EFFECT OF BIHERBAL AQUEOUS EXTRACT OF *Ocimum gratissimum* AND *Psidium guajava* LEAVES ON HEAMAOLOGICAL PARAMETERS

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BY

Augustina IYAHEN (Miss)

SR/2205/RPR/24/51

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY,

FACULTY OF LIFESCIENCES

UNIVERSITY OF BENIN

BENIN CITY,

FEBRUARY, 2025.

EFFECT OF BIHERBAL AQUEOUS EXTRACT OF *Ocimum gratissimum* AND *Psidium guajava* LEAVES ON HEAMAOLOGICAL PARAMETERS

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BY

Augustina IYAHEN (Miss)

SR/2205/RPR/24/51

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, IN PARTIAL FULULLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY

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FEBRUARY, 2025.

CERTIFICATION

We certify that this project work was carried out by **Augustina IYAHEN (Miss)** in the Department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Edo state Nigeria.

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Dr. J.O.Erhabor **Date**

(Project Supervisor)

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Prof. E. D. Vwioko **Date**

(Head of Department)

DEDICATION

This project work is dedicated to GOD ALMIGHTY.

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I am highly grateful to GOD ALMIGHTHY whom in his infinite mercy and love saw me throughout the entire period of this research.

To my supervisor, Dr J.O. Erhabor for his supportive and supervising effort to make this project work achievable. I sincerely appreciate you, Sir.

A sincere gratitude goes to Prof. E. D. Vwioko (The head of the Department of Plant Biology and Biotechnology). Also, to my lecturers that groomed me this far. To Prof. Henry Adewale Akhinibosun, Prof. Aigbokhan, and Dr. Theophilus for their assistance towards this work.

My unreserved appreciation to my lovely parent's Pastor Charles Iyahen and Evangelist Kate Iyahen, for their consistent prayers, financial support and welfare your efforts cannot be undermined. To my colleagues David, Clara, Faith and Grateful, I appreciate you all, for your steady and corporate understanding. Thanks to the academic and non-academic staff of the Department of Plant Biology and Biotechnology for their contribution, in one way or the other, to the success of this program. Many I may acknowledge, but few I can never forget in the midst of many, my special appreciation goes to my beloved brothers and sisters; Destiny, Favour, praise and Light Iyahen,

To my boss, C.E.O Starfield consult; Mr. Bobby Emmanuel Owie for his unceasing financial and moral support throughout this programme. To my special one, G.E. Bienose for your emotional support, welfare and understanding during this programme.

Finally, I will like to appreciate my friends, I say thanks, I am proud to say you are a sign of inestimable value. Thank you all for standing by me.

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ABSTRACT

Psidium guajava L. is a tropical shrub tree and food plant that belongs to the Myrtaceae family. It is an economically important food plant with diverse medicinal properties. In traditional medicine it is used to treat and manage cough, diarrhoea, brain dysfunction, heart diseases, cancer, diabetes and so on. *Ocimum gratissimum* L. of the family Lamiaceae is popularly known as basil and is a shrub found in tropical and subtropical regions. This study aimed to investigate the effect of biherbal aqueous extract of *Psidium guajava* and *ocimum gratissimum* leaves on haematological parameters in rats. The haematological parameters include; white blood cells (WBC) and its differentials, red blood cells (RBC), Haemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RCDW), White blood cells (WBC), Lymphocyte Count (LYM) Platelets (PLT), Platelet crit (PCT), Platelet density width (PDW), and Mean platelet volume (MPV) in Wistar rats. Graded doses (25, 50 and 100 mg/kg) of the biherbal aqueous extract were orally administered to explore the heamatopathological effect of the plant extract. The result from this present study showed a significant increase across graded doses, precisely at 50 and 100 mg/kg in white blood cell count and its differentials (WBC= 6.10, 6.20 10^3/µL; MID = 8.45, 6.50, 9.00 %; LYM = 6.75, 5.45, 5.30 10^3/µL). The result obtained from the red blood cells and its component had a slight significant increase in RBC at (7.66, 7.75, 7.44 10^6/µL; MCV = 54.70, 57.60, 58.10 µM^3; RDWS = 36.30, 39.55, 37.37 µM^3). Also, the platelet and its factors displayed a significant increase across graded doses of the extract onf the PLT (178418.00, 222510.00, 8645.00 10^3 µ/L; PDW (13.80, 12.80, 12.65 %); and PCT (1.59, 2.35, 0.98 %) compared with the control. Conclusively, the biherbal aqueous extract across the graded doses probably has a hematoprotective effect with corresponding ethnomedicinal value.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Globally, medicinal plants and their bioactive constituents are used to treat various diseases. It has been reported that over 80% of the world's population uses medicinal plants or its bioactive compounds to prevent, manage, or treat several diseases (Joshi, 2013; Pant, 2014, Ugbogu *et al.,* 2021). Recently, the uses of medicinal plants or their biologically active compounds have attracted the attention of many scientists/researchers because of their use in drug discovery or the discovery of natural constituents for therapeutics (Dimmito *et al.,* 2021; Sinan *et al.,* 2021) and in ethnomedicinal uses for the treatment of life-threatening diseases such as cancer, diabetes, and hypertension (Sofowora *et al.,* 2013, WHO., 2019).

Plants are used as sources of medicine, and they serve as the oldest health care provider known to man. All cultures throughout history have used herbs and have remained an integral part of the development of modern civilization (Lewis, 2003). The World Health Organization estimated that up to 80% of the world population in developing countries depends on locally available plant resources for primary health care since Western pharmaceuticals are often expensive, inaccessible, or unsuitable (Nostro *et al.,* 2000). Plant phytochemicals have shown a pivotal pipeline in pharmaceutical discovery (Nostro *et al.,* 2000). It stimulated scientific interest in biological activities because of their phytoconstituents (Moghadamtous *et al.,* 2014; Moghadamtous et al., 2015; Al-Daihan *et al.,* 2013). They are used for several curative purposes because of their therapeutic importance (Nostro *et al.,* 2000).

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According to the WHO, herbal medicines are finished and labeled as medicinal products that contain active ingredients. In Nigeria, plants have been widely used as an alternative medicine since the event of orthodox medicine is prone to drug resistance, side effects, non-economical, and non-readily available. Hence, herbal remedies with plant-based medicine have been scientifically proven to have potency, availability, less or no adverse effect, and are very economical (Burke *et al.,* 2017). This classification has helped World Health Organization set a standard in evaluating herbal products' possible safety, efficacy, and quality (Félix-Silva *et al.*, 2014).

Benefits of herbs as medicine serves as the oldest form of healthcare associated with humanity and cultures throughout history (Barnes *et al.,* 2007). Early humans acknowledged their reliance on nature for healthy life, since at that time they solely depended on plant the diversity resources for food, clothing, shelter and medicine to cure myriads of ailments. Led by instinct, taste and experience, primitive men and women treated illness by using plants, animal parts and minerals that were not part of their usual diet. Polyherbal preparations are herbal preparation of multiple herbs.

Plant formulation and combined extracts of plants are used as a drug of choice rather than individual drugs (Chandraprakash and Swarnali, 2013); this could be linked to the fact that the particular plant parts making up the polyherbal formulation could act in synergy or have a potentiating effect. Several works have been reported on the effectiveness of polyherbal formulations. In a work on the development and evaluation of an analgesic polyherbal formulation containing some indigenous medicinal plants, the formulation was found to have a significant ($p < 0.05$) analgesic activity in a dose-dependent manner (Gomase *et al.,* 2011).

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1.2 LITERATURE REVIEW

The use of herbal extracts in traditional medicine has been practiced for centuries, with various plants being utilized for their therapeutic properties. Two of such plant scent leaf (*Ocimum gratissimum*) and Guava (*Psidium guajava*), have been widely used because of their medicinal properties.

Psidium guajava

Psidium guajava L. is commonly known as guava. It is a tropical shrub tree and food plant that belongs to the Myrtaceae family (Ravi and Divyashree, 2014). It grows up to 10 m and is widely distributed in many countries. *Psidium guajava* L. is an economically important food plant with diverse medicinal properties. It has a short trunk, and a patchy, smooth, and peeling bark. The leaves are fleshy dark green with prominent veins (Morais-Braga *et al.*, 2016a, Naseer *et al.*, 2018). It has white flowers, and the fruit contains pulp and small hard seeds.

Plate 1: Leaves and fruit of *Psidium guajava* (Source: Naseer *et al.*, 2018)

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1.3 Taxonomy of Psidium guajava

It belongs to the phylum Magnoliophyta, class Magnoliopsida and Myrtaceae family (Dakappa *et al.,* 2013). It has about 133 genera and more than 3,800 species. *Psidium guajava* and it's all parts have an old history of medicinal value (Nwinyi *et al.,* 2008). The plant is well known by a common name "Guava" in English, guayabo in Spanish, goyaveandgoyavier in French, guyabaorgoeajaab in Dutch, goiaba and goaibeira in Portuguese and jambubatu in Malaya. Pichi, posh and enandi are the names commonly used in Mexico and America (Morton *et al.,* 2004).

1.4 Phytochemical constituents of *Psidium guajava*

Psidium guajava consists of important chemical constituents such as flavonoids, tannins, phenols, alkaloids, triterpenes, saponins, carotenoids, lectins, vitamins, carbohydrates, fibre fatty acids, and glycosides (Gutiérrez *et al.,* 2008, Weli *et al.,* 2019). The leaves have a plethora of beneficial phenolic compounds such as guaijaverin, quercetin, kaempferol, apigenin, catechin, chlorogenic acid, hyperin, gallic acid, epicatechin, myricetin, caffeic acid, and epigallocatechin gallate (Kumar *et al.,* 2021).

1.5 Biological activity of *psidium guajava*

Recent studies have validated the use of *Psidium guajava* plant for traditional medicine, revealing a range of biological activity and phytochemical properties. Below are lists of them:

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1.5.1 Antimicrobial activity

Guava has a high antimicrobial activity. Guava leaf extract doses can reduce the amount of cough due to its anti-cough activity. Aqueous, chloroform and methanol extract of leaves can reduce the growth of different bacteria. Due to its anti-cough activity, it is recommended in the condition of cough (Venkatesan *et al.,* 1999).

1.5.2 Anti-diarrheal activity

Guava has high cytotoxicity (Teixeira *et al.,* 2003). Guava can treat diarrhea caused by E. coli or S. aureus toxins (Vieira *et al.,* 2001). Diarrhea is one of the most common and well-recognized health problems and a global issue. It is very common even in developed countries. It is estimated that about 2.2 million people die annually from diarrhea; most of them are children or infants (Venkatesan *et al.,* 2005).

Guava leaves have quercetin-3-arabinoside and quercetin which can be isolated from leaves. Its leaves contain a compound which has morphine like action It controls the muscular tone. Quercetin repressed intestinal contraction encouraged by enhanced absorption of calcium. Guava extract had anti diarrhoeal activity and it can be used for the treatment and prevention of diarrhoea (Ojewole *et al.,* 2008). Guava have significant antidiabetic and antidiarrhoeal activities in ethanolic extracts (Mazumdar *et al.,* 2015, Birdi *et al., 2010)*.

1.5.3 Anti-inflammatory activity

Extract of guava in ethyl acetate can stop the germ infection and thymus production. It can act as anti-viral agent. It can enhance the mRNA expression. Guava can alter the heme oxygenase-1 protein's work. Due to this reason, it can be used as anti-inflammatory agent for skin. Phenol is an important compound which is present in guava and dependable for the anti-allergic and anti-

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inflammatory activity (Denny *et al.,* 2013). The doses of guava extracts are effective in liver damage inflammation and serum production (Roy *et al.,* 2006)

1.5.4 Antioxidant activity

Guava is highly rich in antioxidants, which help decrease the incidence of degenerative diseases such as brain dysfunction, inflammation, heart disease, cancer, arteriosclerosis, and arthritis (Feskanich *et al.,* 2000). In fruits, the most abundant oxidants are polyphenols and ascorbic acid. The polyphenols are mostly flavonoids and are mainly present in glycoside and ester forms [Fleuriet *et al.,* 2003). The free ellagic acid and glycosides of apigenin and myricetin and are found to be present in guava (Koo *et al.,* 2001).

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1.5.5 Traditional/ethnomedicinal uses

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In ethnomedicine, the various parts of *Psidium guajava* – the stem, bark (Beidokhti *et al.,* 2020), fruits, leaves, and roots (Weli *et al.,* 2019) are used in the treatment of diseases such as diarrhea, rheumatism, and diabetes (Gutiérrez *et al.,* 2008, Morais-Braga *et al.,* 2016b, Díaz-de-Cerio *et al.,* 2017), digestive problems, laryngitis, ulcers, malaria, cough, and bacterial infections (Ravi and Divyashree, 2014, Díaz-de-Cerio *et al.,* 2017), wound healing and pain relief (Metwally *et al.,* 2010). Many natives consume decoction, infusion, and/or boiled preparations of *Psidium guajava* either orally or topically depending on the type of illness (Díaz-de-Cerio *et al.,* 2017).

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For instance, *Psidium guajava* leaves can be applied on wounds whereas aqueous leaf extract can be orally consumed to lower the blood glucose level in diabetic patients (Gutierrez *et al.,* 2008). Different countries have unique applications for medicinal purposes for the various parts (i.e., roots, leaves, bark, stem, and fruits) of the guava plants. These parts have been utilized for

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treating stomach-related diseases, diabetes, diarrhea, and other forms of disease conditions (Kumar *et al.,* 2021). Asian countries have adopted guava leaves to develop traditional medicines for treating diabetes (Kumar *et al.,* 2021). Indonesians use guava leaves, pulp, and seeds to treat respiratory and gastrointestinal disorders and increase blood platelets in dengue fever patients (Laily *et al.,* 2015). Nigerians employ decoction of the plant for treating microbial infections. In Mexico, Brazil, Philippine, and Nigeria, P. guajava is used to prepare a poultice for skin and wound applications (Gutiérrez *et al.,* 2008).

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1.6 Ocimum gratissimum

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Ocimum gratissimum L. belonging to the family Lamiaceae is popularly known as basil and is a shrub found in tropical and subtropical regions of Asia, Africa, and South America (Padalia *et al.,* 2013). It is used as a condiment in cooking, sedative and anxiolytic, and in traditional medicines for headache and abdominal pain, cough, cold, and bronchitis (Silva *et al.,* 2015a; Ashokkumar *et al.,* 2020). Its essential oil has been used as an anesthetic (Silva *et al.,* 2012; Silva *et al.,* 2015a), antinociceptive (Rabelo *et al.,* 2003; Sahouo *et al.,* 2003; Paula-Freire *et al.,* 2013), anti-inflammatory (Sahouo *et al.,* 2003), antioxidant (Pereira and Maia, 2007; Vasconcelos *et al.,* 2021), insecticide (Nguemtchouin *et al.,* 2013), and antimicrobial against bacteria and fungi (Vasconcelos *et al.,* 2021; Franco *et al.,* 2007; Matasyoh *et al.,* 2007; Adjou *et al.,* 2013; Dambolena *et al.,* 2010).

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For scent leaf. Its leaves have been used in local dishes over the years, with documented evidence of varying pharmacological uses of its essential oil. The essential oil of *Ocimum gratissimum* bears the scent of the plant and other important properties such as protectithe plant from pests and attracting insects for cross-pollination. The essential oil of this plant has been

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noted to possess various pharmacological properties which explains its use in inflammatory conditions and the treatment of diarrhoeal disease, wound healing and cerebrovascular disorder, amongst others. *Ocimum gratissimum* is called “Nchanwu” and “scent leaf” in Igbo language and English language and widely available in our locality, used in preparing many local dishes in Nigeria most especially South-eastern Nigeria. It is native to Africa, Madagascar, Southern Asia, Mexico, Panama, Brazil etc. (Orwa *et al.*, 2009). There is the eugenol and the thymol subtypes. The Eugenol subtype being more prevalent in India and while the thymol subtype is prevalent in Africa and parts of South America (De Castro *et al.*, 2019). The various species of *Ocimum gratissimum* usually produce essential oil but are mainly used as vegetables for food in local delicacies.

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Plate 2: Whole plant of *Ocimum gratissimum* (Photo credit: Truelove seeds)

1.7 Taxonomy of *Ocimum gratissimum*

Ocimum gratissimum is a plant of the Lamiaceae family are mostly classified as spices, herbs, and other aromatic variations. The Lamiaceae family comprises 236 genera and 7200 species of vines, shrubs, and trees found all over the globe. The genus *Ocimum* comprises about 60 species, most of which are found in Africa (Tanko *et al.,* 2008). *Ocimum gratissimum* can be found in many forms and oftentimes classified into different species and subspecies.

1.8 Phytochemical constituents of *Ocimum gratissimum*

The phytochemical constituents of *Ocimum gratissimum* vary from country to country and season to season. Joshi (2021) described differences in the constituents of *Ocimum gratisssimum* obtained during summer and winter. The constituents that are central in the essential oil of *Ocimum gratissimum,* irrespective of season or country of origin include tannins, flavonoids and saponins. Other constituents such as eugenol and thymol vary according to the country of location and the season of harvest of the leaves for essential oil extraction.

1.9 Biological activity of *Ocimum gratissimum*

Recent studies have validated the use of *Ocimum gratissimum* plant for traditional medicine revealing a range of biological activity. Below are lists of them:

1.9.1 Antioxidant effect

The antioxidant *Ocimum gratissimum* is proven. In vivo studies on rats and mice showed significant inhibition of peroxidase activity and ferrous sulphate when compared with a known antioxidant like vitamin C (Njoku *et al,* 2011). It may help to reduce the effects of oxidative stress in the body. The antioxidant and anti-inflammatory properties of *Ocimum gratissimum*

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have been ascribed to its therapeutic benefits (Olamilosoye *et al.,* 2019; Oyem *et al.,* 2021). Its leaf extracts have been shown to contain antioxidant vitamins such as alpha-tocopherol and ascorbic acid (Olamilosoye *et al.,* 2019). Previous research has shown that flavonoids and phenols protect against oxidative stress-induced cellular damage.

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1.9.2 Anti-inflammatory/analgesic activity

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Anti-inflammatory/analgesic properties of *Ocimum gratissimum* essential oil have also been demonstrated hitherto (Sahouo *et al,* 2003). This property of *Ocimum gratissimum* may be useful in treating musculoskeletal pain and other inflammatory conditions.

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1.9.3 Immunomodulatory activity

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Some studies have demonstrated the immunomodulatory effect of *Ocimum gratissimum*. A study by Akachukwu *et al.* (2011) showed an increase in the haematocrit level when albino rats were fed with the essential oil of *Ocimum gratissimum.*

1.9.4 Antinociceptive activity

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In traditional medicine, *Ocimum gratissimum* is used to treat painful conditions. In classic pain models, the antinociceptive effects of *Ocimum gratissimum* essential oil and two of its active components (eugenol and myrcene) were investigated (hot plate test and formalin test) on neurogenic and inflammatory pain in murine pain models (Paula-Freire *et al.,* 2013).

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1.9.5 Wound healing

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Ibezim *et al.* (2018) demonstrated that the ointment of *Ocimum gratissimum* showed superior wound healing effect when compared with cicatrin powder. Orafidiya *et al.* (2003) also showed from their studies that topical Ocimum gratisimum promoted better wound healing than cicatrin

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powder used as control and Cetavlex® cream when applied on rabbit wounds for 15 days. *Ocimum gratissimum* ointment even demonstrated further healing properties where Cetavlex® cream did not, after a three-day wash out period. The above were corroborated by studies conducted by Osuagwu *et al.* (2004) and Umeh *et al.* (2022).

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1.10 Ethnomedicinal use of *Ocimum gratissimum*

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Ocimum gratissimum medicinal potential in Africa is incredibly vast and varies by country (Kpoviessi *et al.,* 2014). Its infusions are regarded as tonic and pectoral in Cameroon, and the juice of its sheets is used to treat giddiness, headaches, cold, and cough. In Côte d'Ivoire, several formulations of this plant are used to treat ear infections, dermatoses, and ophthalmias (Kpoviessi *et al.,* 2014).

In Nigeria, it is recommended for diarrhoea therapy (Kpoviessi *et al.,* 2014), while Sofowora (1970) recommended it for respiratory ailments and for use as an anthelminthic. It was also used to treat headaches, fevers, and ophthalmic and skin problems, as well as pneumonitis.In Benin Republic, the aqueous maceration of its pulp or aerial portions is used to treat dystopias, pelvic aches, colic, candidoses, digestive dysmenorrhoea, emesis, haemorrhoid (pile), and diarrhoea. Its stem decoction is used to treat hepatitis, cough, asthma, and wound infections (Chah *et al.,* 2006; Kpoviessi *et al.,* 2014). The juice from its leaves is used to treat angina, cephalgias, headaches, fever, and malnutrition. Its inflorescences are utilized as aromatizers in a variety of meals.

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1.11 Aim of Study

This study aims to evaluate the effect of the biherbal extract of *Psidium guajava* and *Ocimum gratissimum* extract on the complete blood count of Wister rats.

The specific objectives of the study were to:

i. investigate the effect of biherbal extract of Scentleaf (*Ocimum gratissimum*) and Guava (*Psidium guajava*) on Full Blood Count (FBC) of Wister rats.

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CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of plant sample

The leaves of *Ocimum gratissimum* and *Psidium guajava* were sourced from a garden within the University of Benin, Edo State, Nigeria. Subsequent identification and verification were conducted by Prof. Henry Adewale Akinnibosun and Dr. Joseph O. Erhabor of the Department of Plant Biology and Biotechnology, with the herbarium specimen assigned the voucher number UBH-O333 and UBH-P378 *Ocimum gratissimum* and *Psidium guajava.*

2.2 Preparation of Extract

The freshly collected leaves were rinsed with distilled water and air-dried in a shaded place for 14 days. The dried leaves were separated and ground into powder using a local grinding machine. The weight of the powdered leaves was measured in a ratio 1:1 that is 60+60 = 120g. Cold maceration using distilled water (900 ml) was used for the extraction process for 48 hours with intermittent shaking. The macerated mixture was sieved and the filtrate was concentrated using a freeze-drier into powdered form. It was then transferred into a sterilized container for proper storage, at a low temperature (40ºC)

2.3 Experimental animals

The study utilized 15 adult Wistar rats obtained from the Department of Biochemistry, University of Benin. The animals were subsequently housed in a suitable enclosure within the Phytomedicine Unit at the University of Benin and provided with standard feed and water. For acclimatization. They were acclimated for a period of two weeks, and animal handling procedures adhered strictly to standardized laboratory protocols (Yakubu *et al.,* 2005).

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2.4 Experimental protocol

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The animals were randomly selected into five (5) groups of 3 animals each. Group I, II, III were orally pre-treated with 25, 50 and 100 mg/kg of the biherbal aqueous extract for 7 days. Group 1: Received 25mg/kg polyherbal aqueous extract orally. Group 2: Received 50 mg/kg polyherbal aqueous extract orally. Group 3: Received 100 mg/kg polyherbal aqueous extract orally. The positive control group (Group 4) received 2 mg/kg of loperamide. The negative control group (Group 5) received 0.5 ml/kg distilled water orally.

2.5 Haematological Analysis

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Haematological parameters were assessed using the Sysmex KX-21 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). The analyzer measured:

- Red blood cells (RBC)
- Haemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Red cell distribution width (RCDW)
- White blood cells (WBC)
- Differential counts (MO, LY, NEU, EOS, BAS)
- Platelets (PLT)

- Platelet crit (PCT)

- Platelet density width (PDW)

- Mean platelet volume (MPV)

2.6 STATISTICAL ANALYSIS

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Results are presented as mean ± standard error of the mean (SEM). Data analysis employed one-way analysis of variance (ANOVA) and Dunnett's test for mean comparisons. Significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism software (version 7, USA).

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CHAPTER THREE

RESULTS

3.1 Effect of the biherbal leaf aqueous extract on white blood cells and its differentials

The results in Table 3.1 show that there is a significant increase at physiological intervals at graded doses of (25, 50, and 100 mg/kg) treated groups of 50 mg/kg aqueous leaf extract and reference control when compared with the control of the rats' white blood and differentials.

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Table 3.1: Effect of the biherbal leaf aqueous extract on white blood cells and its differentials in rats

Parameters	Negative control (0.5 ml/kg)	Loperamide (2 mg/kg)	Biherbal aqueous extract (25mg/kg)	Biherbal aqueous extract (50 mg/kg)	Biherbal aqueous extract (100 mg/kg)
WBC $10^3/\mu L$	4.00 ± 0.21^a	6.40 ± 0.50^a	7.70 ± 0.14^b	6.10 ± 0.31^b	6.20 ± 0.57^a
LYM %	90.20 ± 2.09^a	85.75 ± 1.31^b	86.00 ± 1.36^b	89.15 ± 1.69^b	85.25 ± 1.78^a
MID %	5.50 ± 0.11^a	9.40 ± 0.36^a	8.45 ± 0.14^a	6.50 ± 0.10^a	9.00 ± 0.35^a
GRAN %	4.05 ± 0.08^a	4.52 ± 0.10^b	3.61 ± 0.06^a	4.35 ± 0.10^b	5.53 ± 0.09^b
LYM $10^3/\mu L$	3.65 ± 0.08^a	5.50 ± 0.14^a	6.75 ± 0.10^b	5.45 ± 0.13^b	5.30 ± 0.17^a
MID $10^3/\mu L$	0.25 ± 0.01^a	0.50 ± 0.02^a	0.52 ± 0.09^b	0.45 ± 0.04^a	0.55 ± 0.02^b
GRAN$10^3/\mu L$	0.10 ± 0.00^a	0.30 ± 0.01^b	0.30 ± 0.01^b	0.20 ± 0.00^b	0.20 ± 0.00^b

The values were expressed in Mean ± SEM and a significant difference was spotted at *p-value* < 0.05. values with different alphabets are significantly different from each other n=3. White blood cells (WBC), lymphocyte count (LYM), Mid-range cell count (MID), Granulocyte count (GRAN).

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3.2 Effect of the biherbal leaf aqueous extract on red blood cells and its component

The result in Table 3.2 shows that the red blood cell and its component of the rat had a significant increase at physiological intervals of graded doses of (25, 50, 100 mg/kg) treatment groups of the biherbal leaf aqueous extract and reference control when compared with the control.

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Table 3.2: Effect of the biherbal leaf aqueous extract on red blood cells and its components in rats

Parameters	Negative control (0.5 ml/kg)	Loperamide (2 mg/kg)	Biherbal aqueous extract (25 mg/kg)	Biherbal aqueous extract (50 mg/kg)	Biherbal aqueous extract (100 mg/kg)
RBC 10^6/µL	5.25 ± 0.23[a]	8.64 ± 0.43[b]	7.66 ± 0.32[b]	7.75 ± 0.40[b]	7.44 ± 0.29[a]
HGB g/dl	13.15 ± 0.47[a]	14.45 ± 0.38[b]	11.00 ± 0.31[b]	12.10 ± 0.33[a]	12.10 ± 0.41[a]
HCT %	44.90 ± 0.63[a]	49.35 ± 0.59[b]	40.20 ± 0.65[b]	44.45 ± 0.47[b]	45.30 ± 0.42[b]
MCV µM^3	54.50 ± 0.61[a]	45.25 ± 0.65[b]	54.70 ± 0.89[b]	57.60 ± 0.81[a]	58.10 ± 0.72[b]
MCH pg	15.90 ± 0.31[a]	16.10 ± 0.29[b]	14.85 ± 0.33[b]	15.55 ± 0.28[a]	16.05 ± 0.42[a]
MCHC g/dl	29.23± 0.31[a]	29.25 ± 0.28[b]	27.25 ± 0.24[b]	27.00 ± 0.37[b]	27.75 ± 0.31[b]
RDWS µM^3	32.00 ± 0.58[a]	45.25 ± 0.62[b]	36.30 ± 0.46[b]	39.55 ± 0.54[b]	37.37 ± 0.47[b]
RDWC %	14.50 ± 0.41[a]	16.05 ± 0.47[a]	16.60 ± 0.45[b]	17.4 ± 0.33[a]	16.40 ± 0.47[a]

The values were expressed in Mean ± SEM and the significant difference was spotted as *p -value* < a 0.05. values with different alphabets are significantly different from each other n=3. Red blood cells (RBC), Hematocrit (HCT), Hemoglobin concentration (HGB), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width-standard (RDWS), Red cell distribution width-coefficient (RDWC).

3.3 Effect of the biherbal leaf aqueous extract on platelet and its factors

The results in Table 3.3 showed that the rat's platelet and factors significantly increased at physiological intervals at graded doses of (25, 50, 100 mg/kg) treated groups of the biherbal leaf aqueous extract when compared with the control.

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Table 3.3: Effect of *the* biherbal leaf aqueous extract on platelet and its factors in rats

Parameters	Negative control (0.5 ml/kg)	Loperamide (2 mg/kg)	Biherbal aqueous extract (25 mg/kg)	Biherbal aqueous extract (50 mg/kg)	Biherbal aqueous extract (100 mg/kg)
PLT10^3/µL	163431.00±31.11^a	107545.00±63.32^b	178418.00±39.99^b	222510.00±48.42^b	8645.00±23.52^b
MPV µM^3	10.50 ± 0.20^a	8.95± 0.09^b	8.95 ± 0.11^b	10.80 ± 0.23^b	9.00 ±0.01^b
PDW %	12.49 ± 0.03^a	12.65± 0.04^b	13.80± 0.05^b	12.80± 0.03^b	12.65 ± 0.04^b
PCT %	0.14 ± 0.01^a	0.96± 0.01^b	1.59± 0.02^b	2.35 ± 0.04^b	0.98 ± 0.02^b

The values were expressed in Mean ± SEM, and the significant difference was spotted as *p - value* < 0.05. Values with different alphabets are significantly different from each other n=3. Platelet volume (PLT), Platelet distribution width (PDW), Platelet crit (PCT).

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CHAPTER FOUR

4.1 DISCUSSION

Haematological indexes are applicable in determining a certain degree of lethal effect on foreign compounds such as plant extract on the blood cells. It can also be used to elucidate the relative blood role of a chemical compound or plant extract (Akindele and Busayo, 2011; Yakubu *et al.,* 2007). One of the earliest immune responses can be seen and calculated by studying the haematological parameters of animals (Yakubu *et al.*, 2007). The first cells to respond to invading exogenous substances are the blood cells. Immuno-modulatory effect of any immune substance is first seen by the white blood cell count and its differential count (Carro-ju'arez *et al.,* 2004).

Accordingly, from the results, the white blood cell count and its differential displayed a significant increase, as observed in the treated groups (25, 50, 100 mg/kg) compared to the control group. This suggests that the biherbal extract has immunomodulatory effects. The extract's ability to enhance white blood cell count may be beneficial in boosting the immune system. The differential count showed significant changes in lymphocyte (LYM) and granulocytes (GRAN) percentages, indicating a shift in immune cell populations. This is represented in table 3.1.

Similar results from the present study (Table 3.2), were observed in the red blood cells, the biherbal extract significantly increased RBC count, haemoglobin (HGB) levels, and hematocrit (HCT) values in treated groups. These findings show that the extract may have erythropoietic effects, enhancing RBC production. The changes in mean corpuscular haemoglobin concentration (MCHC) indicate improvements in RBC morphology in the Wistar rat.

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Also, Studying the immunomodulatory activity of the biherbal leaf aqueous extract in rats showed a significant increase in the platelet count (PLT) and platelet distribution width (PDW) in treated groups. The platelet count and volume (MPV) enhancements show that the extract has thrombopoietic effects. In comparison with loperamide, used as a reference control. showed a significant increase in White blood cell, red blood cell, and platelet parameters. Loperamide is an antidiarrheal medication that is commonly used to treat diarrhea. It works by slowing down gut movement, allowing for more water and electrolyte absorption. It is a suitable control for comparing the effectiveness of the leaf extract. However, the biherbal extract demonstrated more pronounced effects, particularly at higher doses. (50 and 100 mg/kg). The overall results in the present study showed that the immune response is boosted upon the administration of the biherbal leaf aqueous extract. It is evident from the present results that at graded doses of the extract particularly 50 and 100 mg/kg body weight, it has the potential to trigger humoral and cellular response in rats.

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4.2 CONCLUSION

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Conclusively, this study revealed that the biherbal leaf aqueous extract possesses an immunoprotective effect and increased blood properties to enhance normal blood-related function. This study supports that *Ocimum gratissimum* and *Psidium guajava* leaves elicited ethnomedicinal properties of the plant extract in haematological parameters.

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