ANTI-DIARRHEA EFFECT OF BIHERBAL FORMULATION CONSISTING OF *Chromolaena ordata* and *Vernonia amygdalina* IN CASTOR OIL INDUCED DIARRHEA AND TRANSIT TIME IN MICE

BY

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN, BENIN CITY

SEPTEMBER, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES

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SEPTEMBER, 2023

CERTIFICATION

We the undersigned hereby certify that **VICTOR AZUBIKE ODU** with matriculation number **LSC1706091** carried out this work titled '**ANTI-DIARRHEA EFFECT OF BIHERBAL FORMULATION CONSISTING OF *Chromolaena ordata* and *Vernonia amygdalina* IN CASTOR OIL INDUCED DIARRHEA AND TRANSIT TIME IN MICE** ' in the Department Of Science Laboratory Technology, Faculty Of Life Sciences, University Of Benin, Benin City And we approve same as adequate in the scope and quality for the award of bachelor of science degree (B.Sc.) in the Science Laboratory Technology.

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DEDICATION

This project is dedicated to God Almighty for giving me the Grace, Opportunity and Strength to complete undergraduate seminar report.

ACKNOWLEGEMENTS

This project is dedicated to God Almighty for giving me the Grace, Opportunity and Strength to complete undergraduate seminar report. I also like to specifically thank my supervisor. Dr. P.O. Obaro and his wife Dr. (Mrs.) O.E. Obaro-Onezeyi for their support and encouragement. And also, my profound appreciation to the Head of Department, Assoc. Prof. Oshomoh and the entire staff of the Department of Science Laboratory Technology. My esteemed regards to my lovely family Mr. and Mrs. Odu for their unending love and support towards my educational pursuit. And also, to Miss Mawuli Owusu Promise, Oni and my lovely colleagues, thanks for your support and care, you all are the best.

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CHAPTER ONE

1.0. INTRODUCTION

Diarrhea is a common medical condition that is characterized by increased frequency of bowel movements and increased liquidity of stool (Gidudu *et al.,* 2011). Diarrhea is responsible for 2.5 million fatalities worldwide per year. It is responsible for one out of every nine deaths in children globally. When combined with other illnesses, diarrhea is considerably more lethal; the death rate is many times that of the rate without other complications. Unsafe water, insufficient sanitation, and poor hygiene are responsible for about 88% of deaths brought on by diarrhea (C.D.C., 2021).

1.1. Background of study

Diarrhea has been defined in a variety of ways various scientific bodies and health organizations have used terms like "the passage of loose unformed stools" or "three looser-than-normal stools in a 24-hour period" across time, with an emphasis on consistency rather than quantity. The passage of three or more loose or watery stools in a 24-hour period—defined as a loose stool as one that resembles a stool container—is typically considered to be indicative of diarrhea (Gidudu *et al.,* 2011). Basically, Diarrhea refers to the abnormal increase in intestinal peristalsis and stool frequency (Reintam, 2015) although, Diarrhea is a common complication of critically ill patients (Chen *et al.,* 2019).

1.2. Types of Diarrheal Disease

Diarrheal disease is typed based on severity and intensity

1.2.1. Acute diarrhea

Acute diarrhea is defined as stool with increased water content, volume, or frequency that lasts less than 14 days (Guerrant, 2001). In developing countries, contaminated food and Water supplies are crucial in infectious causes of acute diarrhea. Despite greater standards of food production, technical advancement and an increase in mass food production in the industrialized world significantly added to the persistence of severe diarrhea caused by foodborne illnesses. Incidences of acute diarrhea caused by bacteria are seen as a result of increased travel, comorbidities, and foodborne sickness (Barr and Smith, 2014; Farthing *et al*., 2013).

1.2.2. Chronic diarrhea

This is a common yet difficult condition which is characterized by a reduction in amount of stool consistency for more than four weeks. Chronic diarrhea can occur in up to 5% of people at any given time. Patients' definitions of diarrhea vary, but common symptoms include loose stool consistency, increased frequency, urgency of bowel movements, or incontinence (Schiller *et al*., 2017). There are three main types of chronic diarrhea: watery, fatty (caused by malnutrition), and inflammatory (accompanied by blood and pus). Although certain types of chronic diarrhea overlap, not all cases are solely watery, malabsorptive, or inflammatory.

1.3. Causes of Diarrhea

Traveler's diarrhea and medication side effects (many medications can induce diarrhea) are the most frequent causes of acute and chronic diarrhea. Antibiotics, magnesium-containing antacids, cancer medications, and infections are among the medications that might result in diarrhea. Infections that cause diarrhea includes;

1.3.1. Viral infections

Many viruses, such as norovirus and rotavirus, produce diarrhea. Acute diarrhea is commonly caused by viral gastroenteritis.

1.3.2. Infections of bacteria

various types of bacteria can enter your body and produce diarrhea if you consume contaminated food or water. E. coli, Salmonella Shigella, V. parahaemolyticus, Vibrio cholerae, V. Cholerae, Shigella species, and Campylobacter jejuni are a few of the most common bacteria that cause diarrhea as well as Bacteroides fragilis, Yersinia enterocolitica, Yersinia pseudotuberculosis and Clostridium difficile

1.3.3. Infections of parasite.

Parasites route into the body is mainly via food or drink and lodge in your gastrointestinal tract. Parasites that cause diarrhea are protozoans (*Cryptosporidium parvum, Giardia intestinalis*) Microsporida (*Entamoeba histolytica, Isospora belli*), Cyclospora (*cayetanensis Dientamoeba, fragilis Blastocystis hominis*) and Helminths (*Strongyloides stercoralis Angiostrongylus costaricensis Schistosoma mansoni,* S. *Japonicum*) (Farthing *et al*., 2013).

Infections lasting more than 2 weeks and less than 4 weeks can cause persistent diarrhea (Younis *et al.,* 2020; Bharucha *et al.,* 2015; Vernacchio *et al.,* 2006).

Other causes may include;

- Food allergies and intolerance
- Digestive tract problem
- Abdominal surgery

1.4. Management

Because pathogens, which can be easily managed in their spread, are the main cause of diarrheal mortality, they can be avoided. By increasing global access to clean water and

sanitation, oral rehydration treatment, and vaccination, this major cause of death can be reduced substantially (Dadonaite *et al.,* 2018). Oral rehydration therapy (ORT) is central to the management of acute diarrhea, and is sufficient to prevent complications due to dehydration in most patients while the disease runs its course (Dupont and Vernisse, 2009).

1.5. Treatment

Except for oral rehydration therapy, acute diarrhea frequently has a self-limited course and requires little in the way of treatment. There is a longer-term issue with persistent diarrhea. Chronic diarrhea frequently requires long-term symptomatic medication if the underlying etiology cannot be treated.

1.5.1. Use of probiotics

Over the past few years, probiotics have been thoroughly researched for both the prevention and, to a greater extent, the treatment of diarrheal illnesses, especially in pediatric populations (Guarino *et al*., 2015).

1.5.2. Anti-diarrheal drugs

A wide range of new medications have been created in recent years that offer more focused therapy and can reduce diarrhea in particular circumstances.

1.5.3. Opiates

Opiate antidiarrheal medications are the most commonly utilized treatments. These medications work well for a wide range of diarrheal diseases and can typically be taken safely if carefully supervised. They function by reducing motility and extending the time available for absorption (Schiller, 2017).

1.5.4. Diphenoxylate

Diphenoxylate, often known as Lomotil, is a potent synthetic antiperistaltic drug that slows down gastrointestinal motility. Its manner of operation is unknown. Although it shares structural similarities with other narcotic medicines, no appreciable analgesic or addictive effects have been reported. Similar to polycarbophil-thihexinol (Sorboquel), it is highly efficient in reducing functional diarrhea but less so in reducing organic intestinal illness (Kinnear, 1964).

1.5.5. Metronidazole

Metronidazole is the first line medication used against the infection but long-term uses produce several side effects in patients (Ordaz-Pichardo *et al*., 2005)

1.5.6. Anti-parasitic drugs

Albendazole and mebendazole the benzimidazole derivatives, quinacrine (an acridine derivative), paromomycin and nitazoxanides (Reynoldson *et al*., 1992; Escobedo and Cimerman, 2007).

1.5.7. Herbal medication

The utilization of plants for medicinal purposes in herbal medicine dates back to an ancient era. Plants have been used as medications for most of human history, and traditional medicine is still widely used today . In the 19th century, modern medicine started to shift away from herbal remedies in favor of therapies based on data obtained through the scientific method. Examples of herbal medication for treatment of diarrheal include seeds of *Mangifera Indica*, leaf of *Terminalia Catappa* and others (Chaddha *et al.,* 2013)

1.6. Justification

Diarrhea is a commonly encountered gastrointestinal disorder that affects people across the globe. Its presence leads to considerable illness and death particularly in underdeveloped nations. While conventional treatments for diarrhea are accessible. Alternative remedies sourced from nature are also sought after due to their affordability, availability, and potential therapeutic advantages. Therefore, conducting a study to investigate the anti-diarrheal effect of a biherbal formulation consisting of *Chromolaena ordata* and *Vernonia amygdalina* in diarrhea model in mice is justified.

1.7. Scope of study

The study will focus on evaluating the anti-diarrheal effect of a bi-herbal formulation prepared from *Chromolaena ordata* and *Vernonia amygdalina* using diarrhea and transit models in mice

1.8. Aim of Study

The main purpose of this project is to investigate the anti-diarrheal effect of a bi-herbal formulation consisting of *Chromolaena ordata* and *Vernonia amygdalina* in diarrhea and transit models in mice.

1.9. Objectives of Study

The objectives of the study were to:

- Prepare an optimized bi-herbal formulation from *Chromolaena ordata* and *Vernonia amygdalina*.
- Determine the anti-diarrheal effect of the bi-herbal formulation by using castor oil induced diarrhea in mice
- Determine the anti-diarrheal effect of the bi-herbal formulation by using castor oil induced transit in mice
- Compare the anti-diarrheal effects of the biherbal formulation with pre existing medication.

CHAPTER TWO

2.0. Literature Review

Humans have employed plants and their products for thousands of years to treat a wide variety of ailments. Traditional medicine, sometimes referred to as indigenous medicine or folk medicine, is the body of information that predates the advent of modern medicine that was established through many generations in diverse communities (Palombo, 2006). Medicinal plants are those that have demonstrated healing abilities. In the past, these remedies might cure or lessen symptoms. People from all continents have used infusions and poultices made from thousands of plants since prehistory. 60, 000 years ago, Neanderthals treated illnesses with herbs like hollyhock, which are still utilized in many nations today. Actually, traditional medicine still accounts for 80% of medical care in developing nations as medications for their health care (Kim, 2005; Borris, 1996).

Traditional medicine used outside of its traditional culture is referred to as alternative or supplemental medicine in western nations. Nowadays, a wide variety of plant substances are easily accessible as over-the-counter self-medication. Because these formulations are mostly unregulated, herbal suppliers and natural food retailers give their consumers varying amounts of active ingredients with varying degrees of purity. The study of medicinal plants as a source of chemical compounds with pharmacological activity has drawn more attention in recent years on a global scale. The purported biological activity and potential negative impacts of many plants haven't, however, been well investigated. Around 500,000 different plant species are thought to exist on the planet (Dubreuil, 2013).

2.1. *Chromolaena ordata*

The tropical and subtropical species of flowering shrub *Chromolaena odorata*, one of the most utilized plants. It is native to Texas and Florida in North America, as well as in Mexico

and the Caribbean. It then moved to West Africa, South America, Tropical Asia and some regions of Australia. C. odorata is known by many names including Siam weed, Christmas bush, devil weed, camfhur grass, baby tea, cariaquillo, Santa María, fleurit-Noël, crambling shrub and common floss flower. (King, 1970; Lalith, 2009; Howard, 1989; Liogier, 1997). In Nigeria, C. odorata is commonly known as Ewe Awolowo, Siam weed, Elizabeth weed, Obirakara, Olorohuru, and independent weed (Usunobun and Ewere, 2016; Anyanwu *et al*., 2017). The herb gained popularity due to its effective wound healing properties. The anti-microbial properties have made it a popular choice in disinfecting and treating open wounds (Odugbem, 2006). Several studies have shown that it is an excellent treatment for diarrhea, malaria fever, toothache, diabetes, skin problems, dysentery, and colitis. The leaves could be ground and the extracted juice taken to alleviate fever or the treatment of diabetes (Akinmoladun and Akinloye, 2007; Chung and Yun, 2001).

2.1.1. Plant appearance

An erect shrub which forms thickets and grows 1.5 to 3 m tall. It can, however, reach greater heights (6-20 m) when growing over taller plants such as trees. The slender stems are normally yellowish-green and somewhat pubescent (hairy), but develop to become woody near the plant's base. These stems grow up to 7 m or more in length and several are usually produced from the plants long-lived root-stock (CABI, 2011). They are densely branched, with side or lateral branches in pairs on the leaf forks (axils). The leaves placed in opposite directions and are egg-shaped or triangular in outline, with a broad end at the base (ovate) and a acute apex (pointy tip). They have coarsely serrated (toothed) borders and are pubescent on both surfaces. These leaves are borne on petioles (stalks) up to 6 cm long (usually 10-15 mm), and give off a strong odour when crushed (Lazarides *et al*., 1997).

The little flower-heads (capitula) areborne in thick clusters at the terminal panicle's (branches) end and lack 'petals' (ray florets). These flower-heads (about 10 mm long and 3 mm wide) are

pale pink or pale mauve in color (sometimes appearing whitish when older) and consist of numerous (15-30) tiny flowers (Sajise *et al.,* 1974). These flowers of 10-12 mm in length, are surrounded by multiple layers of 8-9 mm long overlapping slender bracts (an involucre). Each flower-head (capitulum) is supported by a 10-30 mm long stalk (peduncle) (Sajise *et al*., 1974). The black or dark brown 'seeds' are 4-5 mm long and topped with a ring of white to brownish coloured hairs (5-6 mm long) (McFadyen, 2004).

In Africa, there are two distinct biotypes. The blooms on the western African type are purplish, whereas the flowers on the southern African form are white.

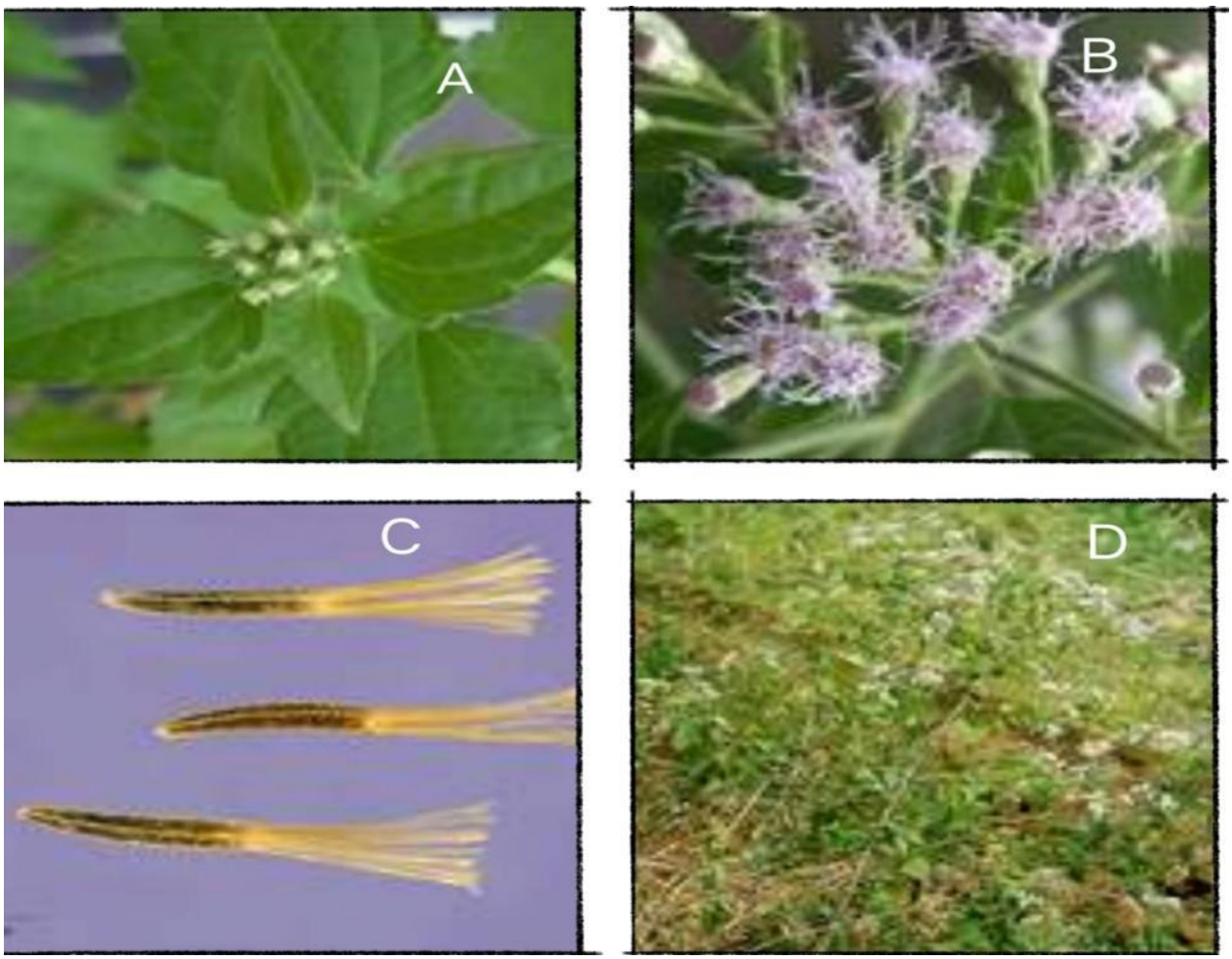

Plate 1. 1A plant leaf, 1B plant flower, 1C plant seed, 1D full photo of plant in habitat (Zahara, 2019).

2.1.2. Taxonomy

Kingdom	Plantae
Division	Tracheophytes
Sub division	Angiosperms
Class	Magnoliopsida
Sub class	Asterids
Order	Asterales
Family	Asteraceae
Genus	*Chromolaena*
Species	***C. odorata***

Table 2.1: Taxonomy of *Chromolaena ordata* (Schmidt and Schilling, 2000).

2.1.3. Phytochemical composition

Studies from Odutayo *et al.* (2017) indicated that terpenoids, steroids, alkaloids and anthraquinones are highly present on both methanolic and ethyl-ether extract, also flavonoids were qualitatively and highly present on methanolic extract but fairly present on ethylether extract. Terpenoids and phenols are relatively abundant in both ethyl-ether and methanolic extracts. Meanwhile, saponins were reasonably only prevalent in the methanolic extract, while phlobatannins were completely lacking in both extracts. Chromolaena odorata leaves contain alkaloids, flavonoids, steroids, tannins, and saponins following a phytochemical

investigation. The presence of secondary metabolites such as alkaloids, tannins, flavonoid, saponins and steroids will contribute to its medicinal value (Nwankpa *et al.,* 2012)

Phytochemicals	*Chromolaena ordata* (Methanolic extract)	Odutayo *et al.*(2017) (Ethyl-Ether extract)	*Chromolaena ordata* leaves
Anthraquinones	++	++	_
Alkaloids	++	++	++
Saponins	+	_	+
Flavonoids	++	+	+
Terpenoids	++	++	+
Steroids	++	++	++
Tannins	_	_	+

Table 2.1 phytochemicals of ethyl-ether, methanolic extract and leaves of *Chromolaena ordata* (Usunobun and Ewere, 2016; Odutayo *et al,.*2017)

Key: + (present), ++ (highly present), - (absent).

2.2. *Vernonia* amygdalina

About one thousand species of shrubs and forbs belongs to the genus Vernonia, and V. amygdalina belongs to the pan-tropical tribe within the Asteraceae family, with it being the most notable species. It grows predominantly in tropical Africa especially in Nigeria,

Zimbabwe and South Africa and it is domesticated in parts of West Africa (Johri and Singh, 1997; Farombi, 2003; Erasto *et al*., 2006).

Vernonia amygdalina, commonly referred to as 'Bitter Leaf,' is a perennial shrub belonging to the Asteraceae family. It earns its nickname because of the distinctly bitter flavor of its leaves. Not only named Bitter Leaf, this plant also has a lot of other local names in different languages of the different regions of the world, such as Ewuro, Onugbu, Oriwo, Etidot and Ityuna in Nigeria, Mululuza and Omubirizi in Uganda, Ebichaa in Ethiopia and Awonwono in Ghana (Yineger and Yewhalaw, 2007; Moshi *et al*., 2010; Farombi and Owoeye, 2011; Komlaga *et al*., 2015; Kiguba *et al*., 2016). This plant is also known as South African leaf even in Malaysia. As the largest genus among Vernoniae tribe, Vernonia has close to 1000 species in its family (Keeley and Jones, 1979; Mahmud, 2019).

Vernonia amygdalina, also known as 'bitter leaf,' is a medicinal plant with fresh leaves that hold great importance in the human diet owing to their rich content of mineral salts and vitamins. It serves as a highly valuable food that contributes to overall health and well-being as well as the prevention and treatment of many ailments. The plant (especially the leaf) has been found useful in the ethno-therapy of diabetes (Nwajo, 2005), asthma, headache (Akah, Okoli, and Nwafor, 2002), skin infections such as ringworm, rashes and eczema, schistosomiasis, malaria (Masaba, 2000), measles, diarrhea, tuberculosis, abdominal pain and intestine complaints as well as fevers, cough, induction of fertility in barren women and hyperlipidemia (Adaramoye *et al*., 2008; Raimi *et al*., 2020).

2.2.1 Taxonomy

Kingdom	Plantae
Division	Angiosperms
Order	Asterales
Family	Asteraceae
Genius	Vernonia
Species	Amygdalina
Botanical Name	*Vernonia amygdalina*

Table 2.2 Taxonomy of *V. Amygdalina.* (Kaur *et al*., 2019)

2.2.2. Habitat

Vernonia amygdalina germinates naturally beside lakes and rivers, in grassland and woodland up to 2800 m in elevation, and in places with an average rainfall of 750-2000 mm. humus-rich soils are favorable for the proper growth of plant but is can adapt in all types of soil and it needs full sunlight and humid environment (Ofori *et al*., 2013).

2.2.3. Plant Appearance

Vernonia amygdalina is a perennial shrub that is locally found in tropical Africa. It is commonly known as omolo, ngogwe, or bitter leaf. The plant grows up to 10 meters tall and has light gray or brown bark. The branches are delicate and the leaves are 10-15 x 4-5 cm in size. The upper surface of the leaves is sparsely hairy, while the lower surface is covered in soft, fine, pale hairs. The leaves have distinctive red veins and their margins can be either

finely or smooth-toothed. The petiole is typically short, but may occasionally reach lengths of 1-2 cm. Flower heads are thistle like, small, creamy white, 10 mm long, grouped in dense heads, axillary and terminal, forming large flat clusters, 15 cm in diameter, sweetly scented (Mahmud, 2019).

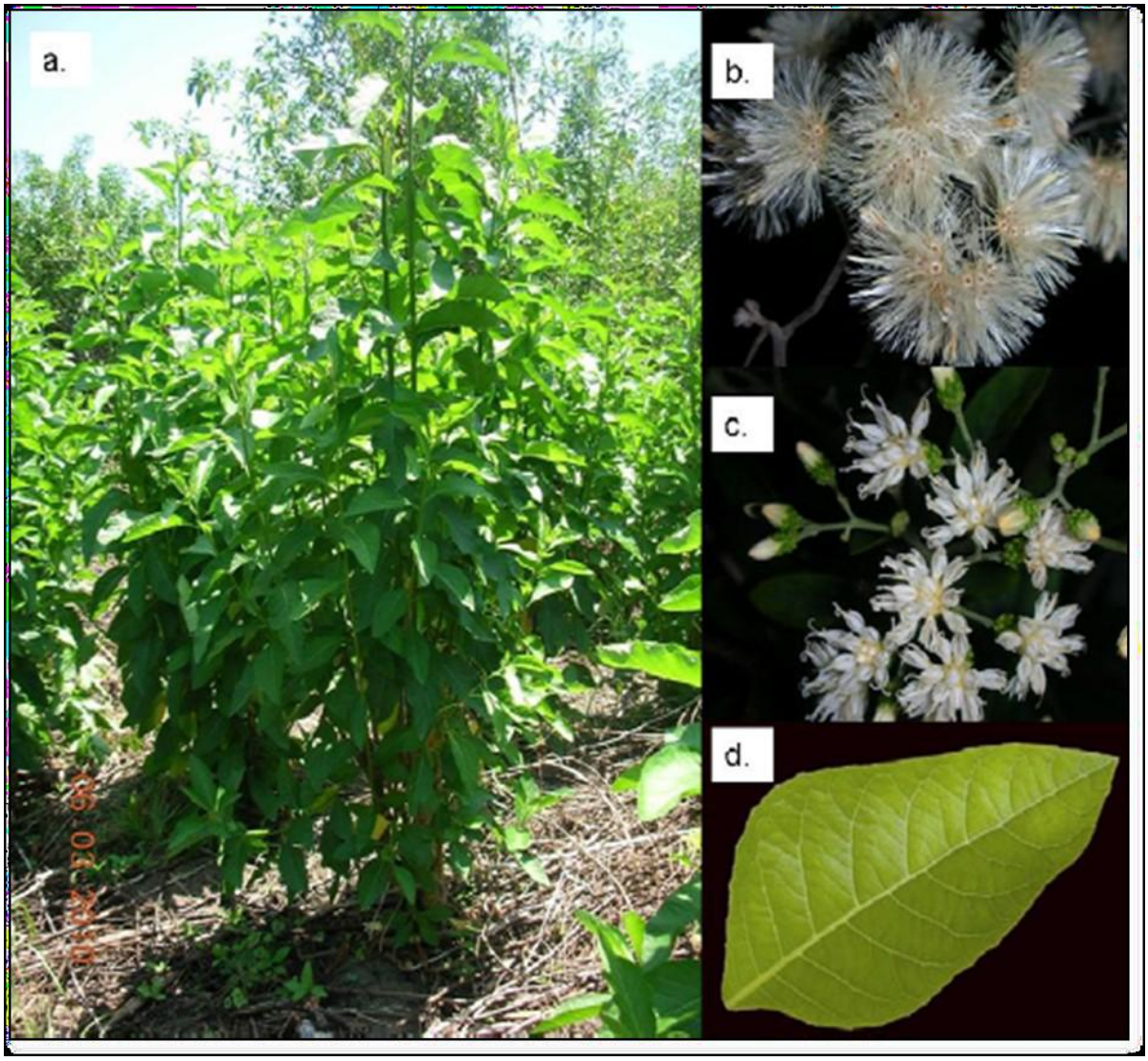

Plate2. 2a: image of *Vernonia amygdalina*. 2b and 2c: *Vernonia amygdalina* flowers. 2d: A leaf of *Vernonia amygdalina* (Yeap *et al*., 2010).

2.2.4. Phytochemical composition

In relation to phytochemicals content, a study conducted by Usunobun and Ngozi (2016) found that saponins, tannins, alkaloids and flavonoids, triterpenoids, steroids and cardiac glycosides were high in *V. amygdalina* which served as a great source of pharmacologically active phytochemicals and effective as supplements in human and animal nutrition.

Phytochemical Constituents	**Results**
Alkaloid	Present
Tannins	Present
Saponin	Present
Phlobatannin	Present
Anthraquinones	Absent
Glycosides	Present
Flavonoids	Present

Phytochemical constituent of *V. Amygdalina* leaves. (Raimi *et al*., 2020)

2.2.5. Mineral constituent

The mineral contents of potassium, zinc, calcium, manganese and chromium are believed to possess beneficial effect in the treatment of diabetes mellitus (Marles and Farnsworth, 1995). Potassium and calcium play important roles to control the glucose level as they help to maintain the normal glucose-tolerance in the human body (Kadiri and Olawoye, 2016). Besides, high sulphur concentration in this herb is essential for detoxification of cyanide while low sodium content is suitable for obese patients (Ifon and Bassir, 1979).

2.2.6. Nutritional constituent

Proximate composition of *V. amygdalina* reveals the presence of protein (62.2%), crude carbohydrate (22%), ash (9.95%), crude fibre (16%) and crude fat (3.45%).High ash content has been found by Yeap *et al.* (2010) and this reflected that *V. amygdalina* leaves contain useful mineral contents. This herb rich in mineral elements including chlorine, copper, ferum, potassium, manganese, nickel, sodium, sulphur phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins such as Vitamin A, C and E (Nwaoguikpe, 2010; Yeap *et al.,* 2010). On the other hand, high sugar (raffinose, lactose, sucrose, glucose, galactose, fructose, maltose and arabinose), vitamin (thiamine, nicotinamide, thiamine, riboflavin, pyridoxine and ascorbic acid), casein, hydrolysate, amino acids (non-essential amino acid: cysteine, glycine and essential amino acid: leucine, valine and phenylalanine), less acid value (10 mg/100 g dry matter) and high iodine (35 mg/100 g) value have promoted in *V. Amygdalina* (Yeap *et al.,* 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant

Fresh *chromolaena* and *vernonia amygdalina* used in this research was collected from Ovbigie community, Ovia South East in Benin City, Nigeria. Dr. H. Akinnibosun, a taxonomist at the University of Benin, Benin City, validated and identified the plant. The leaves of both plants were first shade-dried at (25 - 27^0c)room temperature for 2 weeks, and then completely dried in an oven at 40 degrees Celsius for 24 hours. The dried sample was then grounded and stored in an air tight container for future use (Obaro-Onezeyi and Oshomoh, 2019).

3.2. Preparation of extract

One kilogram (1 kg) of the powder (500:500 g each) was extracted with distilled water using cold maceration method (Oshomoh and Obaro (2019). After weighing the plant samples, they were immersed in 2000 ml of distilled water in a glass jar, covered, and shaken as frequently as possible for 24 hours. The solution was thoroughly macerated for twenty-four (24) hours before being filtered through a cheese cloth. The cheese cloth filtrate was discarded, and the plant extract was placed into crucibles. The resultant extract was dried in an oven at 400 degrees Celsius after being concentrated to dryness using a water bath. The percentage yield was computed using the dried powder used. 20 g of the extract was dissolved in 100 ml of distilled water daily to obtain a stock solution (200 mg/ml) from which dilutions were made and calculated doses administered to the animals during the experimental procedures (Oshomoh and Obaro, 2019).

3.3. Drugs /chemicals

Chloroform (supplied by Fharmatrends Nigeria Ltd), normal saline, activated charcoal and loperamide all of analytical standards and pharmaceutical standards.

3.3.1. Phytochemical screening

The methanolic extract was subjected to qualitative phytochemical screening according to standard methods.

3.3.2. Acute toxicity test

The acute toxicity of a biherbal formulation of *Chromolaena* and *Vernonia amygdalina* extract was assessed in mice using Hilaly et al. approach with minor modifications. Animals that had been starved for 24 hours were randomly separated into three (3) groups of three (6) animals each. Each group of mice were administered a different graded dose of the extract (1000, 2000, and 4000 mg/kg p.o.). The animals were then allowed to eat and drink as much as they wanted for 48 hours, while being observed for any signs of poisoning. During this time period, the number of deaths was recorded.

3.4. Experimental animals

A total of 60 adult albino mice, comprising 15 males and 15 females, each with an average weight falling within the range of 25-30 grams, were retrieved from the animal facility located within the Pharmacology/Toxicology Department at the University of Benin. The mice were housed at room temperature in wooden cages and under typical laboratory settings of 12-hour light and 12-hour darkness. The mice were fed with standard pelletized layers mash and clean water for 14 days as procedure acclimatization period prior to the experimental study.

3.5. Grouping of animals

The mice were partitioned into six groups, each consisting of five mice, denoted as Groups I, II, III, IV, V, and VI for the purpose of conducting experiments related to castor oil-induced diarrhea and transit in mice.

3.6. Castor Oil Induced Diarrhea in Mice

The method, as described by Izzo *et al*. (1992), may be used. Castor oil causes diarrhea by causing ricinoleic acid to enhance the transfer of electrolytes and water and are absorbed into the lumen (intestine). Additionally, it causes inflammatory responses that promote gastrointestinal motility. Mice that get castor oil orally develop severe diarrhea. The technique is appropriate for assessing antidiarrheal medications that affect both gastrointestinal motility and secretion. Randomly selected groups of five mice, regardless of gender, weighing between 25-30 grams, were used in this experiment. The first group was administered distilled water (10 ml/kg) through oral route as the normal control. The biherbal formulation consisting of *chromolaena* and *vernonia amygdalina* was administered orally to three different groups of mice at varying doses: 100 mg/kg for Group I, 200 mg/kg for Group II, and 400 mg/kg for Group III. Group IV, on the other hand, was given loperamide (25 mg/kg through orogastric feeding and this serves as the reference drug. Each group was administered castor oil orally (1 ml) for 30 min and placed in a transparent polypropylene cage, the floor was lined with weighed filter paper (Whatman no. 1). The animals were monitored, and the filter papers were replaced at hourly intervals for up to 4 hours. The beginning of diarrheal stool (the first wet stool that leaves a halo on the filter paper), the quantity and weight of wet stools, the quantity and weight of solid stools, and the overall weight of fecal output are the parameters that are measured.

3.7. Normal Intestinal Transit Time in Mice

This method was first described by Aye-Than *et al.* (1989). The technique is used to assess how test chemicals affect mice's natural intestinal propulsion. The technique can be used to assess the laxative and diarrheic qualities of potential medications.

Mice, weighing between 25-30 grams and of female and male, were randomly divided into groups, each consisting of 5 mice, after an overnight fasting period. The first group received an oral dose of one ml of distilled water and served as the control group (normal). Three different groups (Group 2, Group 3, and Group 4) were orally administered varying doses of the biherbal formulation containing *Chromolaena* and *Vernonia amygdalina* (100 mg/kg, 200 mg/kg, and 400 mg/kg). Group 5 received an oral dose of loperamide (25 mg/kg) and served as the reference drug (positive control). Thirty minutes later, the mice were given a freshly prepared standard charcoal meal (0.2 ml of a 10% activated charcoal suspension in 5% gum acacia) orally. The mice were sacrificed thirty minutes after the coal was fed. The small intestine was dissected, and the distance traveled by the coal powder from the pylorus to the ileocecal junction was calculated. The full length of the small intestine was also measured. The peristaltic index is then calculated for each mouse as a proportion of the distance covered by the charcoal meal in comparison to the entire length of the small intestine. The treated group's peristaltic index is contrasted with that of the control group (which is normal and positive).

3.7. Castor Oil-Induced Gastrointestinal Transit in Mice

This method was also first described by Aye-Than *et al.* (1989). Mice of either sex (25-30 g) are randomly selected and allocated into groups (5 per group) and fasted overnight. The mice in the first group were administered distilled water orally through orogastric feeding (1 ml), and serve as the control. The biherbal formulation of *chromolaena* and *vernonia amygdalina* were administered to three groups (group1, group2, group3) and doses (100 mg/kg, 200

mg/kg and 400 mg/kg) were administered orally according to animal weight respectively. Group 4 were administered loperamide (25 mg/kg, orally), and serves as the reference drug. Each mouse received 0.2 ml of castor oil after 30 minutes. Thirty minutes post-castor oil administration the mice were orally administered freshly prepared standard charcoal meal consisting of 0.2 ml per mouse of a 10% activated charcoal suspension in 5% gum acacia. The mice were humanely sacrificed 30 minutes after receiving the charcoal meal. The distance traveled by the charcoal meal from the pylorus to the ileocaecal junction was measured after the small intestine was cut off. The full length of the small intestine was also measured. The peristaltic index for each mouse was then calculated as a percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine.

3.8. Entero-pooling Assay

Robert *et al*. approach was used to calculate intraluminal fluid buildup. The mice were divided into four groups of six each. Group 1 was given a placebo (distilled water), while Group 2 was given castor oil (2ml). One hour before the oral administration of castor oil, groups 3 and 4 were given extracts of 400, 200, and 100 mg/kg of the plant extract, respectively. The mice were sedated with chloroform and sacrificed two hours later. A thread was tied around the margins of the small intestine, and the intestine was removed and weighed. The volume of the intestinal content was measured after pressing it into a graduated tube. The intestine was reweighed, and the difference in weight between filled and empty intestines was calculated

3.9. Statistical Analysis

Results were expressed as mean ±S.D. Statistical analysis of the data was done using one–way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Graph pad prisms 8.2 software was used for all analysis of data obtained.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis

The preliminary phytochemical analysis study *Chromolaena ordata* and *Vernonia amygdalina* aqueous extract biherbal formulation showed the presence of tannins, saponins, flavonoids, steroids, terpenoids, eugenols, alkaloids, polyphenols, Cardiac glycoside, flavonoids, phenolics, eugenols, and cardiac glycosides were plentiful in the biherbal formulation's aqueous extract as shown Table 4.1.

4.2 Acute Toxicity Test

Oral administration of graded doses (1000, 2000 and 4000 mg/kg) of the aqueous extract biherbal formulation of *Chromolaena ordata* and *Vernonia amygdalina* to mice did not produce any significant change in breathing, behavior, sensory nervous system responses, cutaneous effect or gastrointestinal effect during the observation period. No mortality was recorded in any group after 72h of administering the extract to the animals.

Effect of the Aqueous Extract of biherbal formulation of *Chromolaena ordata* and *Vernonia amygdalina* and Castor oil-induced diarrhea

In the castor oil induced diarrhea experiment where the mice that did not receive the plant extract, the mice showed typical diarrhea sign such as watery and frequent defecation. The extract of the biherbal formulation of *Chromolaena* and *Vernonia amygdalina* produced a marked anti-diarrheal effect in mice. Both the doses of extract significantly decreased (Table 4.3). The average weight of feces in the control group was 7.35 g. Treatment with both doses of extract significantly reduced

CHAPTER FIVE

5.0. DISCUSSION

5.1. Acute Toxicity Study

Acute toxicity assesses the antagonistic impacts that happen subsequently contact of animals with one or different dosages of a test substance in no less than 24 hours by an identified mode of entry (inward breath, dermal and oral) (Saganuwa, 2016). Subsequent administration, of the substance required for the test, is retained and dispersed to different parts of the body before it evokes a fundamental unfavorable impact (Erhirhie *et al*. 2018). Acute toxicity studies involving *Chromolaena* and *Vernonia amygdalina* are essential for comprehending its potential risks and benefits. This typically involve administering various doses of aqueous biherbal formulation of *Chromolaena* and *Vernonia amygdalina* extract to laboratory mice, and closely monitoring their responses. The objective is to establish the LD50 (lethal dose for 50% of the test population) and gain insights into the potential effects on vital organs, biochemical markers, and behavioral changes by helping to establish safe dosages for medicinal applications while also highlighting the hazards associated with misuse. Acute toxicity was really looked at after a portion (1000 and 2000 mg/kg) of mice separated into various section. The mice were kept, an eye on for a time of 72 days. No mortality was documented throughout the said time span, and the mice encountered no unusual changes in their way of behaving. Typical physiology and every one of the faculties was unblemished which showed that biherbal formulation of *Chromolaena* and *Vernonia amygdalina* was protected to up to 4000 mg/kg without any chance of any sort of toxicity. Administration of plant extract leaves at a concentration (1000 to 4000 mg/kg) resulted in no risk of any form of toxicity. The observation of no death in the mice supports their findings.

5.2. Phytochemical Evaluation

A major source of nutrients and a component of the human diet are plants. Carbohydrates, proteins, vitamins, cholesterol-lowering chemicals, antioxidants, and other significant sources of bioactive substances are provided by them. Many of the nutritional qualities of plants have been described in the literature, but little research has been conducted on the bioactive chemicals found in them. Phytochemicals are the name given to these bioactive molecules. These phytochemicals are found in all parts of the plant, including the flowers, stems, fruits, roots, leaves, and seeds. These phytochemicals are employed as is and as source materials for numerous other therapeutically significant substances (Balamurugan *et al*., 2019).

5.2.1. Tannins

They are secondary polyphenolic compounds found in higher plants. The comparable polyphenolic natural compounds lesser plants like algae or the animal kingdom have yet to be identified. The polyphenolic structure of secondary metabolites of higher plants is a necessary but not sufficient condition for the inclusion of tannins (Khanbabaee and Van Ree, 2001). In nature, tannins are found throughout the world in many different families of higher plants (Cuong *et al*., 2019).

5.2.2. Alkaloids

Alkaloids are important in both human medicine and the body's natural defenses. Alkaloids account for approximately 20% of all secondary metabolites discovered in plants (Kaur and Arora, 2015). Alkaloids generally have marked biological activity as a result, they are assumed to play a crucial role in plant-environment interaction. (Defends plants from predators and govern their growth in plants). Alkaloids and plant extracts containing

alkaloids have been used throughout human history as remedies, poisons, psychoactive drugs, and are well known therapeutically as anesthetic, cardioprotective, and anti-inflammatory drugs (Chik *et al*., 2013; fester, 2010) . Morphine, strychnine, quinine, ephedrine, and nicotine are examples of well-known alkaloids utilized in clinical contexts (Heinrich *et al.,* 2021).

5.2.3. Saponins

Saponins are a class of secondary metabolites defined by the presence of a triterpene or steroidal aglycone and one or more sugar chains. Saponins occur naturally compounds and are found in many plants. They have a wide range of biological activities. Saponins occur constitutively in many plant species as part of their defense system (Güçlü-Üstündağ, 2009).

5.2.4. Cardiac glycosides

Cardiac glycosides, which are found in minute levels in plant seeds, leaves, stems, roots, and bark, have a wide geographical range. Many species thrive in tropical locations and were previously utilized for a variety of functions by people in Africa, Asia, and South America. Cardiac glycosides are a distinct class of secondary metabolites that are thought to be among the most effective in treatments and therapeutics (Morsy, 2017).

5.2.5. Flavonoids

Flavonoids, which are secondary metabolites found in high concentrations in plants, fruits, and seeds, are responsible for the color, scent, and flavor of these objects. In plants, flavonoids carry out a variety of tasks including controlling cell growth, luring insects for pollination, and defending against biotic and abiotic stressors (De Luna *et al.,* 2020; Dias *et al*., 2021). These substances have a wide range of bioactive qualities that are linked to health benefits in humans according to recent research, including anti-inflammatory, anti-cancer, anti-aging, cardio-protective, neuroprotective, immunomodulatory, anti-diabetic, anti-

bacterial, anti-parasitic, and antiviral effects (Saini *et al*., 2017; Jucá *et al*., 2020; Fraga *et al*., 2019).

5.2.6. Eugenols

A volatile phenolic component called eugenol is found in the buds and leaves of the plant. It is a useful ingredient of many items that have been used in small quantities in the culinary, cosmetic and pharmaceutical industries. Its derivatives have been used as local anesthetics and antiseptics in medicine. Eugenol has many biological effects, including antioxidant, analgesic, and anti-inflammatory properties. Although eugenol is a substance that is generally considered safe, there have been concerns recently about its toxicity due to its wide range of uses and uses (Nejad *et al*., 2017).

5.2.7. Terpenoids

Terpenes are simple hydrocarbons, whereas terpenoids are terpenes that have been changed with various functional groups. Terpenoids are classified according to their carbon units as monoterpenes, sesterpenes, diterpenes, sesquiterpenes, and triterpenes. The majority of terpenoids with structural modifications are physiologically active and are employed globally to treat a wide variety of diseases. Many terpenoids (Taxol and its derivatives), inhibit a wide range of human cancer cells and are used to treat cancer. Terpenes and derivatives like artemisinin and related chemicals, are employed as anti-malarials. Meanwhile, terpenoids have a variety of roles in meals, medications, cosmetics, hormones, vitamins, and other products. (Perveen and Al-Taweel, 2018).

5.2.8. Phenolics

The antioxidant, structural, attractant, signaling, and protective properties of phenolic compounds (PCs) in plants are crucial for the regulation of growth (Babenko *et al*., 2019). Due of the possible health advantages indicated in a number of studies, phenolic compounds

are among the most researched natural substances. In recent years, natural phenolic compounds have been related to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardio-protective, anti-arthritic and antimicrobial effects (Bhuyan and Basu, 2017).

5.2.9. Steroids

Plant steroids are a distinct group of chemical substances found in both the animal and plant kingdoms. Plant steroids include a variety of medical, pharmacological, and chemical properties, including anticancer, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone, sex hormone, anthelmintic, cytotoxic, and cardiogenic activity (Patel and Savjani, 2015).

5.3. Anti-diarrheal Evaluation

Diarrhea is commonly thought to be caused by impaired motility and fluid collection in the gastrointestinal system. The objective of the diarrheal test is to assess the impact of an aqueous extract derived from a biherbal formulation containing Chromolaena ordata and Vernonia amygdalina on various parameters, including castor oil-induced gastrointestinal transit time, castor oil-induced diarrhea, normal intestinal transit time in mice, and an entero-pooling assay. Castor oil is a triglyceride with a high amount of ricinoleic acid, a hydroxylated unsaturated fatty acid (Saalmüller *et al*., 1846). 90% of the ricinoleate in castor oil is mostly responsible for diarrhea formation (Mckeon *et al*., 1999). After ingesting castor oil orally, lipases present in the intestinal lumen release ricinoleic acid, and this acid is subsequently absorbed in substantial quantities. When ricinoleate is present in the small intestine, the peristaltic activity of the small intestine rises due to changes in the permeability of Na+ and Cl in the intestinal mucosa (Palombo, 2006). Ricinoleate also stimulates endogenous prostaglandin secretion. Prostaglandins belonging to the E series are believed to be potent inducers of diarrhea in both experimental animals and humans. Prostaglandin

biosynthesis inhibitors are thus thought to postpone castor oil-induced diarrhoea (Sorin *et al*., 2012). Prostaglandins are linked to changes in the gut that cause diarrhea.As per a recent research study, ricinoleic acid found in castor oil triggers smooth muscles contractions in the intestines, and this effect is achieved through EP3 receptors activation located on the intestine's smooth muscle. Numerous anti-diarrheal medications function by reducing gastrointestinal motility and/or secretions. Prostaglandin biosynthesis inhibitors postpone castor oil-induced diarrhea (Brijesh *et al*., 2009). Significant anti-diarrheal efficacy is demonstrated by an aqueous extract of a biherbal combination of Chromolaena and Vernonia amygdalina. Anti-diarrheal action has been documented for plant extracts containing alkaloids, steroids, tannin, saponins, and flavonoids (Shemsu *et al*., 2013; Balaji *et al*., 2012).

RECOMMENDATION

The biherbal mixture including *Chromolaena odorata* and *Vernonia amygdalina* has significant anti-diarrheal results. In diarrhea-induced model (castor oil) in mice, the observed reduction in diarrhea severity and improved intestinal transit time are encouraging signs of efficacy. However, the move from preclinical to clinical studies involves more research to determine optimal doses, modes of action, safety profiles, and potential interactions

CONCLUSION

The biherbal mixture including *Chromolaena odorata* and *Vernonia amygdalina* was proven to have significant anti-diarrheal activity. Further research and development in this area have the potential to positively impact public health outcomes and improve the quality of life for individuals suffering from diarrhea.

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