thereby reducing blood sugar levels (Olaniyi *et. al.,* 2017).

2. Infection control: The leaves have antiseptic properties that help to prevent bacterial infections. They are used in traditional medicine to treat various kinds of infections such as boils, cuts, and wounds (Olaniyi *et. al.,* 2017).

3. Respiratory ailments: In many parts of the world, the leaves are used as a natural remedy for respiratory problems such as bronchitis and asthma. It contains compounds that help to clear the air passage and ease breathing (Olaniyi *et. al.,* 2017).

4. Digestive issues: The leaves are also used to treat digestive problems such as diarrhea, dysentery, and indigestion. It contains flavonoids that help to regulate the digestive system and prevent bloating and constipation (Olaniyi *et. al.,* 2017).

Pharmacological uses of *M. indica*

This includes;

1. Antioxidant properties: *M. indica* contains high level of antioxidants that help to prevent cell damage caused by free radicals. They are known to have a higher antioxidant activity than vitamin C.

2. Anti-inflammatory properties: The leaves contain compounds that help to reduce inflammation in the body. This makes them useful in the treatment of conditions such as arthritis, asthma, and other inflammatory disorders (Olaniyi *et. al.,* 2017).

3. Anti-cancer properties: The leaves also contain compounds that have been shown to have anti-cancer properties. Studies have shown that they can inhibit the growth of cancer cells and prevent the formation of tumors (Olaniyi *et. al.,* 2017).

4. Hypocholesterolemia properties: They contain compounds that help to reduce cholesterol levels in the blood. This makes them useful in the prevention and management of cardiovascular diseases.

In conclusion, it is evident that mango leaves have numerous ethnomedicinal and pharmacological uses that make them an essential ingredient in traditional and modern medicine. Further research is needed to fully understand their potential benefits and how to utilize them effectively. (Farombi *et. al.,* 2014).

Figure 7: *M. indica* growing in its cultivated habitat at Isiohor community.

1.2.8. *Azadirachta indica*

A. indica A. Juss. belongs to the Meliaceae family. It is commonly called Neem in English, Akum-shut-up (Igbo), Dogoyaro (Hausa), Dogon yaro (Yoruba), Ebe-eze (Bini) and Uhie (Esan).

Ethnomedicinal uses of *A. indica* in community setting

This includes;

1. Treatment of skin diseases such as rashes, acne and eczema (Li *et. al*., 2019).

2. Treatment of digestive disorders such as constipation, diarrhea and stomach ulcers (Li *et. al*., 2019).

3. Treatment of respiratory disorders such as asthma, bronchitis and coughs (Li *et. al*., 2019).

4. Treatment of parasites such as ringworms, scabies and lice (Li *et. al*., 2019).

5. Treatment of fever and malaria, diabetes and high blood pressure (Li *et. al*., 2019).

Pharmacological uses of *A. indica*

This includes;

1. Antibacterial properties that can treat infections such as *Staphylococcus aureus* and *Escherichia coli* (Li *et. al*., 2019).

2. Antiviral properties that may be effective against viruses such as HIV and herpes (Li *et. al*., 2019).

3. Antifungal properties that may be effective against fungal infections such as *Candida albicans* (Li *et. al*., 2019).

4. Anti-inflammatory properties that may reduce swelling and pain.

5. Immunomodulatory properties that may enhance the immune system's ability to fight infections and diseases (Li *et. al*., 2019).

6. Antioxidant properties that may protect against damage caused by free radicals and reduce the risk of cancer and other chronic diseases.

Figure 8: *A. indica* growing in its cultivated habitat at Isiohor community.

1.2.9. *Psidium guajava*

P. guajava L. belongs to the family of Myrtaceae. It is known as Guava in English. It is also known in various cultures as Uguava (Igbo), Gova (Yoruba), Ya'yan goba (Hausa), Uhe (Bini) and Ora (Esan). P. guajava, commonly known as guava, is a fruit-bearing tree that is native to central and South America.

Ethnomedicinal uses of *P. guajava*

The Ethnomedicinal uses include;

1. Treatment of diarrhea: The leaves of Psidium guajava are used to treat diarrhea by boiling them in water and drinking the solution (Li *et. al*., 2019).

2. Treatment of wounds: The leaves and bark of the tree are crushed and applied topically to heal wounds (Li *et. al*., 2019).

3. Treatment of cough and cold: The fruit and leaves of the guava tree are used to make a tea that is taken for cough and cold.

4. Treatment of diabetes: The leaves of Psidium guajava are used to lower blood sugar levels in diabetes patients (Li *et. al*., 2019).

5. Treatment of hypertension: The leaves of the tree are also used to lower blood pressure in patients with hypertension (Li *et. al*., 2019).

6. Treatment of skin infections: The leaves and bark of the tree are used to treat skin infections such as eczema, psoriasis, and dermatitis.

Pharmacological uses of *P. guajava*

This includes;

1. Antimicrobial activity: *Psidium guajava* has been found to have antimicrobial activity against several microorganisms, including *E. coli, Salmonella, Staphylococcus aureus* and *Candida albicans* (Li *et. al*., 2019).

2. Anti-inflammatory activity: The leaves of the guava tree have been shown to have anti-inflammatory properties, reducing inflammation in animal models (Li *et. al*., 2019).

3. Anti-diarrheal activity: The extract of the guava leaves has been found to have anti-diarrheal activity in animal models.

4. Hypoglycemic activity: The leaves of the tree have been shown to lower blood sugar levels in animal models (Li *et. al*., 2019).

5. Cardiovascular effects: The extract of the guava leaves has been found to have cardiovascular effects, including lowering blood pressure and decreasing heart rate (Li *et. al*., 2019).

Overall, *P. guajava* has numerous ethnomedicinal uses in community settings and has been researched for its pharmacological properties, showing potential as a natural remedy for various ailments.

Figure 9: *P. guajava* growing in its cultivated habitat at Isiohor community.

1.3. Traditional Uses of Herbal Remedies

Herbal remedies have been used for centuries to heal and alleviate various health issues. Historically, the use of herbal remedies has been a significant aspect of traditional medicine in various cultures around the world. Indigenous people have used medicinal plants and herbs to treat various diseases, ailments, and support general health. Many of these natural remedies remain popular in modern times. Here are some traditional uses of herbal remedies:

1.3.1 Managing Digestive Problems:

Traditional herbal remedies used for digestive problems include ginger, peppermint, chamomile, and fennel. These plants help to soothe stomach cramps, improve digestion and relieve constipation.

1.3.2 Relieving Pain:

For centuries, indigenous cultures have used herbs such as willow bark, devil's claw and arnica to alleviate pain, reduce inflammation and promote healing. In modern times, these herbs can be found in capsules, teas and creams

1.3.3 Boosting Immune System:

Echinacea, elderberry and ginseng are herbs regularly used to boost the immune system. Traditional practices that involve infusing these herbs in tea are still common. Most people use these herbs to reduce the symptoms of various infections, illnesses and diseases (Olaniyi *et. al*., 2017).

1.3.4 Addressing Sleep Problems:

Lavender, chamomile and valerian root are common herbal remedies used for insomnia relief. They have calming effects and help to promote better sleep quality.

1.3.5 Reducing Anxiety and Stress:

Herbs commonly used as natural remedies for managing anxiety and stress includes ashwagandha, chamomile, lemon balm and passionflower. People practice ingesting them using tea or supplements.

In conclusion, traditional herbal remedies have been used for centuries to help alleviate various ailments. Although the remedies may lack scientific evidence, they remain highly valued alternatives to conventional medicines for many people. However, individuals are advised to seek medical advice before applying any remedies (Olatunji *et. al.,* 2019).

1.4. Safety studies of polyherbal remedy.

Medicinal plants have been used in healthcare since time immemorial. In Africa and other developing countries, a large population relies on traditional practitioners and their medicinal plants to meet their increasing healthcare needs. In most cases, modern medicines may exist side by side with such traditional practices. Generally, these plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Although many of the phytochemicals present in plants have beneficial effects on long term health when consumed by humans and can be used to effectively treat human diseases. Studies however need to be carried out globally to verify their safety.

Herbal remedies do not differ greatly from conventional drugs in terms of how they work. This enables herbal remedies to be as important as conventional medicines, but also gives them the same potential to cause harmful side effects and other toxic effects (Tapsell 2006). Also, diagnosis, method of administration, irregularity in dosage regimen of these medicines by the herbalists and traditional healers have been highly unscientific due to their little knowledge on the pharmacology of the plant, anatomy of the human body and poor personal hygiene. The plant kingdom has many genera and species of plants containing substances of medicinal values which are yet to be discovered. Though many of such plants already worked on have been found to have pharmacological activity, studies need to be carried out to know if the beneficial effect is more than the toxic effects. Polyherbal remedies are becoming increasingly popular due to their potential efficacy and fewer side effects compared to conventional drugs. However, before any herbal remedy can be considered safe and effective for use, rigorous safety and toxicology studies must be conducted (Marwa *et. al.,* 2023).

Safety studies of polyherbal remedies involve assessing their potential adverse effects on various systems of the body. This includes evaluating the effects on vital organs such as the liver, kidneys, heart and lungs. Researchers also examine the impact on the immune system, reproductive system and gastrointestinal system. Animal models are often used to study the short-term and long-term effects of various herbal remedies (Singh *et.al.,*2023).

Toxicological studies often involve administering the herbal remedy at different doses to animals, closely monitoring their health status and analyzing various indicators of toxicity, such as biochemical markers and histopathological changes in tissues. These studies provide valuable information about the safety profile and potential adverse effects of the herbal remedy. They provide crucial information to assess the safety profile of herbal remedies before they can be

considered for integration into malaria treatment strategies. It is vital to conduct these studies in a rigorous and standardized manner to ensure the safety and efficacy of herbal remedies for malaria treatment.

In a study conducted by Shehri *et. al*., (2023), on evaluating the impact of an antimalarial herbal remedy on liver function in rats, 15% of the experimental group showed mild elevation in liver enzyme levels compared to the control group. Also, a study that involved administration of a standardized herbal extract for a duration of 90 days showed that 90% of the animals had no significant adverse effects, while 10% experienced mild gastrointestinal disturbances such as diarrhea. Another study involving the administration of an antimalarial herbal remedy to pregnant rats found that 80% of the offspring exhibited normal development without any observable abnormalities, indicating a relatively low risk of teratogenicity (Shehri *et.al*. 2023).

In a toxicological study that evaluated the effect of long-term exposure to an herbal remedy on reproductive parameters in male rats showed that 30% of the exposed rats had a decrease in sperm count and motility, indicating a moderate adverse effect on male fertility. The effects of an antimalarial herbal remedy versus a placebo in a randomized controlled trial showed that 25% of participants in the herbal remedy group had mild gastrointestinal symptoms such as nausea and bloating, compared to only 10% in the placebo group (Shehri *et.al*. 2023).

In a survey-based study conducted among individuals who regularly used antimalarial herbal remedies, it was revealed that 70% of the participants reported improvements in their malaria symptoms, such as fever reduction and reduced frequency of attacks (Shehri *et.al.,* 2023).

While antimalarial herbal remedies have the potential to offer alternative treatments for malaria, it is important to note that they should not replace standard medical care and should be used

under the guidance of a qualified healthcare professional. Additionally, the safety and efficacy of these remedies may vary depending on the region, preparation and dosage used.

1.5. HPLC Analysis of Herbal Remedies

High-performance liquid chromatography (HPLC) analysis is a powerful analytical technique widely used in pharmaceutical and biotechnology industries for the identification, separation and quantification of various components in a sample. In recent years, HPLC analysis has also been applied in the field of natural products, particularly in the characterization and standardization of herbal medicines. HPLC is especially useful for analyzing complex mixtures such as plant extracts, where multiple compounds may be present in varying concentrations. In recent times, HPLC analysis has become an essential tool in the analysis of herbal remedies. The quality and efficacy of herbal remedies are dependent on the active principles present in them. HPLC analysis can be used to determine the active components along with their concentration in herbal remedies (Farombi *et. al.,* 2014).

The HPLC analysis of herbal remedies involves the use of a stationary phase (column) and a mobile phase (solvent) along with a detector to separate and identify the different components present in the sample. The stationary phase used in HPLC is packed with a high surface area porous material that helps in the separation of the analytes based on their polar and non-polar properties. The mobile phase that is used in HPLC is usually a mixture of water and organic solvents such as acetonitrile or methanol (Farombi *et. al.,* 2014).

The HPLC analysis of herbal remedies requires sample preparation that involves the extraction of the active components of the herb using a suitable solvent. The extracted sample is then filtered and injected into the HPLC system. The sample is separated based on the polarity and solubility of the components; and the separated components are detected using a UV detector or

a Mass Spectrometer. The data obtained from HPLC analysis helps to identify the different components present in the herbal remedy and their concentration, which can be used to determine the quality and potency of the herbal product (Farombi *et. al.,* 2014).

The HPLC method is advantageous as it is a reliable, reproducible, and highly sensitive analytical technique for the analysis of herbal remedies. The information obtained from HPLC analysis of herbal remedies can be used to evaluate the safety and efficacy of herbal remedies, which can help in ensuring the quality of herbal products in the marketplace (Jegede *et. al.,* 2020).

1.6 Effect of Herbal Remedies on Biochemical Parameters

Biochemical parameters are essential measurements that help to assess the health of an organism or diagnose disease. Biochemical tests involve the measurement of various chemical substances in the body, such as proteins, enzymes, hormones, sugars, lipids, electrolytes and metabolites that play a crucial role in various physiological processes. These tests provide useful insights into the functioning of various organs and identify any abnormality or deviation from the normal range. There are a wide variety of biochemical parameters that are used in clinical settings and research laboratories. Some common biochemical tests include liver function tests, kidney function tests, lipid profile tests, glucose level tests, electrolyte tests and blood protein tests. These tests can be performed using various methodologies, including spectrophotometry, chromatography, immunoassays and electrochemical techniques (Oyeyipo *et. al.,* 2018).

The results obtained from these biochemical tests can help clinicians in diagnosing various health conditions and monitoring the progression of treatment. For example, elevated levels of enzymes such as alanine transaminase (ALT) or aspartate aminotransferase (AST) are indicative

of liver damage or disease, while abnormally high levels of cholesterol and triglycerides in the blood may be an indication of an increased risk of heart disease (Oyeyipo *et. al.,* 2018).

The increasing interest in the safety of natural remedies has led to a surge in studies on their effects on biochemical parameters in the human body. These investigations aim to understand the significant effects of the herbal medicines and to identify their potential toxic benefits. Herbal remedies have been shown to modulate various biochemical pathways in the human body. Studies have revealed that they can alter the levels of various biomarkers in the blood, such as blood glucose, lipid profile, liver enzymes, and antioxidant parameters. For example, research has shown that turmeric, green tea and flaxseed help to improve lipid profile parameters in individuals with dyslipidemia (Salaudeen *et. al.,* 2014). However, herbs such as *Tripterygium wilfordii* Hook (thunder god vine) contain diterpenoid epoxide, which induces apoptosis and has been reported to cause kidney damage. *Averrhoa carambola* L. (star fruit) contains oxalate in high quantity which can cause acute nephropathy (Allard T et.al.,2013). *Guaiacum officinale* L. (rough bark) and *Arctostaphylos uva-ursi* (cranberry) increase stone formation.

Overall, studies on the effect of herbal remedies on biochemical parameters may have shown promise in the management of various health conditions. It is however important to note that herbal remedies can have adverse effects and may interact with other medications. Therefore, it is necessary to consult a healthcare professional before including herbal remedies as part of a treatment plan. Also, the need for studies that focus on the safety and toxicity of herbal remedies in various patient populations so as to establish evidence-based guidelines for their use, should be seriously considered.

1.7. AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to evaluate the polyherbal remedy and to determine its safety profile. Specific objectives were to;

1. collect and properly identify the different medicinal plants used in this study,

2. identify the chemical constituents present in the polyherbal remedy and determine their concentration using HPLC, and

3. evaluate the effect of the herbal remedy on biochemical parameters such as liver function, kidney function and lipid profile of male Wistar rats.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1. LABORATORY EQUIPMENTS

Volumetric flask, measuring cylinder, beakers, digital weighing scale, syringes (1, 2, 5, 10 mL), oro-gastric tube, surgical dissecting kits, plain bottles, sample bottles, cotton wool, surgical gloves, ruler, pot, filter, gas cylinder, distilled water, rat cages, forceps, thimble, evaporating dish, electric water bath, e.t.c.

2.2 METHODOLOGY

2.2.1 Plant Collection and Preparation

Leaves of nine (9) plants were collected in the month of April 2023 from Isiohor community Ovia North East of Edo state. The collection took place in the morning and the leaves of the following plants were collected; *Moringa oleifera, Anacardium occidentale, Carica papaya, Azadirachta indica, Mangifera indica, Cymbopogon citratus, Justicia carnea, Ocimum gratissimum* and *Psidium guajava*. The plants were carefully selected based on their medicinal properties and use (in combination) by herbal practitioners in the treatment of various ailments including malaria. Each plant was identified by a taxonomist, Prof. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life

Sciences, University of Benin, Benin City; and its common and botanical names were recorded to ensure accuracy in subsequent analysis after which they were transported to the Laboratory of the Department of Pharmacognosy for further processing.

2.2.2 Plant Extraction

The plants were rinsed with water to remove any dirt or debris. Fifty (50) grams of each plant were weighed and chopped into smaller pieces and placed in a pot. Three (3 L) liters of water was added and boiled for 30 minutes. This procedure was repeated for multiple extractions. After boiling, the plant extracts were cooled, filtered and the filtrates evaporated to dryness in porcelain dishes, over a boiling water bath to obtain a yield of 39.21g of plant extract. The plant extract was scrapped into an amber coloured bottle and preserved in a refrigerator for subsequent reconstitution when needed.

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS

A Schimadzu (Nexera Mx) type HPLC equipment with column (ubondpak C18, 100mm long, 4.6mm internal diameter and 7um thickness), UV 254nm detector and 15mpa pump pressure was used to perform the HPLC analysis. Acetonitrile was used to extract about 10g of the material and ethyl acetate was used to stabilize the extract. It was prepared to specification by making up to the mark in a 25ml steward flask. At a flow rate of 2ml/min, five (5) milliliters of the stabilized extract was injected. A mixture of acetonitrile and water (70:30) was used as the carrier (mobile phase).

2.4 ANIMALS

Male Wistar rats weighing (180 - 200g) were obtained from the Animal house of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept in the Laboratory of the Department of Pharmacognosy for a 14-day

acclimatization period. The animals were maintained under standard conditions of temperature (26^{o}C – 28^{o}C) and natural day/night light cycle. They were kept in plastic cages, fed pelletized feed (Top feeds limited, Ibadan, Nigeria) and water allowed *ad libitum*. The bedding materials of the cages were changed daily. Ethical approval for the use of laboratory animals (Wistar rats) was sought from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin city. All experiments were carried out in accordance with the National Institute of Health Guidelines for the care and use of laboratory Animals (NIH publications No. 80-23) revised in 2002.

2.4.1 SUBACUTE TOXICITY TESTS.

Twenty (20) male Wistar rats were randomly divided into four groups (A - D) of five rats per group. The first group (A) served as the control and received water (10 mL/kg) throughout the treatment period. Group B animals (low dose) were administered 200 mg/kg/day body weight of the plant extract. Group C animals (medium dose) were administered 400 mg/kg/day body weight of the plant extract. Group D animals (high dose) were administered 800 mg/kg/day body weight of the plant extract. All administrations were done via the oral route using oro-gastric tube for 14 days. Daily observations were made for any toxic effects.

2.4.2 SPECIMEN COLLECTION

At the end of the treatment period, the rats were fasted overnight and anaesthetized in a chloroform saturated chamber. They were removed from the jar and dissected. Blood samples were collected through cardiac puncture with the aid of a 10 mL hypodermic syringe and needle. The blood samples were collected into plane bottles and taken to the Department of Chemical Pathology, University of Benin Teaching Hospital (UBTH), Benin city, Edo state where biochemical analysis of the samples was done. Biochemical parameters studied were;

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total bilirubin (TB), Conjugated bilirubin (CB), Total protein (TP), Albumin (ALB) and Globulin (GLO), Sodium ion (Na^+), Potassium ion (K^+), Bicarbonates (HCO_3^-), Chlorides (Cl^-), Urea and Creatinine.

2.5 STATISTICAL ANALYSIS

Graph Pad Instat Version 2.05 software (UK) was used. The values were expressed as Mean ± S.E.M. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett comparism tests. P values < 0.05 were considered significantly different from control.

CHAPTER THREE

3.0 RESULTS

3.1 RESULT OF EXTRACTION

Upon concentration of the extract, an extract yield of 39.21g was obtained..

3.2 RESULT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF THE SAMPLE

The results of the HPLC analysis are shown in Figure 10 and Table 1 below. The extract contained twenty (20) compounds of which the most abundant were quercetin, chlorogenic acid, apigenin, mangiferine and guaijaverin, respectively.

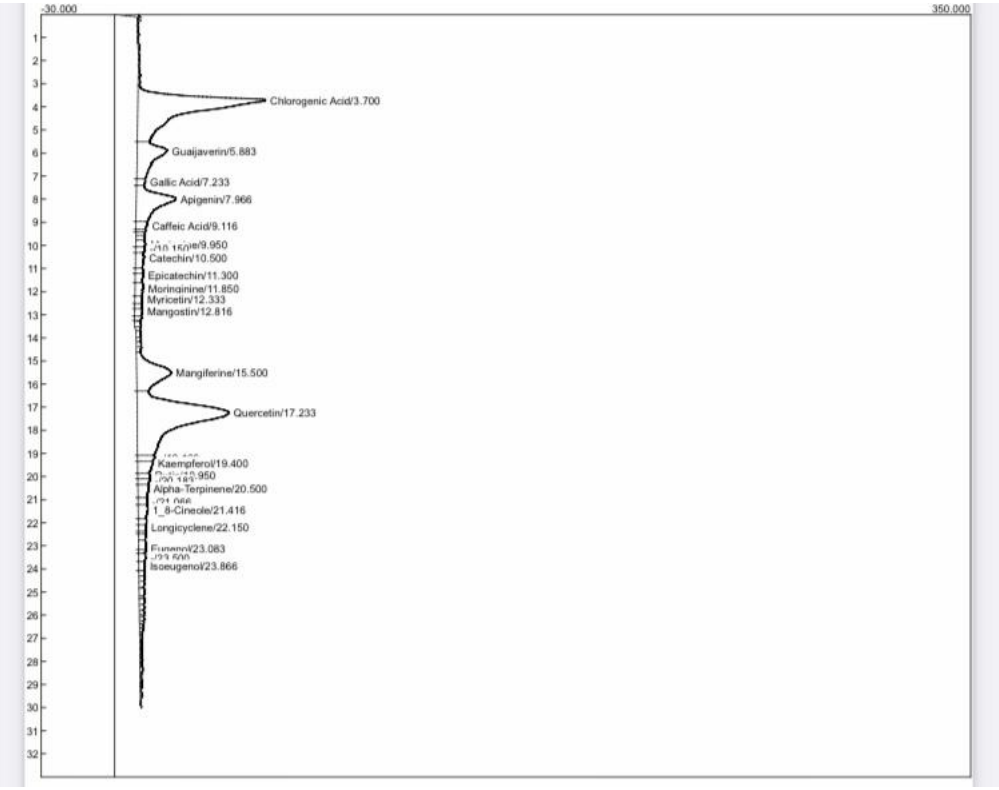

Figure 10: HPLC chromatogram of the polyherbal extract showing the presence of twenty compounds.

Table1: Retention times, areas and heights of the constituents present in the polyherbal extract chromatogram of the HPLC

Component	**Retention**	**Area**	**Height**
Chlorogenic Acid	3.700	2554.3730	52.191
Guaijaverin	5.883	680.5950	12.352
Gallic Acid	7.233	57.4905	3.633
Apigenin	7.966	803.7650	16.191
Caffeic Acid	9.116	86.5150	4.690
Moringine	9.950	67.7590	4.080
Catechin	10.500	126.7400	3.697
Epicatechin	11.300	74.0100	3.359
Moringinine	11.850	107.5940	3.510
Myricetin	12.333	58.8340	3.075
Mangostin	12.816	56.3985	3.211
Mangiferine	15.500	802.9260	14.229
Quercetin	17.233	2828.8535	37.633
Kaempferol	19.400	174.2350	6.396
Rutin	19.950	73.4300	5.139
Alpha Terpinene	20.500	138.2840	4.473
1_8-Cineole	21.416	133.0180	4.019
Longicyclene	22.150	52.6995	3.269
Eugenol	23.083	73.1080	3.090
Isoeugenol	23.866	55.5920	2.638

3.3 SUBACUTE TOXICITY TEST

This includes effects of the polyherbal extract on biochemical parameters such as liver function and kidney function parameters.

3.3.1 EFFECT OF POLYHERBAL EXTRACT ON LIVER FUNCTION PARAMETERS

From Table 2 below, it could be said that the extract did not significantly affect the values of the various liver enzymes when compared to control. However, a significant difference ($p < 0.05$) was observed when the high dose of the extract was compared with the control, for total bilirubin.

Table 2: Effect of the polyherbal extract on Liver function parameters of male Wistar rats

Parameters	TREATMENT GROUPS			
	10ml/kg (Control)	200mg/kg (Low dose extract)	400mg/kg (Medium dose extract)	800mg/kg (High dose extract)
ALP	624.4±76.70	637.4±76.40	753.2±90.47	447.6±22.58
ALT	94.8±12.80	93.8±13.04	86.4±13.20	71.4±10.76
AST	219.6±21.74	207±18.22	215.4±14.88	181.6±21.99
TB	0.36±0.02	0.3±0.04	0.3±0.03	0.22±0.02*
CB	0.12±0.02	0.14±0.02	0.12±0.02	0.1±0.00
TP	6.68±0.12	6.34±0.37	6.28±0.38	6.58±0.41
ALB	3.36±0.08	3.2±0.24	2.82±0.16	3.16±0.25
GLO	3.32±0.07	3.14±0.14	3.46±0.30	3.42±0.21

Key: Values are Mean ± SEM. N = 5. *$p < 0.05$, significantly different from control.

3.3.2 EFFECT OF THE POLYHERBAL EXTRACT ON UREA, ELECTROLYTE AND CREATININE

From Table 3 below, it could be said that the extract did not significantly affect the values of the various kidney parameters when compared to control. However, a significant difference ($p <$

0.05) was observed when the medium and high doses of the extract were compared with the control, for urea.

Table 3: Effect of the polyherbal extract on kidney parameters of male Wistar rats

Parameters	TREATMENT GROUPS			
	10ml/kg (Control)	200mg/kg (Low dose extract)	400mg/kg (Medium dose extract)	800mg/kg (High dose extract)
Urea	43.6±1.25	41.6±2.77	34.8±1.43*	35±1.87*
Na^+	141.2±0.97	139.8±0.97	140.2±0.73	139.4±0.68
K^+	5.4±0.26	4.98±0.14	5.34±0.21	4.96±0.19
Cl^-	103.2±0.58	102.2±1.16	101.6±1.03	100±0.84
HCO_3^-	19.6±0.68	20.6±0.93	20.8±0.73	20.4±0.81
Creatinine	0.94±0.02	0.88±0.08	0.78±0.07	0.76±0.06

Key: Values are Mean ± SEM. n = 5. *$p < 0.05$, significantly different from control.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

High Performance Liquid Chromatography (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. From the results, the following components were identified in the polyherbal extract.

Quercetin belongs to a group of plant pigments called flavonoids that gives many fruits and vegetables their colours. Flavonoids, such as quercetin are antioxidants that can neutralize free radicals. Quercetin may help protect against heart disease and cancer. Quercetin can also help stabilize the cells that release histamine in the body and thereby have an anti-inflammatory and antihistamine effect (Anand *et al.,* 2022). Chlorogenic acid is the ester of caffeic acid and quinic acid, found in high concentrations in coffee. Chlorogenic acid and its related compounds exhibit activities in extensive biological profiles such as antidiabetic effect, DNA protective effect and neuroprotective effect. Moreover, chlorogenic acid also shows inhibitory activity against hepatitis B virus (HBV). As an antioxidant, it also possesses liver protection activity and suppresses carcinogenesis (Wang *et al.,* 2016). Apigenin is a dietary flavonoid found in many plants such as parsley, celery, basil, chamomile tea and kumquats like fruits and vegetables. It has antioxidant, antiproliferative, antimicrobial and anti-inflammatory activities which are the basis for its therapeutic potentials. Apigenin is a flavone considered to be safe even in a high dose and so far, no toxicity of the molecule has been reported (Kaul & Kaur, 2022)

Mangiferine is a xanthone present in significant levels in higher plants and in different parts of the mango fruit. It is a promising antioxidant with health-related properties such as antiviral,

anticancer, antidiabetic, antioxidative, antiaging, immunomodulatory, hepatoprotective and analgesic effects (Zhang *et al.,* 2020). Guaijaverin is a flavonoid compound found in guava leaves. It has several health benefits such as antioxidant, antibacterial, anti-inflammatory and anti-diabetic properties. It can be used as a natural remedy for a variety of conditions including infections, inflammation, diabetic nephropathy and cancer (Kumar & Pandey, 2013). Gallic acid is a phenolic compound found both as a free state and as a constituent of tannins, namely, gallotannin. Gallic acid and its derivatives are present in nearly every part of a plant such as bark, wood, leaf, fruit, root and seed. Gallic acid is a well-known natural antioxidant and a very important common antioxidant tea formulation, known as an Ayurvedic herb. The antihyperlipidaemic action of gallic acid in high-fat diet induced mice revealed a decrease in triglyceride and low-density lipoprotein-cholesterol; and an increase in high-density lipoprotein-cholesterol. Also, the cardioprotective action of gallic acid in isoprenaline-induced cardiotoxicity and streptozotocin-induced diabetic rats revealed a protective effect with respect to several biochemical and histopathological parameters (Zanwar *et al.,* 2014).

Caffeic acid is an organic compound. It is a type of polyphenol, a class of compound known for their antioxidant properties. It can be found naturally in a wide range of plants. The compound is claimed to have many health benefits including anti-inflammatory, anticancer and antiviral potentials. It may help boost the performance of athletes. However, it isn't considered "essential" for human health. In other words, you don't need it to survive. The most common source of caffeic acid in the human diet is from drinking coffee. It's also found in certain vegetables, fruits and herbs. Catechins are polyphenolic compounds belonging to the flavanol subfamily of flavonoids. The most abundant dietary sources of catechins are green tea, cocoa products and wine. Catechins have been shown to possess beneficial effects in many pathological conditions

including cardiovascular diseases, which are currently one of the main causes of mortality in the world. Catechins are potent antioxidants, although under pathological conditions, they act as prooxidants, modulating signal transduction, inflammation and cell death regulation pathways. Furthermore, catechins may regulate metabolic processes via direct effects on mitochondria, which are responsible for energy supply in the cells. Epicatechin is a dietary polyphenol exerting beneficial effects on the cardiovascular system. The most prominent sources of epicatechin are cocoa products, red wine as well as green and black tea. Epicatechin has certain beneficial effects which include improving insulin sensitivity, regulating blood sugar levels and stimulating muscle protein synthesis. It helps in lowering cholesterol due to its antioxidant properties and improves brain and heart health (Adebayo *et al.,* 2020).

Moringine is a stable isothiocyanate compound found in the leaves and seeds of the *M. oleifera* plant. Isothiocyanates are a class of plant compounds that have been shown to have a variety of biological activities including antioxidant, anti-inflammatory and anticancer effects. Moringine has been shown to have a number of potential health benefits including; lowering blood sugar levels, protecting against heart disease, boosting immunity, relieving pain and improving skin health. Moringine is generally safe for most people to consume in moderate amounts. However, it is important to note that moringine can interact with certain medications such as blood thinners and diabetes medications (Morinaga & Nishisaka, 2011). Myricetin is a flavonoid compound found in a variety of fruits, vegetables, berries, teas and red wine. It is a powerful antioxidant with a wide range of potential health benefits which includes; reducing the risk of cancer, protecting against heart disease, improving cognitive function and boosting immunity. Mangostin is a polyphenolic compound found in the rind of mangosteen fruit. It is a member of the xanthone family of compounds, which are known for their antioxidant and anti-inflammatory

properties. Mangostin has been shown to have a number of potential health benefits including; reducing inflammation, protecting against cancer, improving gut health, boosting immunity and improving skin health (Adebayo *et al.,* 2020).

Kaempferol is a flavonoid compound found in a variety of fruits, vegetables and herbs; including broccoli, onions, apples, grapes and green tea. It is a powerful antioxidant with a wide range of potential health benefits as it reduces the risk of cancer, protects against heart disease, improves cognitive function, boosts immunity and reduces inflammation. Alpha-terpinene is a monoterpene, which is a type of organic compound found in many plants, including cannabis. It has a number of potential health benefits including; anti-inflammatory, antibacterial and antifungal, anxiolytic and analgesic properties. Alpha-terpinene is also thought to have potential benefits for respiratory health. It may help to clear mucus from the lungs and airways; and it may also help to reduce inflammation in the respiratory system. Rutin is a flavonoid glycoside, which is a type of plant compound that is made up of a flavonoid and a sugar molecule. Rutin is specifically composed of the flavonoid; quercetin and the disaccharide, rutinose. Flavonoids are a large group of plant compounds that have many health benefits. They are antioxidants; which means they can help to protect cells from damage caused by free radicals. Free radicals are unstable molecules that can damage cells and contribute to the development of chronic diseases such as cancer and heart disease. Rutin has a number of specific health benefits as it strengthens blood vessels and reduces the risk of bruising and bleeding, improves circulation and reduces the risk of blood clots. It has anti-inflammatory and antioxidant properties. It may help to protect against cancer and heart diseases; and help to improve blood sugar control in people with diabetes (Li, & Yao, 2013).

1_8-Cineole also known as eucalyptol, is a natural organic compound found in many plants including eucalyptus, mint and rosemary. It is the main component of eucalyptus essential oil, which is used for a variety of purposes, including medicine. 1, 8 - cineole has a variety of biological properties including; anti-inflammatory, antimicrobial, mucolytic, bronchodilator and analgesic properties. It can be used to treat a variety of conditions including respiratory illnesses, pain, infections and Nausea and vomiting. 1, 8 - cineole is generally considered safe to consume in moderate amounts. However, there are some potential side effects such as headache, nausea and vomiting, diarrhea and allergy. Longicyclene is a sesquiterpene, a type of organic compound found in many plants, including cannabis. It has a number of potential health benefits, including; Anti-inflammatory, analgesic, antioxidant and anticancer properties. However, more research is needed to confirm the safety and efficacy of longicyclene in humans (Adams *et al.,* 1963).

Eugenol is a natural compound found in many plants, including cloves, cinnamon, nutmeg, and basil. It has a strong, spicy aroma and is often used as a flavoring agent in food and beverages. Eugenol also has a number of potential health benefits, including anti-inflammatory, antioxidant and Anticancer properties (Owolabi *et al.,* 2011). Isoeugenol is a naturally occurring organic compound that is found in the essential oils of many plants, including ylang-ylang, nutmeg, cinnamon, and cloves. It is a colorless liquid with a sweet , spicy aroma. Isoeugenol has a number of medicinal properties, including antimicrobial, anti-inflammatory, and analgesic activity. It is used in some topical over-the-counter medications to relieve pain and inflammation. Isoeugenol is also being investigated for its potential use in treating a variety of other medical conditions, including cancer, Alzheimer's disease, and depression. Isoeugenol is generally considered safe for human consumption when used in the amounts typically found in food and beverages. However, some people may experience allergic reactions to isoeugenol. High doses

of isoeugenol can also cause side effects such as nausea, vomiting, and dizziness (Obame *et al*., 2015).

From the sub-acute toxicity study carried out, results from the liver function tests clearly showed that the polyherbal extract of the plant when administered to male Wistar rats at doses of 200 (low), 400 (medium) and 800 mg/kg/day (high) body weight of the extract, respectively; did not cause any significant changes in the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), conjugated bilirubin (CB), total protein (TP), albumin (ALB) and globulin (GLO). This however, was not the case with total bilirubin (TB) which was significantly different ($p < 0.05$) from control, at the 800 mg/kg dose.

The liver function test is a test carried out to ascertain the health state of the liver by measuring the levels of proteins, liver enzymes or bilirubin in the blood. Their levels in the blood are a reasonably sensitive indicator of liver damage or injury from different types of diseases or conditions. However, it must be emphasized that higher-than-normal levels of these liver enzymes should not be automatically equated with liver diseases. They may or may not mean liver problems (Lala *et al*., 2023).

Although it was observed that there was a significant difference when the high dose of extract was compared with the control for total Bilirubin, lower-than-usual bilirubin levels are usually not a concern. In fact, since higher levels of bilirubin may indicate liver disease as the liver is not clearing bilirubin properly, it can be said that this plant extract help to reduce bilirubin levels. Hence, it may be hepatoprotective though further studies still needs to be carried out to ascertain this claim. These findings align with a previous study in 2018 on the safety of *Cymbopogan citratus,* as it was found to significantly reduced liver enzymes (Adebayo *et al*., 2013)

Also, the levels of electrolytes and creatinine did not show any significant difference between the control and treated groups; except for urea, which was significantly different ($p < 0.05$) from control, at medium dose (400 mg/kg) and high dose (800 mg/kg). This may indicate malnutrition or starvation, due to increase metabolic activity, resulting in increase demand for nutrition. However, hepatic function was well preserved by the polyherbal extract, in the rats. This was indicated by the serum levels that were comparable to that of the control (Tarkang *et al*., 2014).

4.2 CONCLUSION

In conclusion, the plant extract contains various useful phytochemicals such as phenolics, terpenes and xanthones, that maybe responsible for their use in the treatment of various ailments like malaria. The results of the toxicity study and effects of the extract on biochemical parameters showed that, the polyherbal extract of the various plants maybe relatively safe on short term use. However, caution should be exercised during the use of the extract for long term purposes.

4.3 SUGGESTIONS FOR FURTHER WORK

To fully validate the toxicity profile of the polyherbal remedy used in this study, further studies on the subchronic and chronic toxicities profiles of the extract should be done. Haematological, biochemical and histological evaluations should be done during the long term exposure of rodents (both sexes) to the polyherbal remedy.

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